Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2008

If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October.
If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number: **DAQ131**

Project Title: Improved understanding of the damage, ecology and management of mirids and stinkbugs in Bollgard® II

Project Commencement Date: 1 July 2004 **Project Completion Date:** 30 June 2008

CRDC Program: 3 Crop Protection

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Part 3 – Final Report Guide (due 31 October 2008)

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

Background

1. Outline the background to the project.

In recent years mirids and stinkbugs have emerged as important sucking pests in cotton. While stinkbugs are causing damage to bolls, mirids are causing damage to seedlings, squares and bolls. With the increasing adoption of Bollgard®II and IPM approaches the use of broad-spectrum chemicals to kill *Helicoverpa* has been reduced and as a result mirids and stinkbugs are building to levels causing damage to bolls later in crop growth stages. Studies on stinkbugs by Dr Moazzem Khan revealed that green vegetable bug (GVB) caused significant boll damage and yield loss. A preliminary study by Dr Khan on mirids revealed that high mirid numbers at later growth stages also caused significant boll damage and that damage caused by mirids and GVB were similar. Mirids and stinkbugs therefore demand greater attention in order to minimise losses caused by these pests and to develop IPM strategies against these pests to enhance gains in IPM that have been made with *Bt*-transgenic cotton. Progress in this area of research will maintain sustainability and profitability of the Australian cotton industry.

Mirid damage at early growth stages of cotton (up to squaring stage) has been studied in detail by Dr Khan. He found that all ages of mirids cause damage to young plants and damage by mirid nymphs is cumulative. Maximum damage occurs when the insect reaches the 4th and 5th nymphal stages. He also found that mirid feeding causes shedding of small and medium squares, and damaged large squares develop as 'parrot beak' bolls. Detailed studies at the boll stage, such as which stage of mirids is most damaging or which age boll is most vulnerable to feeding, is lacking. This information is a prerequisite to developing an IPM strategy for the pest in later crop growth stages. Understanding population change of the pest over time in relation to crop development is an important aspect for developing management strategies for the pest which is lacking for mirids in Bollgard®II.

Predators and parasitoids are integral components of any IPM system and play an important part in regulating pest populations. Some generalist predators such as ants, spiders, damsel bugs and assassin bugs are known to predate on mirids. Nothing is known about parasitoids of mirids. Since green mirid (GM), *Creontiades dilutus*, is indigenous to Australia it is likely that we have one or more parasitoids of this mirid in Australia, but that possibility has not been investigated yet.

The impact of the GVB adult parasitoid, *Trichopoda giacomelli*, has been studied by Dr Khan who found that the fly is established in the released areas and continues to spread. However, to get wider and greater impact, the fly should be released in new locations across the valleys.

The insecticides registered for mirids and stinkbugs are mostly non-selective and are extremely disruptive to a wide range of beneficial insects. Use of these insecticides at stage I and II will minimise the impact of existing IPM programs. Therefore less disruptive control tactics including soft chemicals for mirids and stinkbugs are necessary.

As with soft chemicals, salt mixtures, biopesticides based on fungal pathogens and attractants based on plant volatiles may be useful tools in managing mirids and stinkbugs with less or no disruption. Dr Khan has investigated salt mixture against mirids and GVB. While salt mixtures are quite effective and less disruptive, they are quite chemical specific. Not all chemicals mixed with salt will give the desired benefit. Therefore further investigation is needed to identify those chemicals that are effective with salt mixture against mirids and

GVB. Dr Caroline Hauxwell of DPI&F is working on fungal pathogen-based biopesticides against mirids and GVB and Drs Peter Gregg and Alice Del Socorro of Australian Cotton CRC are working on plant volatile-based attractants against mirids. Depending on their findings, inclusion of fungal-based biopestcides and plant volatile-based attractants in developing a management system against mirids and stinkbugs in cotton could be an important component of an IPM approach.

Objectives

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

    1. Investigate mirid damage at boll formation stage
    2. Investigate population dynamics of mirid in Bollgard®II and alternative hosts
    3. Investigate selective management of mirids and GVB
    4. Rear, release and monitor *Trichopoda*
    5. Investigate plant volatile based attractants and sex pheromones (in collaboration with Drs Gregg and Del Socorro)
    6. Investigate fungal pathogen based biopesticides for mirids (depending on the findings of Dr Hauxwell)
    7. Disseminate mirid and stinkbugs ecology, biology and management information to the industry and to the scientific community

All of these objectives excepting number 6 have been achieved. Objective 6 was not achieved due to the lack of pathogen-based biopesticide material to evaluate in trials. Additional trials were conducted to evaluate kaolin film technology against mirids and stinkbugs and to understand damage caused by pale cotton stainer (CSB).

Methods

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

Damage assessment of mirids in Bollgard®II

A series of trials were conducted both in field and in an outdoor insectary at Dalby, Macalister and Jimbour and at J. Bjelke Petersen Research Station, Kingaroy (KRS) to understand mirid damage at boll formation stage in Bollgard®II, to determine critical damage stage and action thresholds at different crop stage, to compare damage potential of green and brown mirid and to determine susceptible boll age and the most damaging GM stage.

Field trials were conducted in three irrigated and raingrown cotton fields during 2004-06 seasons and in one irrigated cotton field during 2006-07 season. Details of these sites are given in Table 1. Damage was assessed at three different crop growth stages (treatments). There were two more treatments - unsprayed and sprayed throughout the season (fully protected). Plots were sprayed if mirid numbers reached >1/m following an IPM strategy. Treatments were replicated three times. Plot sizes for each trial site are given in Table 1. Sizes of the plots were determined according to the grower's spray boom length for ease of management operations. The stages were squaring (from seedling to 60% plants reached first flower), early boll (from 60% plants reached first flower to 60% bolls reached 20 days old) and late boll (from 20 day old bolls to cut out). Each stage was left unsprayed during the defined time period, but before and after that time treatment plots were sprayed to keep mirids and other insects under control.

Fields were sampled once a week. Up to the 8/9 node plant stage, one 20 m row section/replication was sampled with a suction machine. Collected insects were returned to the laboratory for further processing and for recording data. After the 9 node plant stage, sampling was done on 3 x 1 m row sections/replication using a beat sheet. The beat sheet method was used at this stage since this method was more efficient than any other method for sampling mirids and other predatory insects. For each stage, estimated fruit loss for all stages and boll damage for early and late boll stage, were assessed twice (3 x 1 m row section/replication); one at the last sampling date of the stage and the other at cut out stage. In unsprayed and fully sprayed treatments, damage was assessed every time as with other treatments. Yield was assessed by hand picking, three randomly selected 1 m row sections in each plot. Tight lock and brown lint portions were discarded from the hand harvest since mechanical pickers can not pick tight locks and brown lint has quality implications (Lei *et al*. 2002).

To determine the action threshold at different crop stages, replicated cage trials were conducted on Bollgard®II at KRS with different densities of GM on 1 m row cotton using 1 m x 0.8 m x 1.2 m field cages at different stages of crop growth for three seasons. The treatments (stages) were the same as in the field trial and densities of GM were 0, 1, 2, 3 and 4/cage with 5 replications. Caged plots were marked just after emergence for each stage and sprayed in accordance with an IPM strategy (such as reduced rate fipronil (40 mL/ha Regent®) plus salt) to keep plots insect free until caging. Insects were allowed to feed throughout the stage. Thereafter cotton was sprayed until maturity to avoid further damage. Male GM were used to avoid further build up of the population and dead individuals were replaced. Fruit loss and boll damage were assessed twice for each stage as described for the field trial. Plots were hand-harvested and cotton weight was recorded as in the field trial.

To determine susceptible boll age a replicated field trial using a foam cup cage was conducted at Byee. Boll age was determined by tagging at bloom and plots were sprayed with low rate fipronil to keep bolls free of any damage. Four age groups of bolls - 10, 15, 20 and 25 days old with 10 replications were used. Individual bolls were caged 3 days before treatment application to allow time for any boll drop due to handling. One 5th instar GM nymph from the laboratory culture was confined on a boll for 3 days. Thereafter another 7 days were allowed to develop symptoms and bolls were brought back to the laboratory and checked thoroughly for the number of black spots, warts and damaged locks.

The most damaging stage of mirid was determined using all five stages of nymphs and adult male and females in a replicated trial using foam cup cages in the field at Byee. Bolls were tagged at bloom as described above and sprayed with low rate of fipronil to keep bolls free of any damage. One GM growth stage per boll was confined on a 15 day old bolls for 3 days, with 11 replications per GM growth stage. Damage assessed as black spots on the boll and warty growth inside the boll wall were assessed in the laboratory.

A replicated trial was also conducted to compare GM and brown mirid (BM) damage at the boll and squaring stage. For the boll stage, one GM or one BM male was confined on a 15 day old boll in the glasshouse with 15 replications per treatment. Insects were allowed to feed for 5 days and damage was assessed as described above. For squaring plants the trial was conducted in an outside insectary using whole plants at 14 node growth stage. The trial was replicated 5 times and insects (4 adults, 2 male and 2 female) were confined onto plants using field cages as described above. Insects were allowed to feed for 7 days. Plants were thoroughly mapped both pre and post treatment.

Population dynamics of mirids in Bollgard®II

Regular suction or beat sheet sampling was conducted at six sites representing irrigated and raingrown Bollgard®II on the Darling Downs and at one site at Byee, 60 km north of Kingaroy, during 2004 to 2006 seasons. Because of the drought during the 2006 to 2008 seasons, sampling was conducted only at one irrigated site on the Darling Downs. Drought also prevented to sampling at KRS in 2004 – 05 season and Byee in 2006 – 07 season. At KRS sampling was conducted during 2005 to 2008 seasons. 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 plants grew tall enough to use the beat sheet.

Table 1. Description of trial sites

Growing System	Seasons	Trial site	Variety	Plot size (per replication)	Date of planting
Irrigated	2004-05	1. Glen Fresser (Mayfield), Dalby	Sicot 71BR	100 m X 24 rows	26 Oct. 2004
		2. Peter Bailey, Macalister	Sicot 71BR	100 m X 18 rows	24 Oct. 2004
		3. Neval Walton, Macalister	Sicot 289BR	120 m X 36 rows	25 Oct. 2004
	2005-06	1. Glen Fresser (Mayfield), Dalby	Sicot 289BR	50 m X 24 rows	3 Nov. 2005
		2. Peter Bailey, Macalister	Sicot 71BR	50 m X 24 rows	2 Nov. 2005
		3. Neval Walton, Macalister	Sicot 71BR	50 m X 24 rows	30 Oct. 2005
	2006-07	Glen Fresser (Mayfield), Dalby	Sicot 71BR	64 m X 24 rows	27 Oct. 2006
Raingrown	2004-05	1. Richard Dowsett (Wyobie), Jimbour	Sicot 80B	150 m X 18 rows	22 Oct. 2004
		2. Neil Wegener, Macalister	DP576 BGII	100 m X 20 rows	5 Nov. 2004
		3. Ross Cameron, Nandi	Sicot 289BR	100 m X 24 rows	15 Nov.2004
	2005-06	1. Richard Dowsett (Wyobie), Jimbour	Sicot 289BR	50 m X 24 rows Single skip row	31 Oct. 2005
		2. St. John Kent (Coondarra), Jimbour	Sicot 289BR	20 m X 18 rows Single skip row	1 Nov. 2005
		3. Neil Wegener, Macalister	Sicot 71BR	50 m X 20 rows Double skip row	2 Nov. 2005

In addition, less regular sampling was conducted at St George, Goondiwindi (Carrington), Boggabilla (Morella), Moree (Norwood), and Narrabri (ACRI) to determine species composition. At ACRI a replicated trial with randomised complete block (RCB) design was conducted in collaboration with Dr Robert Mensah to determine if species of mirids varied with different crops. The crops used in the trial were Bollgard®II and conventional cotton, lucerne, soybean, mungbean, pigeon pea, sunflower and sorghum. Mirids, both adults and nymphs, were collected, once in a month, from the above sites to rear at KRS to identify parasitoids.

Selective management options for mirids and stinkbugs

Two trials using dimethoate (Rogor®) at 200 mL/ha (Trial 1) and fipronil (Regent®) at 40 mL/ha were conducted to determine the optimum rate of table salt (NaCl) mixed with reduced rates of chemical. The salt rates were 0, 2, 5, 7 and 10 g/L of water. Four more trials were conducted to evaluate different chemicals mixed with salt against mirids and stinkbugs at KRS, Byee and Warra. Treatments of the trials were summarised in Table 2. In all trials treatments were replicated 3 times in RCB design. Each replication measured 12 to 24 rows wide and 15 to 25 m long. The chemicals were applied with ground rig using 100 to 140 L/ha water depending on the crop stage. Pre-treatment counts (0 days after treatment (DAT)) were made the day before treatments were applied. Post treatment observations were made at 3 and 7 DAT except in one trial during 2004 – 05 season at Byee where post treatment observation was made only at 4 DAT. Pest and beneficial insects were sampled using the beat sheet method on three 1 m sections of row per replication.

Table 2. Treatments and rates used in chemical trials (Trials 1 – 3 against mirids and Trial 4 against pale cotton stainer)

Trial	Treatment	Formulation (g/L)	Rate (mL/ha)
1	Control	Untreated	
	Intruder	Acetamiprid 225 SL	100
	Intruder + Salt	Acetamiprid 225 SL + NaCl	100 + 10 g/L of water
	Rogor	Dimethoate 400 EC	150
	Rogor + Salt	Dimethoate 400 EC + NaCl	150 + 10 g/L of water
	Rogor	Dimethoate 400 EC	500
	Canopy	Parafinic Oil 792 EC	2% v/v
	Canopy + Salt	Parafinic Oil 792 EC + NaCl	2% v/v + 10 g/L of water
	Steward	Indoxacarb 200 SC	400
	Steward + Salt	Indoxacarb 200 SC + NaCl	400 + 10 g/L of water
	Steward	Indoxacarb 200 SC	800
	Regent + Salt	Fipronil 200 SC + NaCl	40 + 10 g/L of water
	Salt	NaCl	10 g/L of water
2	Steward	Indoxacarb 150 EC	350
	Steward	Indoxacarb 150 EC	800
	Steward + Salt	Indoxacarb 150 EC + NaCl	350 + 7 g/L of water
	Intrepid	Chlorfenapyr 360 SC	250
	Intrepid	Chlorfenapyr 360 SC	500
	Intrepid + Salt	Chlorfenapyr 360 SC + NaCl	250 + 7 g/L of water
	Regent + Salt	Fipronil 200 SC + NaCl	40 + 7 g/L of water
	Control	Untreated	
3	Calypso	Thiacloprid 480 SC	100
	Calypso + adjuvant	Thiacloprid 480 SC + pulse	100 + 0.2% v/v
	BAS 320	Unregistered product	1500
	Regent + Salt	Fipronil 200 SC + NaCl	40 + 7 g/L of water
	Control	Untreated	
4	Control	Untreated	
	Decis	Deltamethrin 27.5 EC	700
	Decis	Deltamethrin 27.5 EC	400
	Decis + Salt	Deltamethrin 27.5 EC + NaCl	400 + 10 g/L of water
	Steward	Indoxacarb 150 EC	850
	Steward	Indoxacarb 150 EC	400
	Steward + Salt	Indoxacarb 150 EC + NaCl	400 + 10 g/L of water
	Regent	Fipronil 200 SC	125
	Regent	Fipronil 200 SC	60
	Regent + Salt	Fipronil 200 SC + NaCl	60 + 10 g/L of water

Salt mixture verification trial

A large scale trial involving a consultant and grower was conducted to verify salt mixture technology. The non-replicated trial was conducted in a 67 ha irrigated cotton field near Dalby. The field of skip row Bollgard®II cotton, variety Sicot 71BR, was planted on 17 October, 2007 and divided into three blocks to accommodate 3 treatments. Blocks 1 and 2 were each 4 ha and block 3 comprised the remainder of the field. Treatments 1 and 2 were predetermined salt/insecticide mixtures and treatment 3 was the grower's commercial practice (Table 3). Cotton was sprayed 3 times (Table 4) according to consultant's and grower's decision. All other operations such as irrigation, fertilization, etc. were constant across each of the three blocks.

The field was sampled weekly using a suction machine across 5 X 20 m lengths of row in each of block 1 and 2 and 8 X 20 m lengths of row in block 3, until the crop was at the 8 node stage. Thereafter a beat sheet was used to sample 8 X 1 m lengths of row in each of block 1 and 2 and 12 X 1 m lengths of row in block 3. Plants were mapped on 3 x 1 m lengths of row in each block, to assess fruit loss and boll damage at squaring stage on 18/12/07 and 02/01/08; at early boll stage on 15/01/08 and 21/2/08; at late boll stage on 25/3/08 and at harvest on 17/4/08.

Since the field was also infested with GVB and CSB in the later part of the season, damage assessments were made on 12/03/08 to determine if damage from these pests varied with the treatments. First position bolls on the 7th node (counted from 1st unfolded leaf), 100 bolls per treatment, were randomly selected and assessed in the laboratory for damaged locks. Lock damage was recorded in 3 categories as low (≤ 25% lint of a lock is damaged), medium (25 -50% lint of a lock is damaged) and high (≥ 50% lint of a lock is damaged). Data were analysed as percentage of boll damage for each category if at least one lock was damaged for such a category.

Cotton was harvested with a 4 row picker on 09/05/08 from 2.76, 2.72 and 2.70 hectares in treatments 1, 2 and 3 respectively.

Table 3. Treatments used in the salt mixture verification trial

Treatments	Comments
T1. Dimethoate 250 mL/ha plus salt (10 g/L) Fipronil 40 mL/ha plus salt (10 g/L) Fipronil 50 mL/ha plus salt (10 g/L)	Fourth spray would be grower's practice if needed. However, previous experience showed that 2 to 3 sprays were required to manage mirids in the field.
T2. Dimethoate 250 mL/ha plus salt (10 g/L) Dimethoate 250 mL/ha plus salt (10 g/L) Fipronil 50 mL/ha plus salt (10 g/L)	
T3. Fipronil 50 mL/ha Fipronil 50 mL/ha Fipronil 80 mL/ha	Grower's practice

Table 4. Spray detail in the salt mixture verification trial

Date of spray	Crop stage Days after sowing (DAS)	Treatment	Chemical	Rate (mL/ha)	Spray volume (L/ha)
15/12/07	Early Squaring 59 DAS	1 2 3	Dimethoate 400EC Dimethoate 400EC Fipronil 200SC	250 250 50	80 80 80
31/12/07	Squaring 75 DAS	1 2 3	Fipronil 200Sc Dimethoate 400EC Fipronil 200SC	40 250 50	80 80 80
20/02/08	Maximum Boll 126 DAS	1 2 3	Fipronil 200SC Fipronil 200SC Fipronil 200SC	50 50 80	100 100 100

Release and monitor GVB parasitoid

The GVB parasitoid, *Trichopoda giacomellii*, was reared and multiplied at KRS to make further releases in areas where activities of the parasitoid had not been previously recorded, such as St George. The rearing was initiated from parasitised GVB collected at Byee. During 2004 - 06 seasons three releases were made in St George. Both adult fly and parasitoid pupae were released in a pigeon pea field where GVB were present. Each time at least 20 pairs of adults and 3 lots of pupae (25 per container) were released. Parasitoid activities were monitored in the established areas by regular sampling of the GVB population at several locations on the Darling Downs and South Burnett district.

Evaluate plant volatile based attractant and GM sex pheromone

Trials were conducted in collaboration with Drs Peter Gregg and Alice Del Socorro of UNE. One replicated trial with attractant (Magnet®) was conducted at Byee during 2004-05 season. Since this attractant was found to be ineffective for mirids, trial work was discontinued. Two trials with GM sex pheromone were conducted at Byee and Dalby during 2005-06 and 2007–08 seasons to evaluate if pheromone can be used as a monitoring tool. Four traps (lure plus pesticide strip) were put in four corners (at least 50 m in from edges) above the plant canopy. Traps were cleared twice a week and catch was recorded. The lure and pesticide strip were replaced after 6 weeks. Six 1 m visual counts and 6 X 20 m suction samples were made twice a week. Collected mirids were kept on 70% alcohol and was sent to Dr Del Socorro for further assessment.

Evaluate kaolin based particle film technology

Two choice tests in the insectary and one small scale field trial at KRS were conducted to evaluate the effectiveness of kaolin mixed with petroleum spray oil (PSO) against mirids and stinkbugs.

The kaolin formulation used in these trials was Surround™ WP (SWP) from USA (Englehard Corp., Iselin, NJ) and the PSO was *n*C27 petroleum spray oil (Caltex Canopy®). The treatments used in the trials are presented in Table 5.

Table 5. Treatments used in the kaolin film technology trials

Treatments	Rate
1. SWP	60 g /L of water
2. SWP + PSO	60 g/L of water + 2% v/v
3. PSO	2% v/v
4. Regent + salt	40 mL/ha + 10 g/L of water
5. Unsprayed control	

<u>Quadruple Choice Tests</u>

Two trials, each for mirids and GVB, were conducted using treatments 1, 2, 3 and 5 as described in the Table 5 with 10 replications. Bolls aged 10–15 days were coated uniformly with the products using a hand sprayer. After drying for an hour, treated bolls were placed, each into one corner of a feeding arena (aquarium 31 cm long X 20 cm wide X 19 cm deep with a nylon mesh lid). One adult mirid or GVB was confined into the feeding arena to select bolls on which to feed. Insects were starved overnight before release. Observations were made every hour after release for 6 hours and daily thereafter for 3 days for mirids and 5 days for GVB.

<u>Field Trial</u>

The trial was conducted in Bollgard®II using five treatments (Table 5) with three replications. Each replication measured 5 m X 4 rows. The products were applied with a knapsack sprayer using 250 to 500 L/ha water. Treatments were applied four times starting from squaring (when 50% plants had first square) to maturity (first boll open in any of the treatment plots). External boll damage (black spots) was assessed twice and internal damage (lint damage/tight lock) was assessed once just before harvest. Mirids and stinkbugs numbers were assessed 6 times after first spray using a beat sheet, 2 X 1 m per replication. Yield was assessed by hand picking 3 X 1 m row sections in each plot. Tight lock and brown lint portions were discarded from the hand harvest as mechanical pickers can not pick tight locks and brown lint has quality implications.

Pale cotton stainer damage

Pale cotton stainer became a major problem during 2007–08 seasons and for the first time in Australian cotton reached damaging levels. As a quick response to this problem, I conducted a foam cup cage trial in KRS using bolls of different age to understand their damage. Three age groups of undamaged bolls, young (≤ 15 days), mature (≥ 25 days) and open bolls were selected and 4 densities (0, 1, 3 and 5 per boll) of adult CSB were confined onto bolls for 7 days. Young and mature bolls were selected by feeling with hand (soft/hard) and for each age group 10 bolls were used. Lint damage was assessed as the number of locks damaged per boll. Each damaged lock was assessed in 3 categories such as ≤ 25%, 25 – 50 % and >50% damage. For easy analysis each damage category was assigned damage score as follows:
No damage = 0; each lock ≤ 25% damage = 1; each lock 25 – 50% damage = 2; each lock >50% damage = 3.

Statistical analysis

Data on damage assessment were pooled for each season and then for the 3 years for analysis. Unless otherwise stated all data were subjected to one-way analysis of variance and where necessary means were separated by using Fisher's least significance difference test at the 5% level (MINITAB statistical package, Ryan *et al.* 1992). Regression analysis was also performed on some data set combinations as appropriate.

Results

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

Damage assessment of mirids in Bollgard II

Irrigated cotton

The results on three years pooled data showed that mirid numbers in the unsprayed control at squaring and early boll stage were significantly higher (*P<0.05*) than the sprayed control. At late boll stage difference between late boll and sprayed control was not significant (*P>0.05*) (Figure 1). When data were analysed within seasons, differences between treatments were significant only at early boll stage in all seasons and during 2005-06 season at squaring stage (Figure 1). At late boll stage and during 2004-05 and 2006-07 seasons at squaring stage mirid numbers was low, less than 1/m. At late boll stage the highest mirid number was 0.75/m in the unsprayed control during 2005-06 season. At early boll stage, high mirid number in all three seasons perhaps is indicative of their preference for squares and small bolls.

At squaring stage both for three years pooled and within season results showed that percentage fruit loss at observation 1 in squaring and unsprayed control was significantly higher (*P<0.05*) than sprayed control (Figure 2). Percent fruit loss at squaring stage in different seasons was 10 to 35%. Highest fruit loss occurred at this stage in 2005-06 season as with mirid number (see Figure 2 and 1). For observation at cut out (Obs. 2) percent fruit loss was significantly lower (*P<0.05*) at squaring and sprayed control than unsprayed control (Figure 2) indicating the plant's ability to compensate completely. However, plant compensation was partial when percent fruit loss at observation 1 was ≥ 30% as in 2005-06 season (Figure 2).

Three years pooled data showed that at early boll stage in observation 1, highest fruit loss was 29% in early boll stage and was significantly higher (*P<0.05*) than the sprayed control (Figure 3). Within season analysis showed that fruit loss at early boll stage was higher (35 – 40%) in 2004-05 and 2005-06 seasons than in 2006-07 season when fruit loss was 12% and in that season at both observations differences between treatments were not significant (*P>0.05*). Within season analysis showed that for observation 1 percent fruit loss was significantly higher (*P<0.05*) in early boll stage and unsprayed control than sprayed control in all seasons except in 2006-07 season and in observation 2 (at cut out) this trend remained the same (Figure 3). Thus even after plant compensation for damage at early boll stage, plants could not recover fully.

At late boll stage, both three years pooled data and within season data showed that fruit loss both at observation 1 and 2 between late boll and sprayed control was not significantly different (*P>0.05*) except in 2005-06 season when at observation 1 percent fruit loss in late boll was significantly higher (*P<0.05*) than sprayed control (Figure 4). At late boll stage overall mirid number was low (see Figure 1 and population dynamic section). Fruit loss was compounded by other factors such as crop physiology, water stress and temperatures.

Figure 5 shows yield (bales/ha) at different stages for each year and for three years pooled data. The results showed that yields at squaring and late boll stage were significantly higher (*P<0.05*) than unsprayed control and almost similar to sprayed control. At early boll stage, yields were significantly lower (*P<0.05*) than sprayed control in all seasons. Except in 2005-06 season, yield difference was not significant (*P>0.05*) between early boll and unsprayed control. The results suggest the plant's inability to compensate fully for damage incurred at the early boll stage.

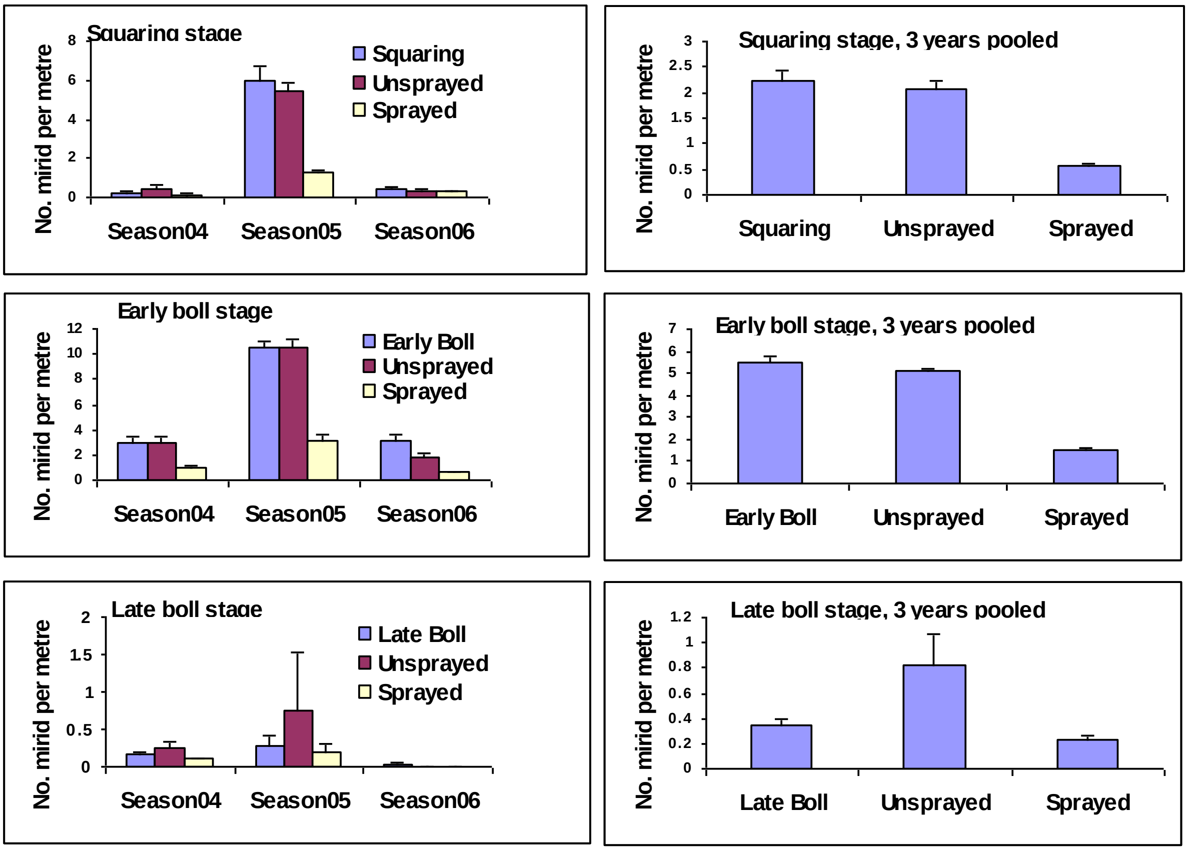


Figure 1. Number of mirids in different treatments at different crop stage in irrigated Bollgard®II cotton. Error bars indicate standard error of means.

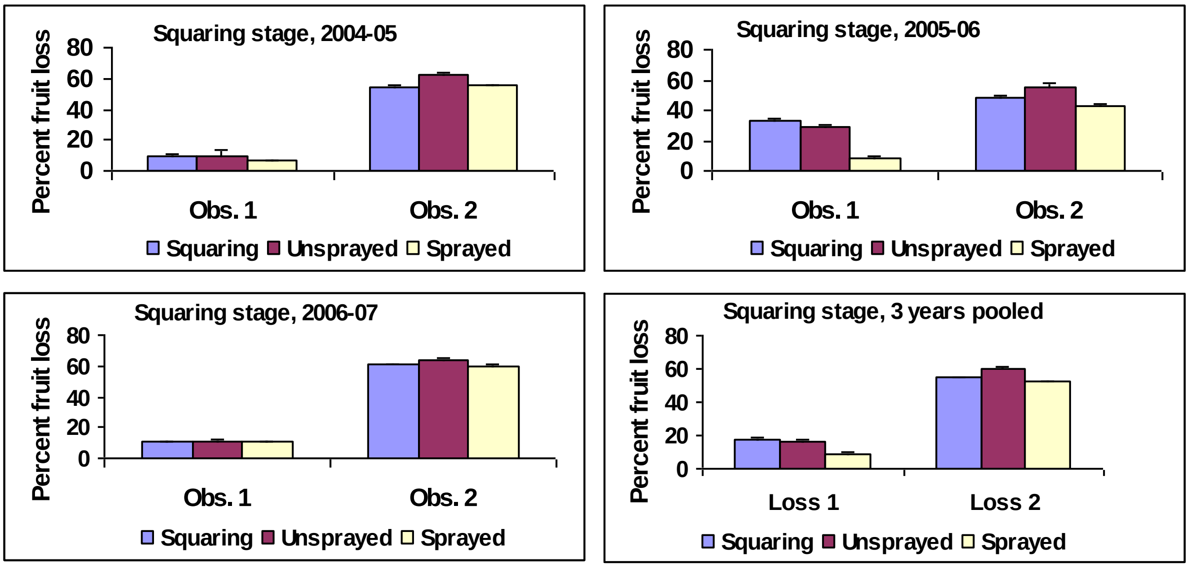


Figure 2. Percent fruit loss at squaring stage (obs. 1) and at cut out (obs. 2) in irrigated Bollgard®II cotton. Error bars indicate standard error of means.

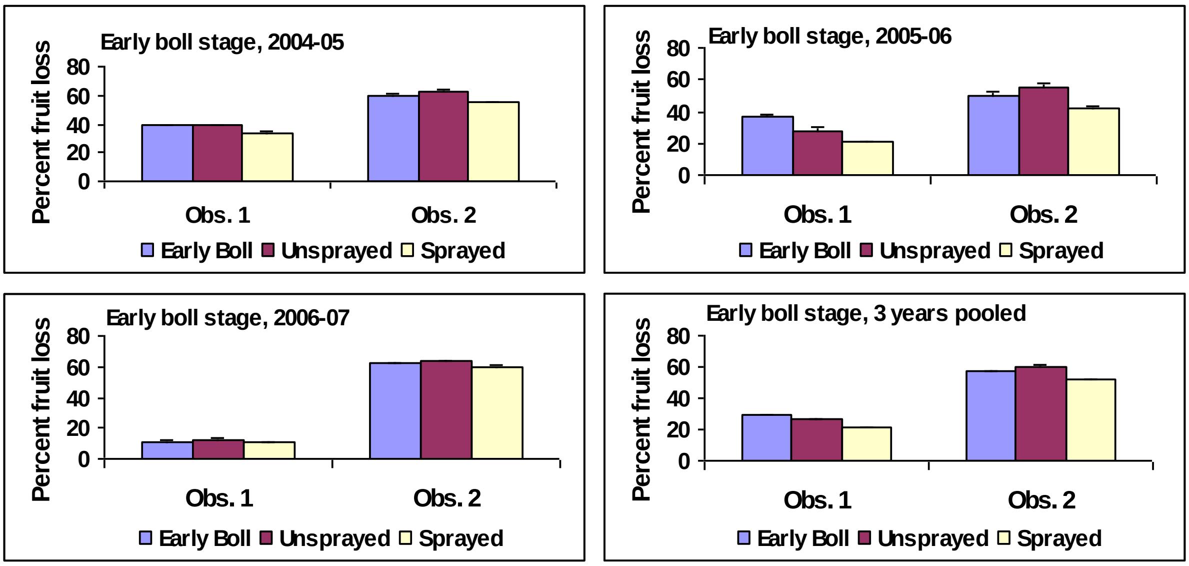


Figure 3. Percent fruit loss at early boll stage (obs. 1) and at cut out (obs. 2) in irrigated Bollgard®II cotton. Error bars indicate standard error of means.

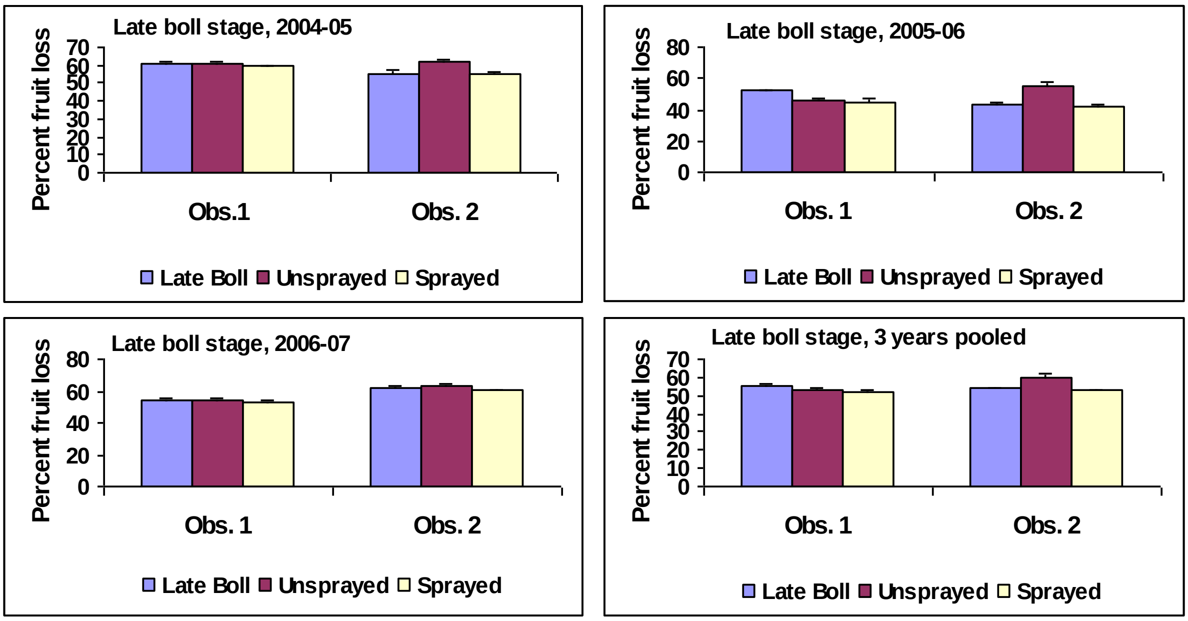


Figure 4. Percent fruit loss at late boll stage (obs. 1) and at cut out (obs. 2) in irrigated Bollgard®II cotton. Error bars indicate standard error of means.

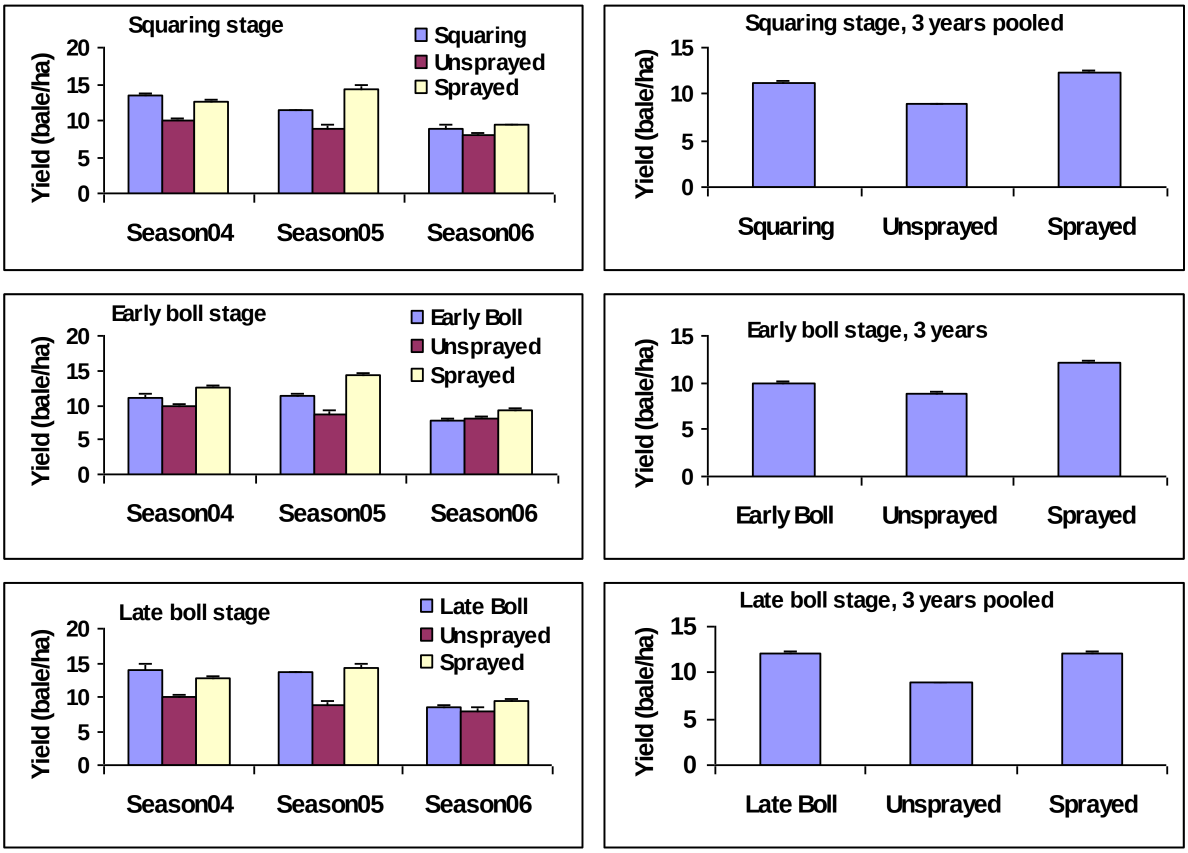


Figure 5. Yield (bale/ha) at each stage in different seasons. Error bars indicate standard error of means.

Dryland cotton

Mirid numbers for different seasons and two years pooled data are given in Figure 6. Mirid number in dryland cotton was lower than in irrigated cotton except in squaring stage during 2004-05 season. At squaring stage in both seasons mirid numbers were significantly higher ($P<0.05$) in squaring and unsprayed control than sprayed control. At early and late boll stage, however, numbers in unsprayed control were significantly higher (*P<0.05*) than the other two treatments in all seasons. Two years pooled data also show a similar trend.

Both at squaring and early boll stage in most cases the percent fruit loss at observation 1 in squaring and unsprayed control was significantly higher (*P<0.05*) than the sprayed control. The trend remained the same at observation 2 (Figure 7). However, during 2004-05 season in observation 1 at squaring and early boll stage and at observation 2 in early boll stage, differences between treatments were not significant (*P>0.05*). Both two years polled and within season data showed that at late boll stage at both observation 1 and 2 percentage fruit loss in unsprayed control was significantly higher (*P<0.05*) than late boll except in observation 1 during 2004-05 season and in observation 2 during 2005-06 season (Figure 7).

Both 2 years pooled and within season data showed that at squaring stage yield in squaring and sprayed control was significantly higher (*P<0.05*) than unsprayed control (Figure 8). At early boll stage however yield in sprayed control was significantly higher (*P<0.05*) than early boll and unsprayed control and there was no significant difference (*P>0.05*) between early boll and unsprayed control (Figure 8). At late boll stage 2 years pooled data analysis showed that yield in late boll and sprayed control was significantly higher (*P<0.05*) than unsprayed control. Within season analysis showed that in both seasons yield in late boll was not significantly different (*P>0.05*) from unsprayed or sprayed control (Figure 8).

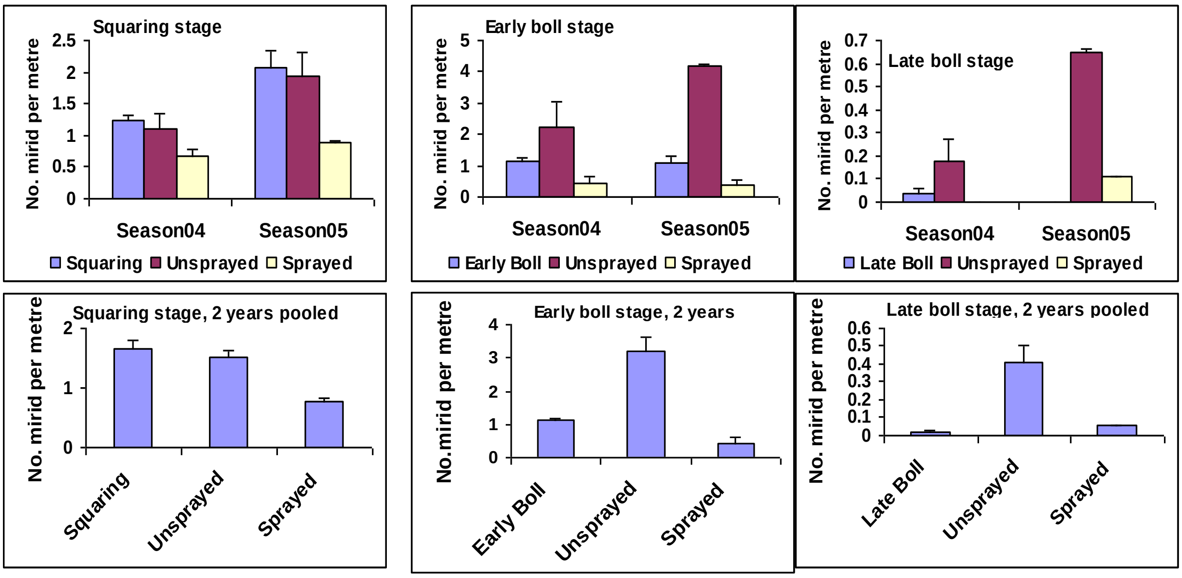


Figure 6. Number of mirids in different treatments at different crop stage in dryland Bollgard®II cotton. Error bars indicate standard error of means.

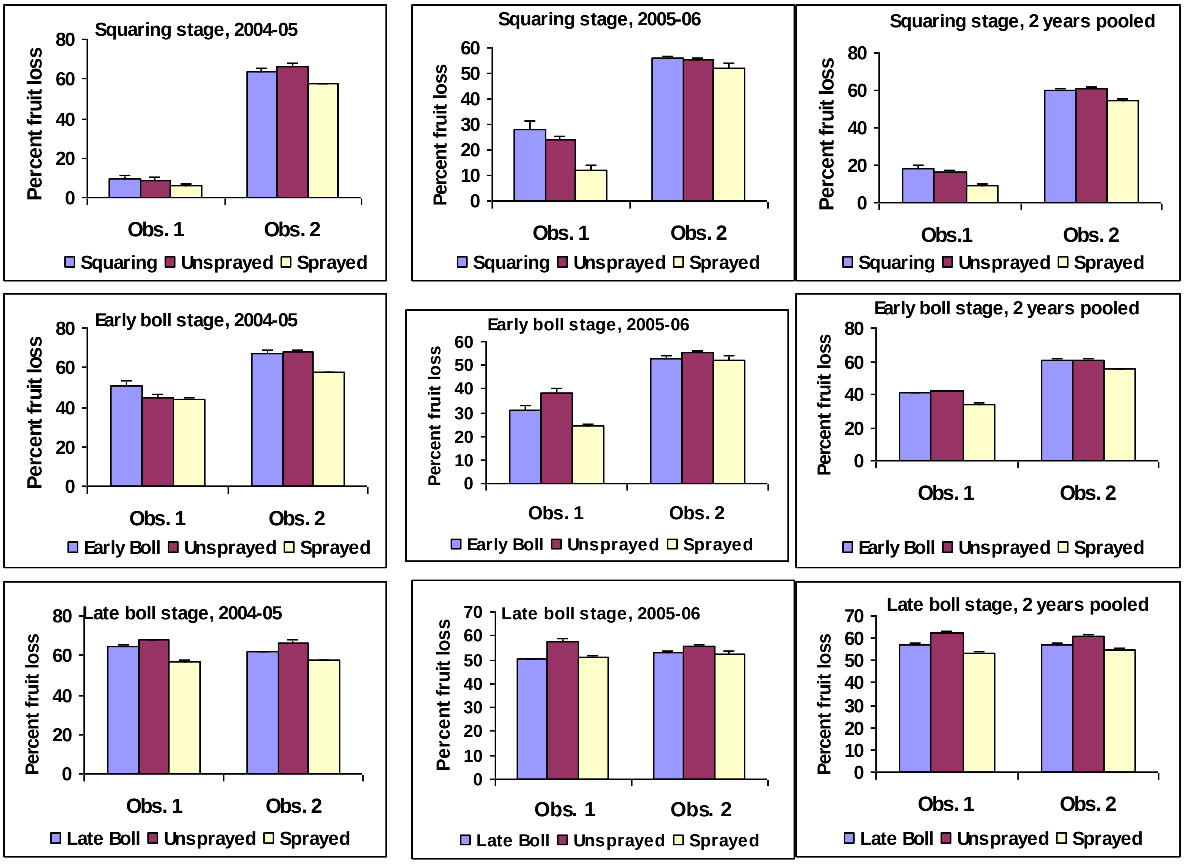


Figure 7. Percent fruit loss at each stage (obs. 1) and at cut out (obs. 2) in dryland Bollgard®II cotton. Error bars indicate standard error of means.

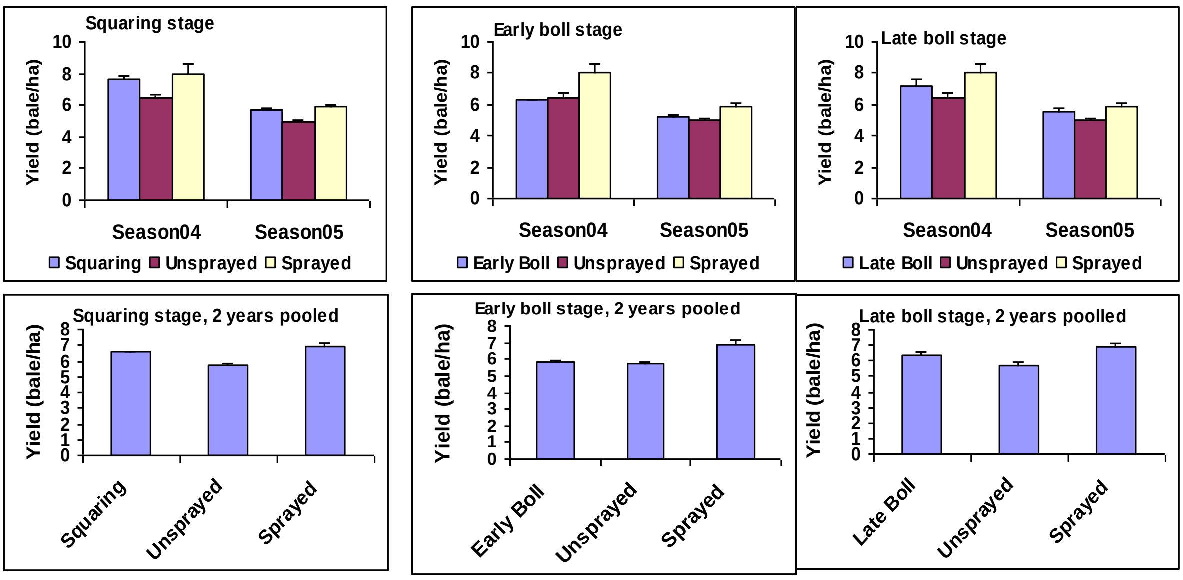


Figure 8. Yield (bale/ha) at each stage in different seasons. Error bars indicate standard error of means.

Action threshold for mirids at different crop stage

Three years pooled data on mirid density and damage at different crop stage from field cage trials are presented in Table 6. Analysis revealed that differences between treatments for both squaring and early boll stage are highly significant (*P<0.05*). At squaring stage 3 mirids and at early boll stage 2 mirids per metre caused significantly less yield compared to control (no mirid). At late boll stage however differences between treatments were not significant (*P>0.05*) and therefore further analysis was not carried out for this stage.

Table 6. Yield (bale/ha) at different crop stage from field cage trial. Three years data pooled.

Mirid per metre	Yield (bale/ha) ± SE Squaring stage	Yield (bale/ha) ± SE Early boll stage	Yield (bale/ha) ± SE Late boll stage
0	6.9 ± 0.20 a	5.6 ± 0.11 a	8.0 ± 0.45 a
1	6.7 ± 0.18 a	5.4 ± 0.07 a	7.9 ± 0.47 a
2	6.4 ± 0.13 ab	5.1 ± 0.05 b	7.7 ± 0.19 a
3	6.0 ± 0.19 bc	4.6 ± 0.07 c	7.4 ± 0.29 a
4	5.7 ± 0.22 c	4.2 ± 0.12 d	7.3 ± 0.30 a

Means in a column followed by different letter are significantly different at *P<0.05*

When regression was performed on the data at squaring and early boll stage a significant relationship (*P<0.05*) between mirid density and yield was obtained (Figure 9). The regression equations for squaring and early boll stage were Y = - 0.30X + 6.96 (R^2 = 0.59) and Y = - 0.37X + 5.74 (R^2 = 0.88) respectively. For both squaring and early boll stage insects were allowed to feed for 6 weeks. The damage factors in the equations were for 42 days feeding by a mirid i.e. for one day feeding damage factors are 0.0073 and 0.0088 for squaring and early boll stage respectively. Using these damage factors in a classical ETL model which considers two more factors such as spray cost ($15/ha, reduced rate fipronil plus salt) and value of the cotton ($450/bale), I calculated ETL for mirids as 4.6 and 3.8 mirids per metre for squaring stage and early boll respectively. Since sometimes in the field the relationship between mirid numbers and damage is unreliable, I propose mirid action thresholds for irrigated cotton of 4 and 3 mirids per metre at squaring and early boll stage respectively along with 65% retention (since ≥ 30% fruit loss at squaring stage plants failed to recover fully, see Figure 2). Since dryland cotton performance is dependent on weather conditions which can be very unreliable, I propose 3 mirids per metre as the action threshold for both squaring and early boll stage along with 65% fruit retention. Such estimate should be made with beat sheet sampling.

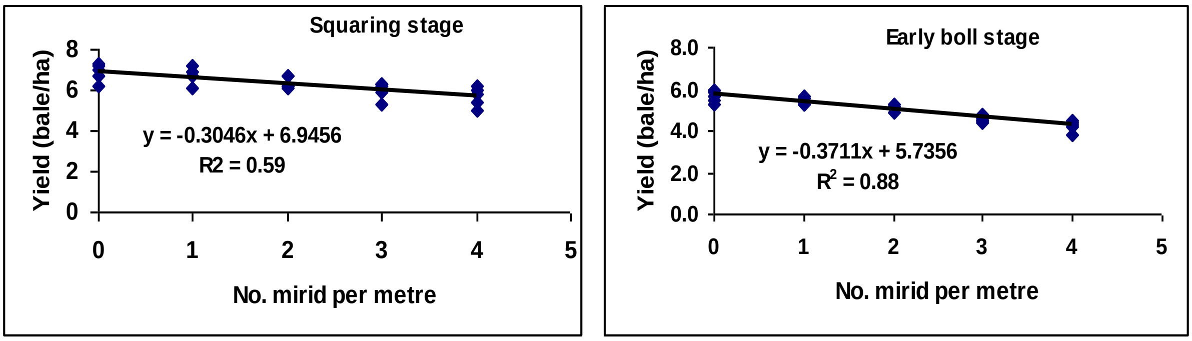


Figure 9. Relationship between number of mirids per metre and yield (bale/ha) in Bollgard®II cotton.

Mirid damage to different age of boll

The results clearly showed the relationship between mirid feeding and boll age (Figure 10). For all damage categories such as black spots on the boll, warty growth inside the boll wall and lint damage, younger bolls were preferred by mirids to older bolls. Mirid feeding on 10 day old bolls produced significantly more black spots (*P<0.05*) than any other age group. The black spots produced on 15 and 20 day old bolls were significantly different ($P<0.05$) from 25 day old bolls. However, difference between 15 and 20 day old bolls was not significant ($P>0.05$). Ten, 15 and 20 day old bolls incurred significantly more wart and lint damage ($P<0.05$) than 25 day old bolls. Wart and lint damage was not significantly different ($P>0.05$) between 10, 15 and 20 day old bolls (Figure 10). Younger bolls, up to 15 days old, can be judged by their soft feel when handled. Because of the toughness of the older bolls, mirids may find it difficult to penetrate their stylets into the bolls and hence produced less damage.

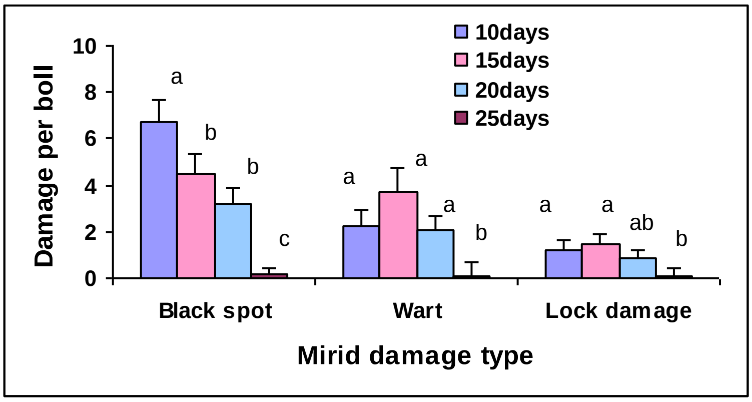


Figure 10. The relationship between mirid damage and boll age. Error bars indicate standard error of mean. Bars with the same letter for each damage type are not significantly different ($P>0.05$); Fisher's least significance difference test.

Damage caused by different stages of mirids

All stages of mirid caused damage to bolls and the number of black spots and warts per boll increased during successive stages (Figure 11). Fourth and 5th instar nymphs and adult male and female caused significantly ($P<0.05$) more damage (both black spots and warts) than other stages but differences between them were not significant ($P>0.05$). The damage caused by 1st, 2nd and 3rd instar nymphs was not significantly different ($P>0.05$). Third instar caused 1/3 to ½ and 1st and 2nd instar caused ¼ of the damage caused by 4th instar. Adult male caused the highest damage. Less damage caused by female than male might be due to the fact that they spend time laying eggs.

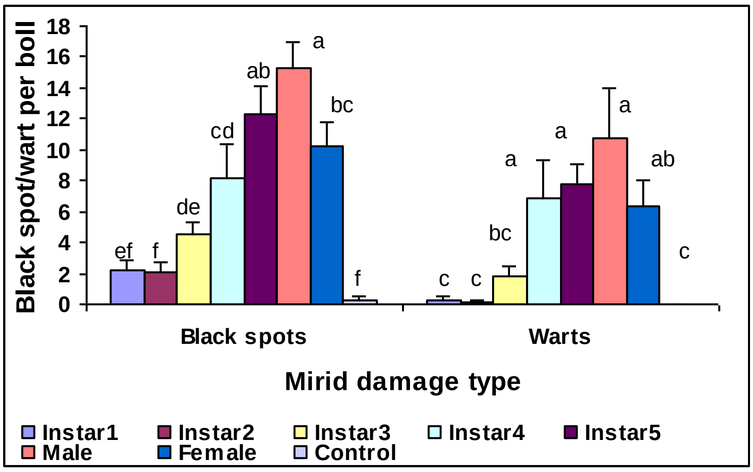


Figure 11. Damage caused by different stages of mirid to 15 day old bolls. Error bars indicate standard error of mean. Bars with the same letter for each damage type are not significantly different ($P>0.05$); Fisher's least significance difference test.

Comparing damage between mirids

Results for green mirid (GM) and brown mirid (BM) damage to squaring plants and bolls are presented in Figure 12. At squaring stage GM caused significant ($P<0.05$) square loss compared to BM. On the other hand BM produced significantly more ($P<0.05$) wart growth compared to GM.

However, the difference between the black spots (external damage) produced by GM and BM were not significant (*P>0.05*).

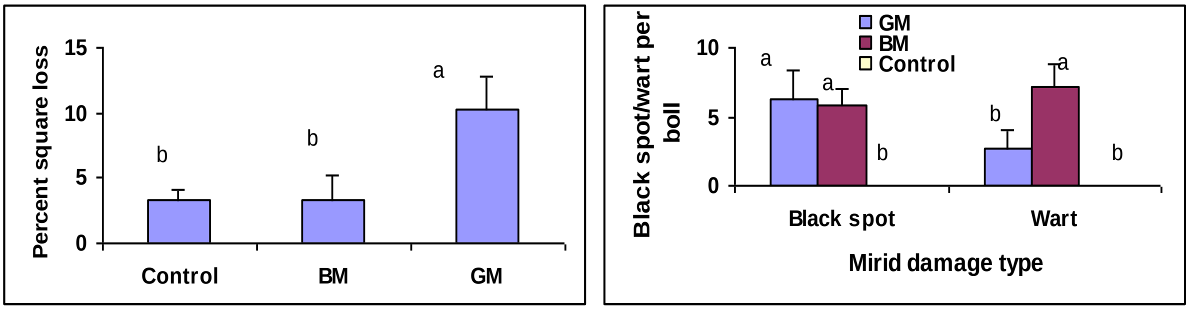


Figure 12. Comparing damage between green and brown mirid to squaring plants and bolls. Error bars indicate standard error of mean. Bars with the same letter for each damage type are not significantly different (*P>0.05*); Fisher's least significance difference test.

Population dynamics of mirids in Bollgard®II

Seasonal changes of mirids on Bollgard®II cotton

Results are summarised in Figure 13 for irrigated cotton and in Figure 14 for dryland cotton. For both irrigated and dryland systems high (3/m at the peak) to very high (20/m at the peak) numbers of mrids were found in 2004-05 and 2005-06 seasons. During 2006-07 season mirid number reduced to 3-4/m at the peak. Least numbers of mirids (1 – 2/m) were found in 2007-08 season. Subsequent reduction from 2006-07 season was perhaps due to the dry winter, spring and early summer contributing fewer plant hosts to build the initial population. Irrespective of growing systems the figures shows that in all sites mirids moved to cotton by the 2nd week of December and reached their peak between late January and 2nd week of February. By the end of February to early March the population died down except at 1 site (Nev Walton) during 2005-06 season where the population peaked twice, once in 2nd week of December and the other in 1st week of February. During 2006-07 season in Mayfield population peaked in 3rd week of February.

Species composition of mirids

Two species of mirids, *Creontiades dilutus* (green mirid = GM) and *C. pacificus* (brown mirid = BM) are found in cotton in Australia. Data form 8 sites in different cotton growing regions revealed that GM was the dominant species (Figure 15). In all sites GM population was >96% except at Byee. In Byee GM and BM population was 79 and 21% respectively. Higher percentage of BM population in Byee was perhaps due to the fact that Byee is a mixed cropping area and quite a few growers grow early mungbeans, soybeans and sorghum where BM population build up. Once these hosts, mature BM move to cotton around early to mid January. Seasonal abundance data also showed that BM moved to cotton in early to mid January and unlike GM, BM populations continued in cotton (though low in number) even in the later part of the season (Figure 16). Results of the replicated trial on different hosts showed that species composition varied with crop hosts (Figure 17). Cotton (both conventional and Bollgard®II) and lucerne was significantly (*P<0.05*) preferred by GM while soybean, mungbean and pigeon pea were equally preferred by both species.

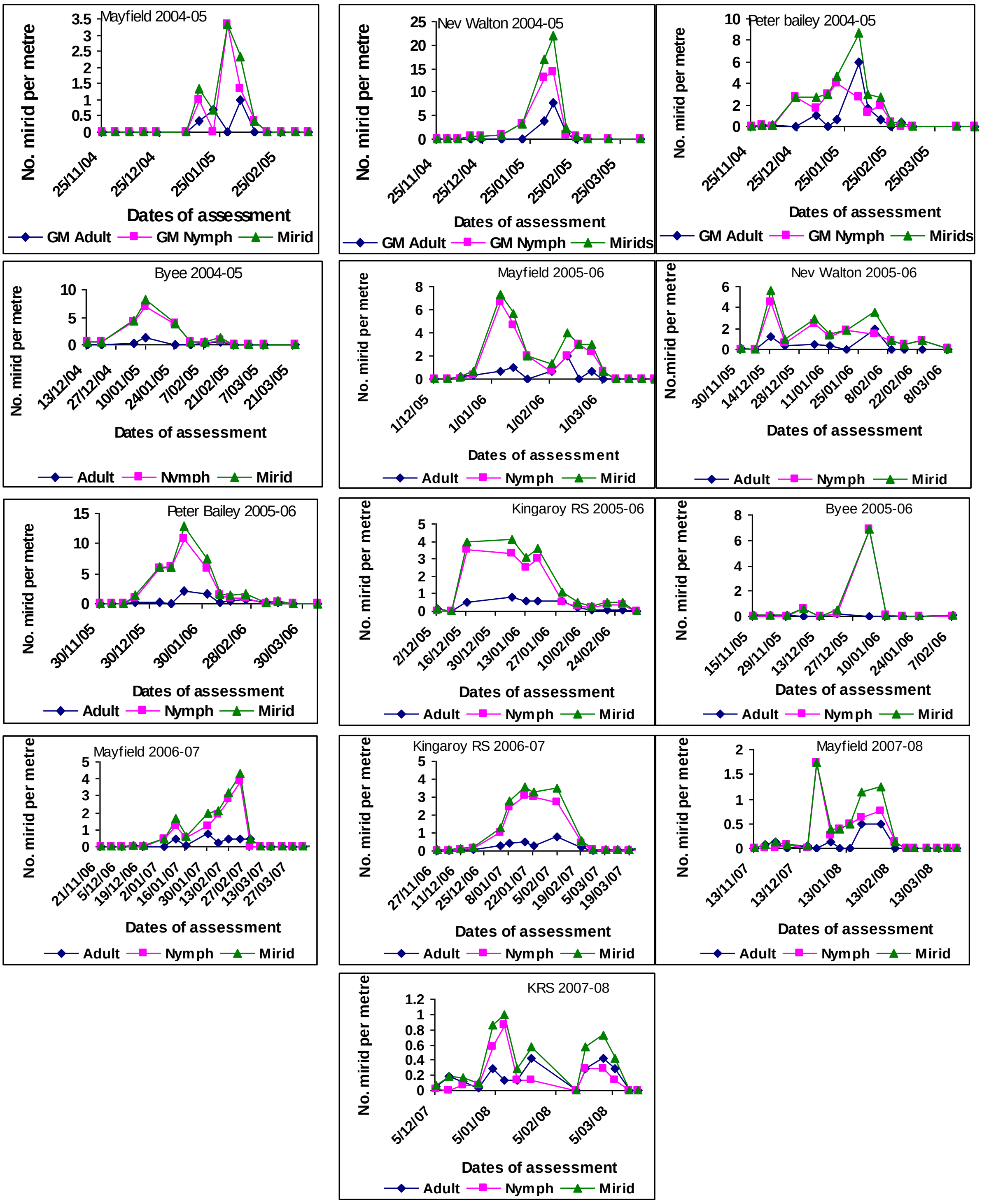


Figure 13. Seasonal abundance of mirids in irrigated Bollgard®II cotton

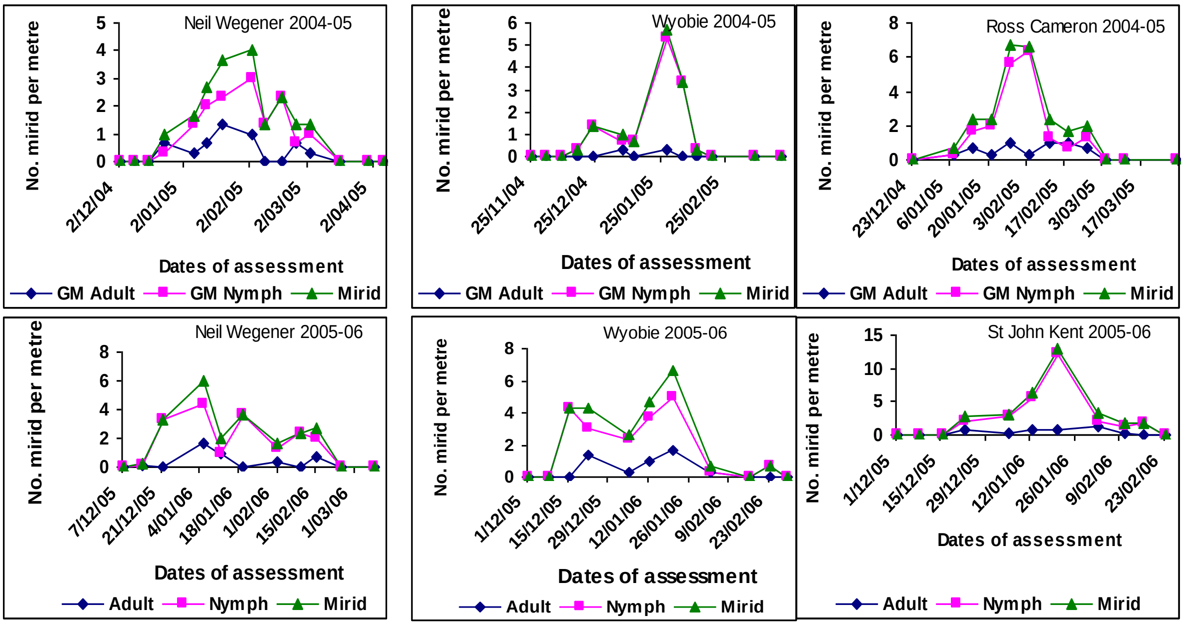


Figure 14. Seasonal abundance of mirids in dryland Bollgard®II cotton

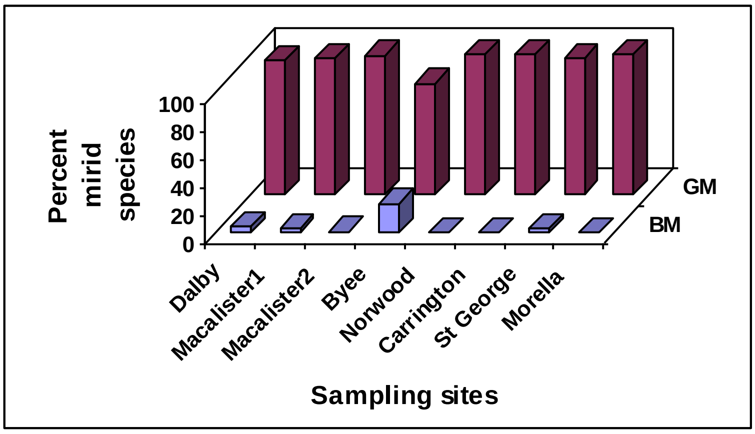


Figure 15. Species composition of mirids at different sites

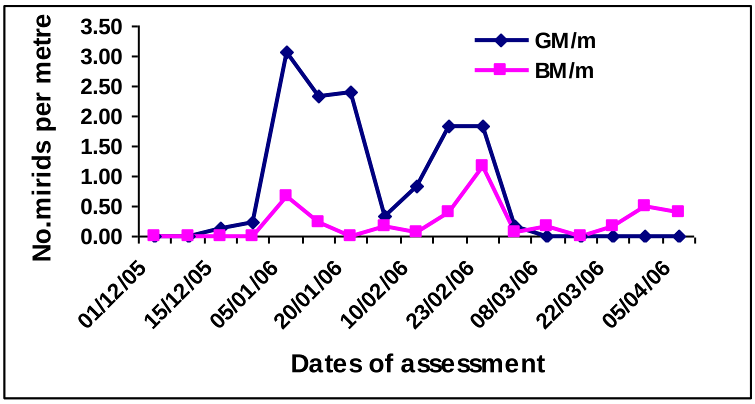


Figure 16. Seasonal abundance of GM and BM in Bollgard®II cotton at Byee during 2005-06 season.

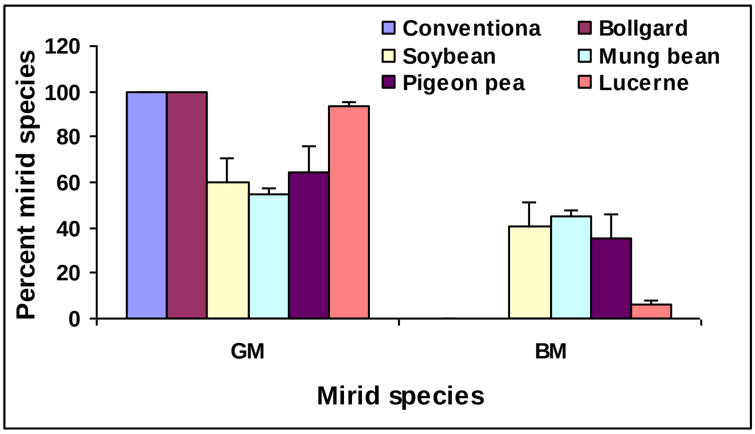


Figure 17. Species composition of mirids in different hosts. Error bars indicate standard error of mean.

Parasitoid of mirids

Altogether 1278 mirid nymphs and adults were reared in the laboratory and no parasitoids were detected. However, one parasitic mite, *Nabiseius melinae* sp. n. was recorded in mungbean in KRS and some observations were made (Plate 1). This was the first such record from southern cropping area. However, in 1994 Melina Miles of QDPI&F first found this mite at Biloela and it was described by Halliday. From the observations it was revealed that the mite was species and gender specific. In the field the BM to GM population ratio was 74 to 26% but the mite was only GM. For GM, the male to female ratio was 25.9 to 74.1% but the mite infestation ratio was 15 to 85% (Figure 18). It is yet to be determined how mite infestation affects GM females.

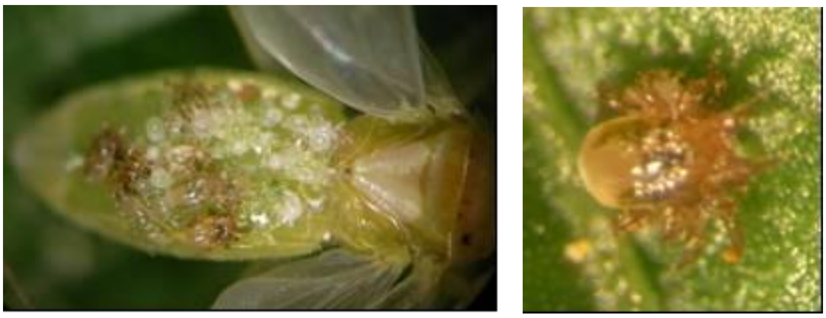


Plate 1. Parasitic mite, *Nabiseius melinae* sp. n.

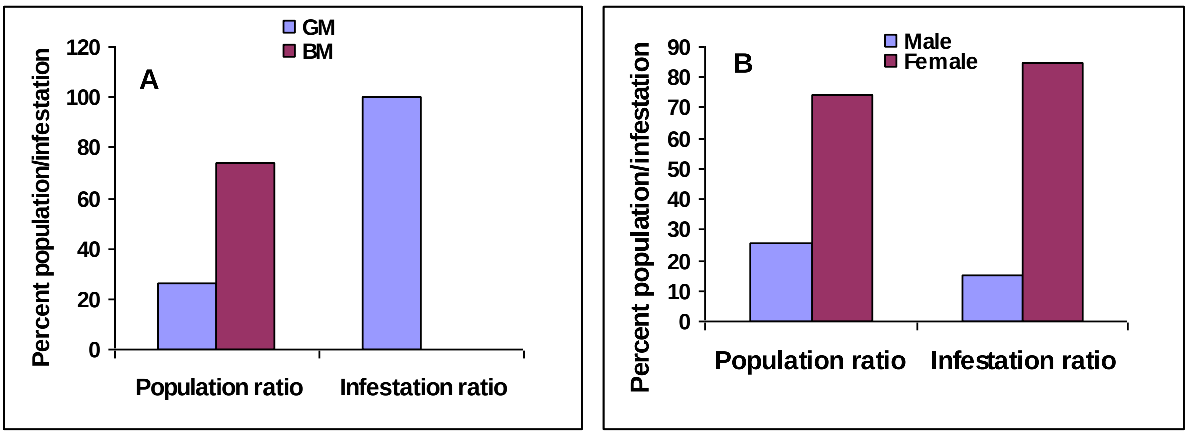


Figure 18. Parasitic mite, *Nabiseius melinae* sp. n. infestation on mirid showing GM and BM population and infestation ratio (A) and GM male and female population and infestation ratio (B).

Selective management options for mirids and stinkbugs

<u>Trial on determining optimum salt rate to mix with reduced rate of chemical</u>

<u>Salt Rate Trial 1</u>

Pre-treatment mirid numbers were moderate, with 2 to 5/m, with 4 to 10 beneficial arthropods/m. More than 80% of the mirid population were nymphs. Among the beneficial arthropods, spiders were the dominant group (47%) followed by damsel bug (DB) (26%), lady beetles (14%) and brown smudge bug (BSB) (9%).

The results showed that mirid numbers decreased for different rates of salt when mixed with dimethoate at both 3 and 7 DAT (Figure 19). Population reduction was greater with salt rates of 7 and 10 g and the difference was significant (*P<0.05*) at 3 and 7 DAT compared to other salt rates and dimethoate alone. There was no significant difference (*P>0.05*) between 7 g and 10 g of salt.

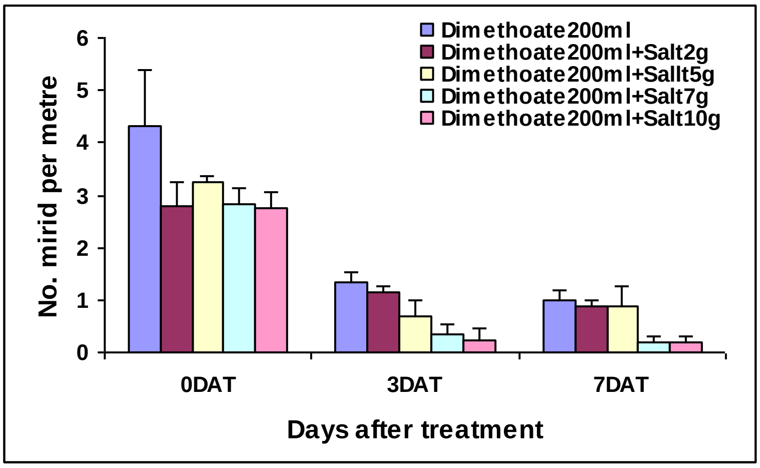


Figure 19. Effect of different rates of salt mixture with dimethoate against mirids in Bollgard®II. Error bars indicate standard error of mean.

Figure 20 shows that for different rates of salt, the impact on beneficial arthropods was low to moderate except on lady beetles and brown smudge bug for 10 g salt mixture where impact was high. However, differences between treatments were not significant (*P>0.05*).

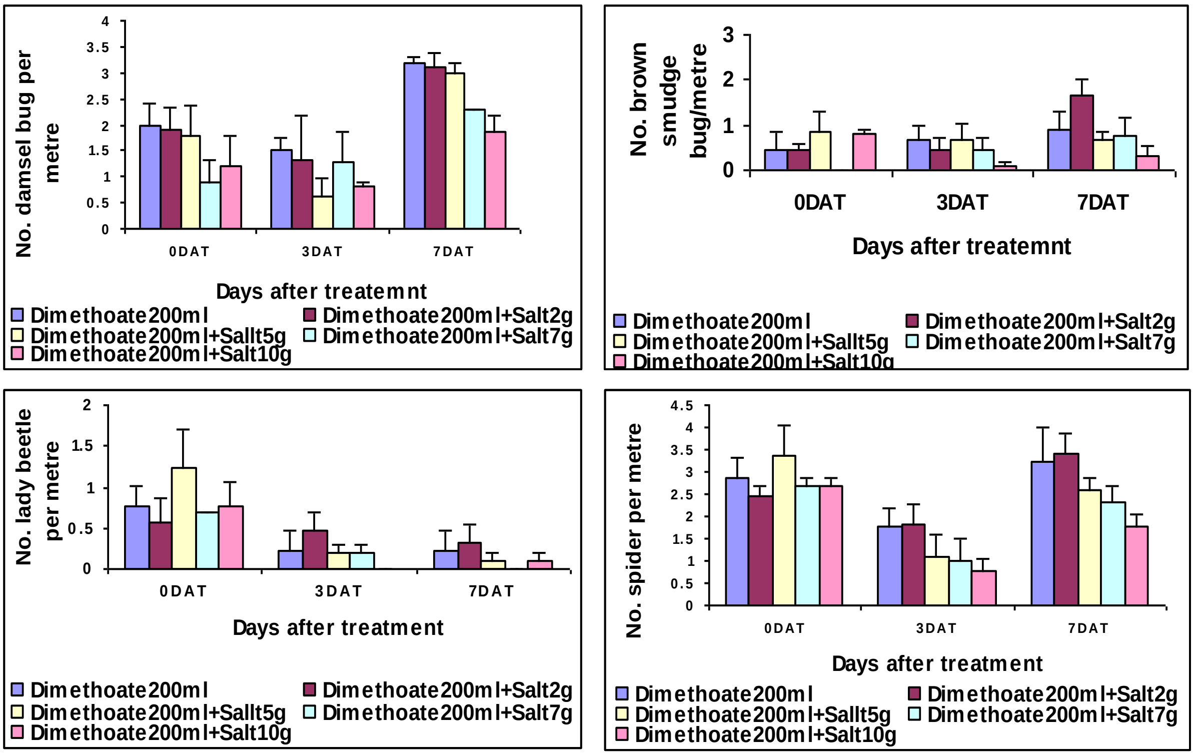


Figure 20. Effect of different rates of salt mixture with dimethoate against beneficial arthropods in Bollgard®II. Error bars indicate standard error of mean.

Salt Rate Trial 2

In this trial pre-treatment mirid numbers were low, 0.3 to 2/m, as were beneficial arthropods except spiders with 7/m. Mirid adults represented 79% of the population. Among the beneficial arthropods spiders were the dominant group (69%) followed by red and blue beetle (RBB) (23%).

Figure 21 and 22 summarises the effect of fipronil (Regent® at 40 mL/ha) plus different rates of salt against mirid and impact on beneficials respectively. The figure shows that higher salt rates substantially reduced the mirid population and 7 and 10 g salt mixture at 7 DAT killed significantly (*P<0.05*) more mirids than fipronil alone.

Figure 22 shows that while fipronil alone reduced RBB population by 45–58%, the effect of different rates of salt mixture was negligible. Spider populations were unaffected either by fipronil alone or salt mixtures.

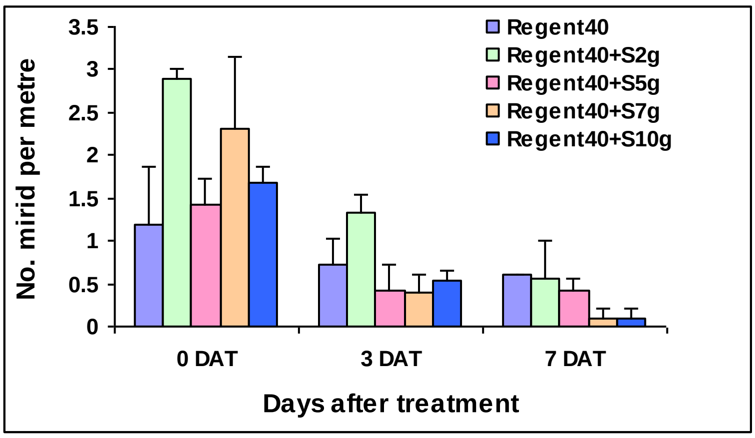


Figure 21. Effect of different rates of salt mixture with Regent® against mirids in Bollgard®II. Error bars indicate standard error of mean.

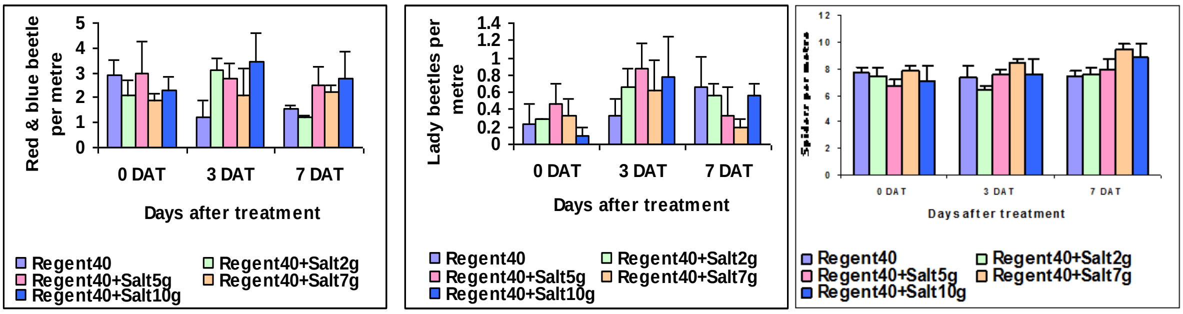


Figure 22. Effect of different rates of salt mixture with Regent® against beneficial arthropods in Bollgard®II. Error bars indicate standard error of mean.

Trial 1

Pre-treatment mirid numbers were moderate to high, ranging from 2 to 7/m. Seventy percent of the mirid population were nymphs. The most abundant beneficials were spiders (64%) followed by damsel bugs (32%). Other beneficials were red and blue beetles and lady beetles in low number.

The results showed that Steward® (200 SC) (indoxacarb) full rate (800 mL/ha), Steward® ½ rate plus salt and Regent® plus salt killed significantly (*P<0.05*) more mirids than other treatments (Figure 23). Intruder® (acetamiprid) was the least effective against mirids, killing only 33–36%. Salt mixed with reduced rates of chemical increased mortality substantially except for Intruder®.

Steward® full rate had moderate impact on both damsel bugs and spiders (Figure 24). Regent® plus salt had high impact on damsel bugs (Figure 24). Other treatments had no or very low impact on both damsel bugs and spiders.

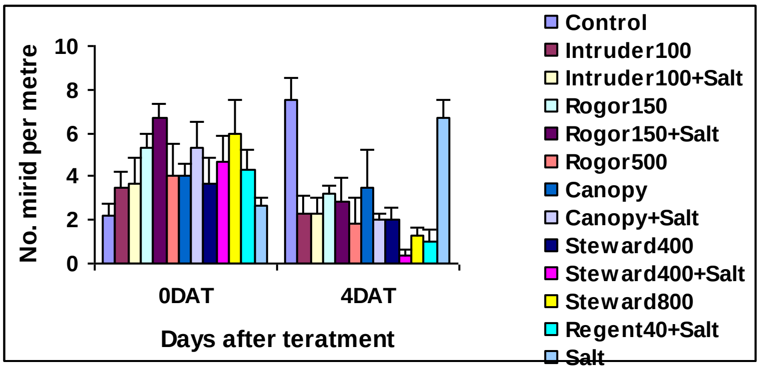


Figure 23. Effect of Steward® (200 SC), Intruder® and other chemicals against mirids in Bollgard®II. Error bars indicate standard error of mean.

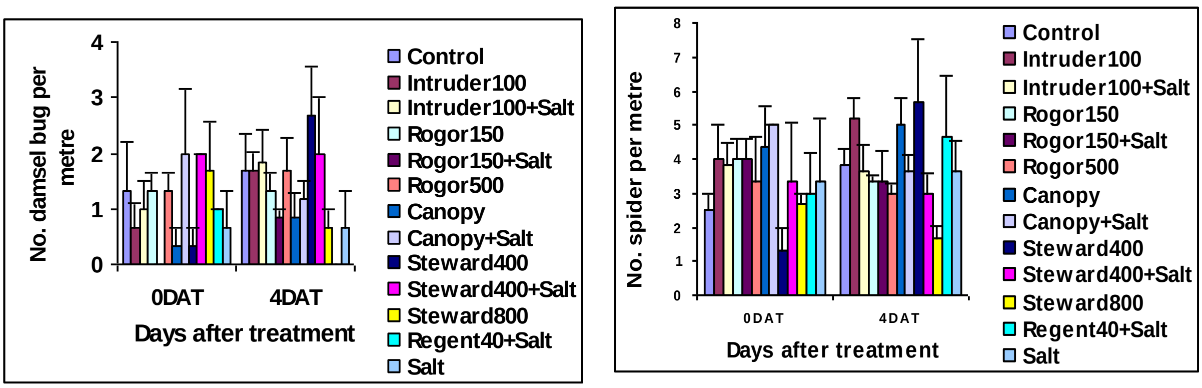


Figure 24. Impact of Steward® (200 SC), Intruder® and other chemicals on beneficials in Bollgard®II. Error bars indicate standard error of mean.

Trial 2

In this trial pre-treatment mirid numbers were high (4–7/m) and >95% were nymphs. The most abundant beneficials were spiders and red and blue beetles followed by brown smudge and damsel bugs.

The results showed that irrespective of rates, the effect of Steward® EC formulation and Intrepid® (chlorfenapyr) was low to moderate compared to standard control (Regent® plus salt) (Figure 25). While Regent® plus salt killed 73-94%, Intrepid® plus salt killed 65-66% and Steward® full rate killed 62-64%. Reduced rate of Steward® plus salt killed only 45%. Control mortality in this trial, however, was quite high at about 30%.

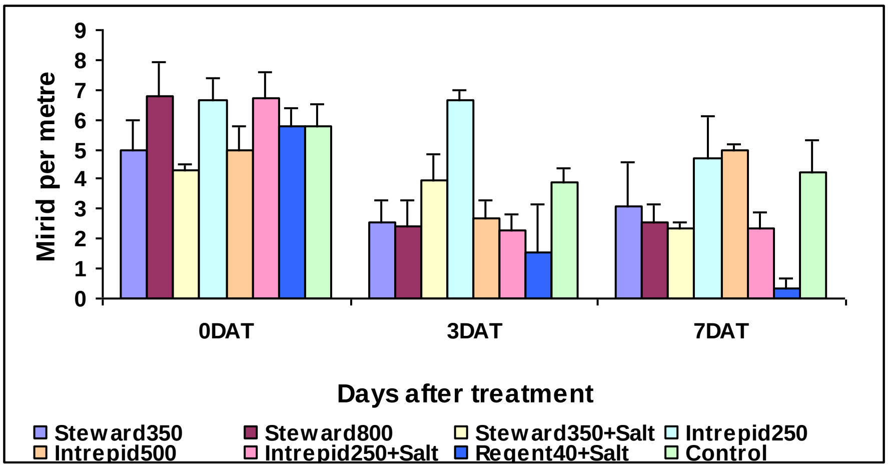


Figure 25. Effect of Steward® (150 EC) and Interpid® against mirids in Bollgard®II. Error bars indicate standard error of mean.

Impact of Steward® EC formulation and Intrepid® on benefcials are summarised in Figure 26. Overall impact on brown smudge bugs and spiders was very low. However, impact of low rate Intrepid® and standard control on damsel bugs and red and blue beetles was moderate to high.

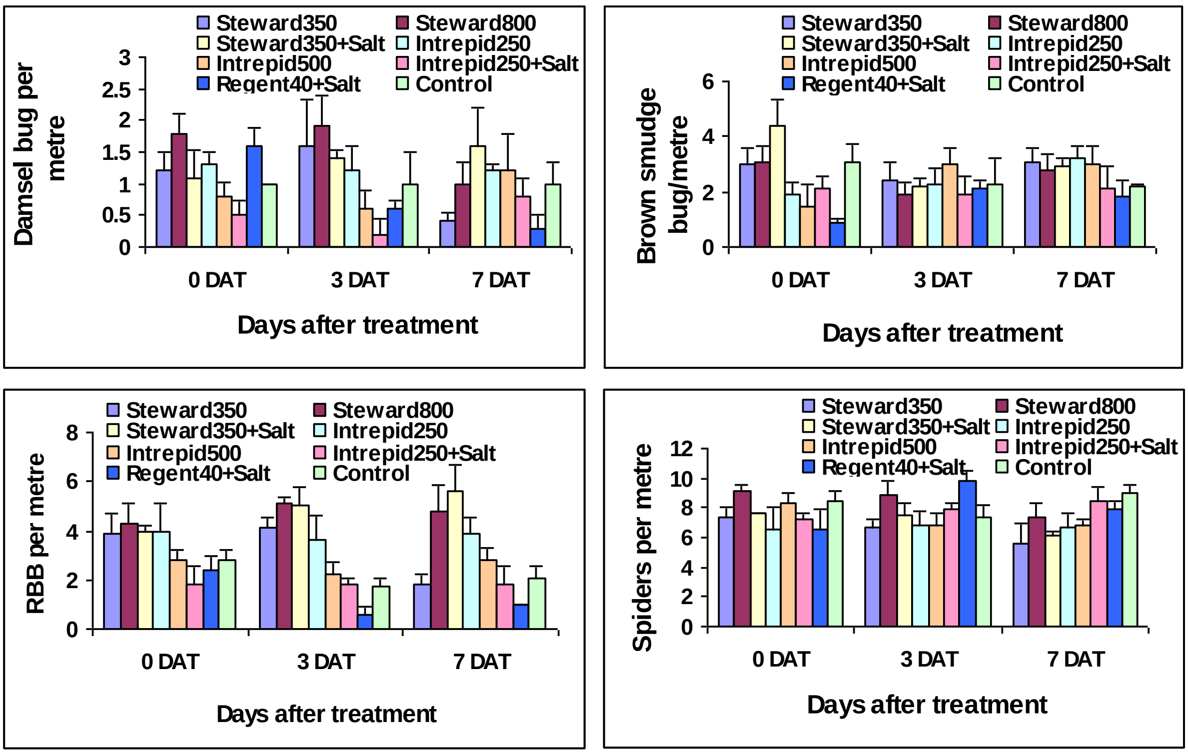


Figure 26. Impact of Steward® (150 EC) and Intrepid® on beneficials in Bollgard®II. Error bars indicate standard error of mean.

Trial 3

Pre-treatment mirid numbers were low (1–2/m) and the nymph to adult population ratio was 4:1. The most abundant beneficials were spiders (70%) followed by red and blue beetles (20%) and brown smudge bugs (7%). The remainder were lady beetles, big eyed bugs and lacewings.

The results showed that Calypso® (thiacloprid) alone and Calypso® plus Pulse® and Regent® plus salt (standard control) killed significantly more mirids (*P*<0.05) than BAS 320 both at 3 and 7 DAT (Figure 27). Calypso® alone and Calypso® plus Pulse® killed 50-59% of mirids at 3 DAT and 80-86% at 7 DAT. Standard control killed 68 and 94% at 3 and 7 DAT respectively. BAS 320 killed 39 and 69% of mirids at 3 and 7 DAT respectively.

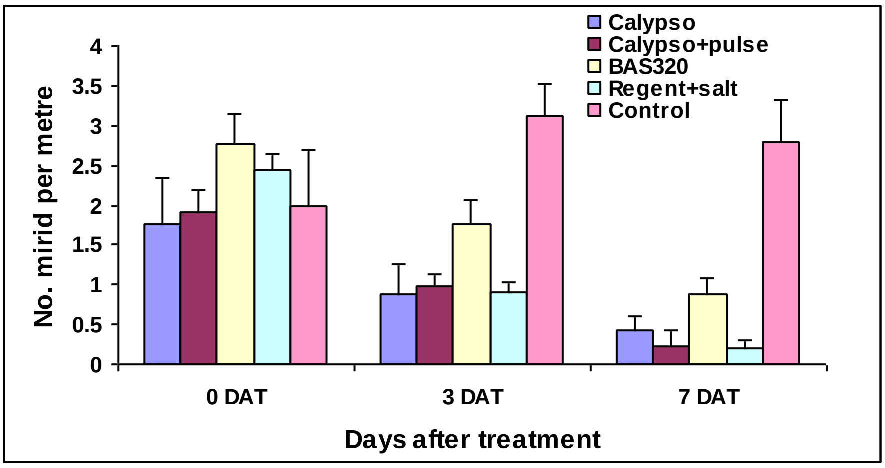


Figure 27. Effect of calypso and BAS320 against mirids in Bollgard®II. Error bars indicate standard error of mean.

Figure 28 shows that the impact of Calypso® and BAS 320 on red and blue beetles and spiders was negligible. However, Calypso® had moderate to very high impact on brown smudge bugs. While Calypso® plus Pulse® killed 76-100% and Calypso® alone killed 59-82%.

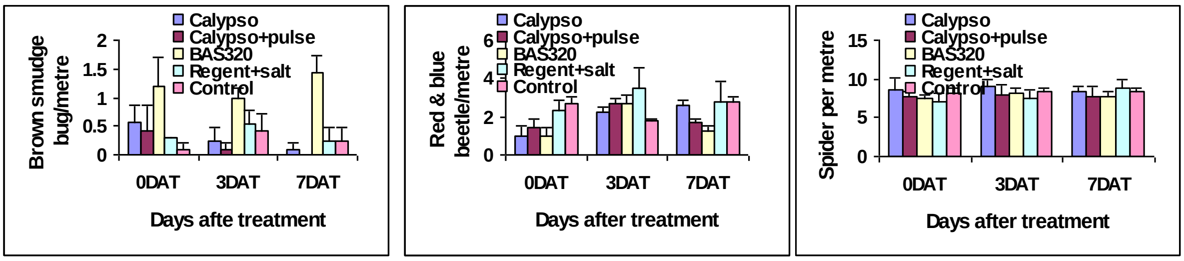


Figure 28. Impact of Calypso® and BAS320 on beneficials in Bollgard®II. Error bars indicate standard error of mean.

Trial 4

This trial was conducted against pale cotton stainer (CSB). Pre-treatment CSB numbers were moderate, ranging from 1-9/m and all of them were adults. The most abundant beneficials were spiders (59%), followed by brown smudge bug (BSB) (37%).

The effect of the treatments on the CSB is presented in Figure 29. Decis® full rate, 400 and 400 mL plus salt reduced the population of CSB at 3 DAT by >95% and the efficacy of full rate and salt mixture increased up to 100% at 7 DAT. Steward® 400 mL plus salt reduced the CSB population by 71 and 98% at 3 and 7 DAT, respectively. Efficacy of Steward® 400 mL was poor both at 3 and 7

DAT. Full rate Steward® gave 64% kill at 7 DAT. Regent® full rate, 60 and 60 mL plus salt gave 71, 63 and 73% kill at 3 DAT and 74, 80 and 92% kill at 7 DAT, respectively.

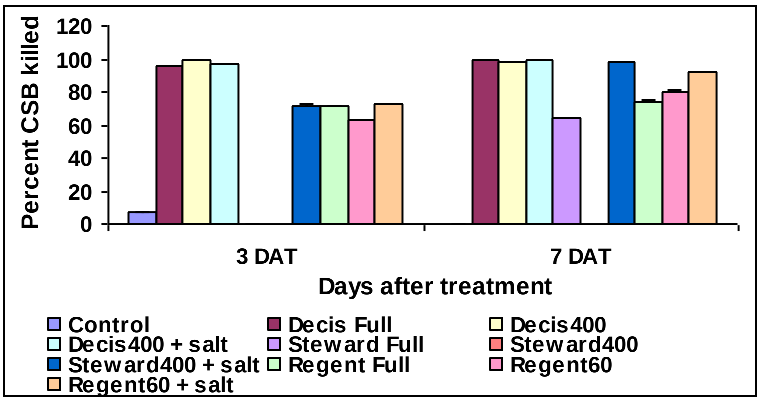
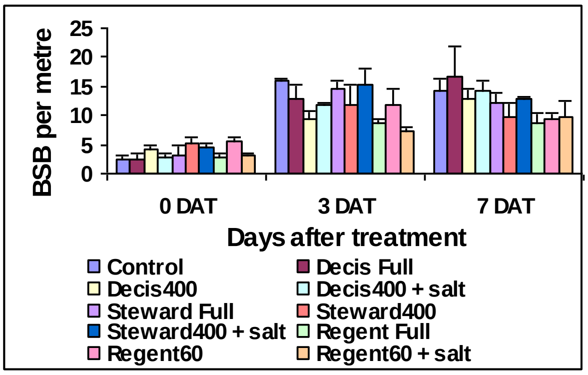


Figure 29. Effect of different chemicals against pale cotton stainer in Bollgard®II. Error bars indicate standard error of mean.

The impact of tested chemicals on beneficials was low (Figure 30). The population of BSB and spiders increased for all treatments except for Decis® full rate and Decis® 400 mL plus salt at 3 DAT where the spider population reduced by 8 and 20%, respectively.



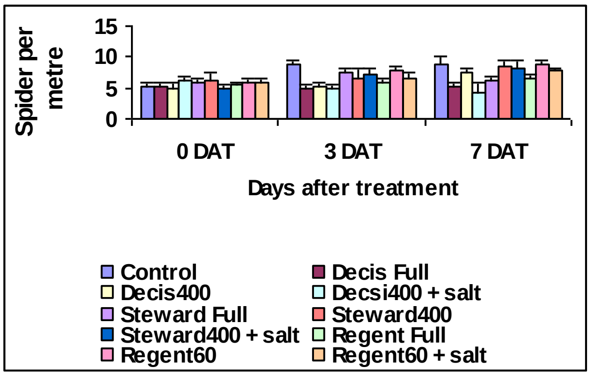


Figure 30. Impact of different chemicals on beneficials in Bollgard®II. Error bars indicate standard error of mean.

<u>Salt mixture verification trial</u>

<u>Mirid, GVB and cotton stainer bug population</u>

Weekly data of mirid, GVB and CSB populations are presented in Figure 31. Mirid numbers were low irrespective of treatments. The highest numbers, 1.17, 1.75 and 2.13/m in treatments 3, 2 and 1 respectively, were found during the squaring stage on 24/12/07. The trends of the mirid population for different treatments were more or less similar except on 2 occasions. On 04/02/08 in T1 and T3 mirid numbers reduced to zero and to 0.92/m respectively whereas in T2 mirid number increased to 1.63/m. On 13/02/08 mirid numbers reduced in T2 and T3, whilst in T1 mirid number increased from their previous date. From the third week of February 2008, the mirid population declined, coinciding with the third spray targeting stinkbugs.

Trends of GVB and CSB populations in relation to treatments were inconsistent throughout the season. On one occasion in one treatment the population was higher while on another occasion the population was higher on the other treatment.

When data were analysed for the whole season, the number of mirids and GVB did not vary significantly between treatments (Figure 32). Cotton stainer bug number, however, was 3 – 4 times lower in T3 than T1 and T2 (Figure 32).

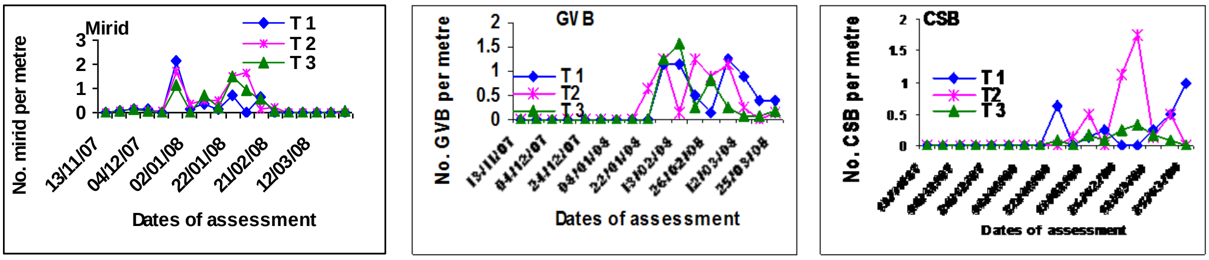


Figure 31. Mirid, green vegetable bug (GVB) and cotton stainer bug (CSB) at different sampling dates in different treatments.

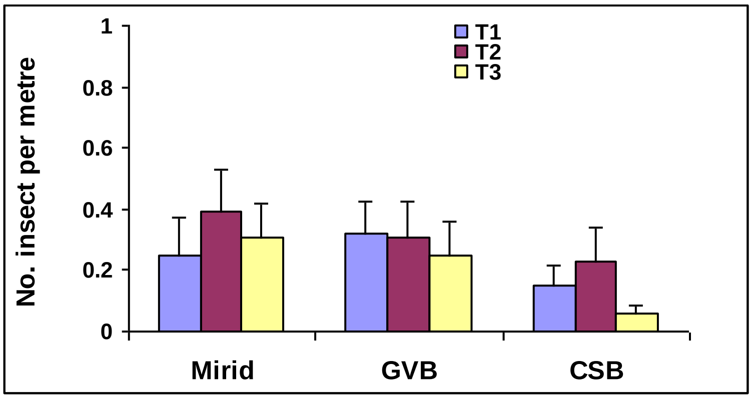


Figure 32. Mirid, green vegetable bug (GVB) and cotton stainer bug (CSB) number per treatment. Error bars indicate standard error of mean.

Beneficial population

The major beneficials were apple dimpling bugs, red and blue beetles, brown smudge bugs, lady beetles and spiders and their numbers in different treatments on each sampling occasion were more or less similar (Figure 33). However, the apple dimpling bug population in T2 on 13/02/08, the lady beetle population in T3 on 15/01/08, and the red and blue beetle population in T1 on 04/02/08 and the brown smudge bug population in T3 on 19/03/08 were significantly higher than other treatments.

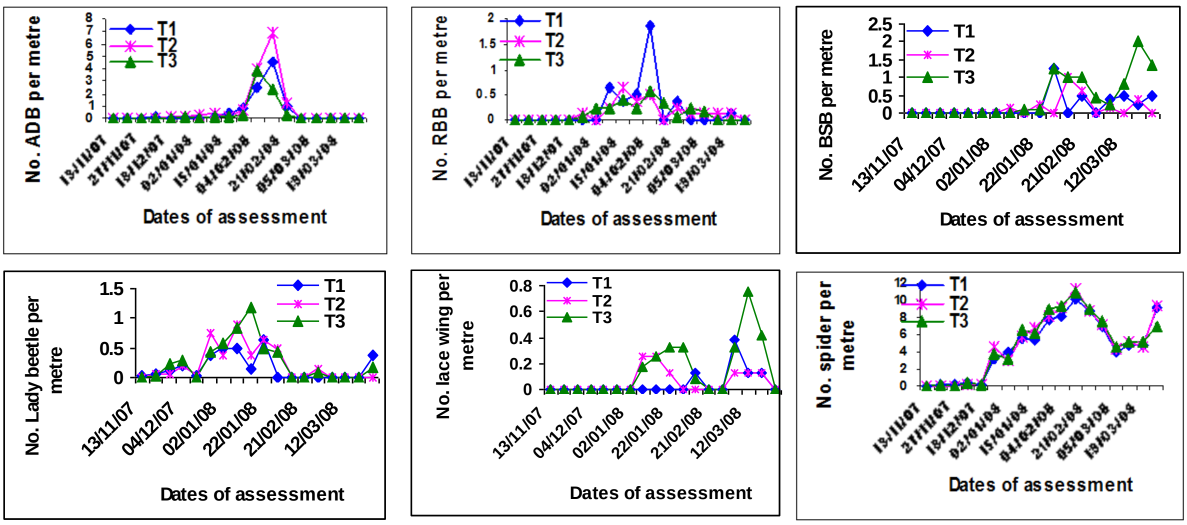


Figure 33. Beneficial numbers at sampling dates. ADB - apple dimpling bug, RBB - red and blue beetle, BSB - brown smudge bug.

Damage and yield

Percent fruit loss at early squaring (18/12/07) and squaring stage (02/01/08) were low to medium (9 - 31%), however the loss in T3 was significantly lower than other treatments (Figure 4). At maximum boll stage (21/02/08) percent fruit loss was 39 to 42 percent and the difference between treatments was not significant.

Percent boll damage in the low category was 48-64% and the damage in T3 was significantly lower than other treatments (Figure 34). However, for the medium and high category damage there was no significant difference between treatments. The percent of damage was very low, 3-7% for medium and 1-3% for high category.

There was no significant difference between treatments in yield. Lint yield for T1, T2 and T3 were 9.3, 9.1 and 8.9 bales/ha respectively.

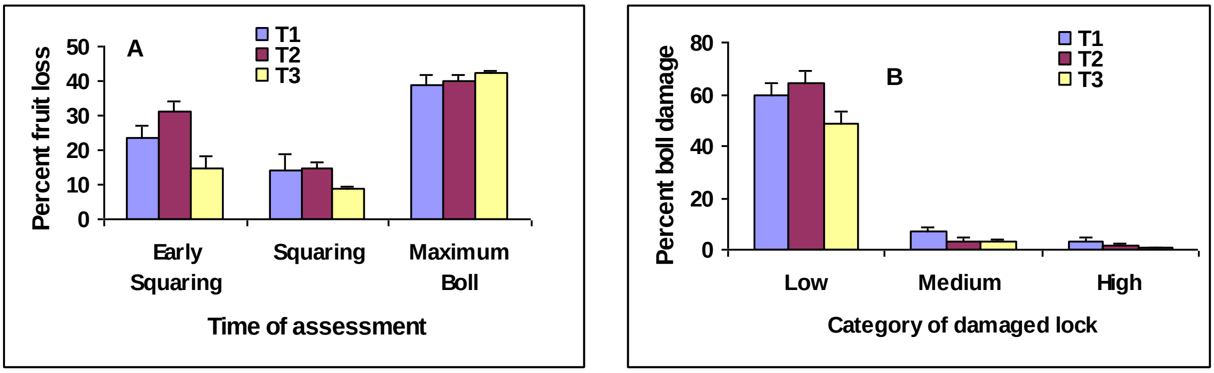


Figure 34. Fruit loss immediately after or before spray (A) and boll damage at late boll stage (B). Error bars indicate standard error of mean.

The study clearly showed that reduced rate of chemical plus additive was as good as grower practice if not better. Grower practice used similar chemicals at a marginally higher rate than the predetermined (T1 and T2) rate, which limited the scope of the study to show significant differences between treatments. Perhaps for this reason the study also did not show differences between treatments in terms of impact on the beneficials. Only apple dimpling bugs, lady beetles, red and blue beetles and brown smudge bugs at the peak population time showed differences between treatments (see Figure 33).

Other than mirid, GVB and CSB were the major pests. Most of the GVB during early to mid February were adults, possibly having moved from an adjacent sorghum field after harvest. Significantly more CSB numbers in T2 on 26/02/08 and 05/03/08 were not due to treatment difference, but rather that most of the population was 1st instar nymphs which were found crowded inside open bolls.

Cotton was invaded with whitefly and aphids from the middle of February. Perhaps, beneficials which survived because of the low chemical rate (including grower practice) kept this insect under control for the rest of the season. It is possible that mild seasonal temperatures also contributed to the low whitefly population build up.

Boll damage data presented here were probably caused by GVB and CSB, since at the time of damage assessment mirid number had already declined. Percent boll damage in the low category, at least one lock had $\leq$ 25% lint damage, was quite high (see Figure 34). However, this damage diminished at harvest. Bolls with such damage were still harvestable by the pickers and did not contribute to any yield loss. Medium and high categories of lock damage, some of which were tight locked (un-harvestable by the pickers), usually contribute yield loss. In this trial, however, medium and high categories of lock damage were very low and hence did not show any significant yield difference between treatments. A slightly lower yield in T3 might be due to herbicide contamination.

Release and monitor GVB parasitoid

Overall GVB and *Trichopoda* populations were low across the valleys (Table 7). The low populations were mainly due to lack of alternative hosts during spring and early summer because of drought. Also chemical sprays for mirids throughout the season in the survey area kept the GVB population down. The result showed that in cotton monthly parasitism rate in the South Burnett (Byee and Kingaroy) was higher than on the Darling Downs (Dalby and Macalister). At St George, *Trichopoda* failed to establish even after several releases. One of the reasons may be lack of hosts to support GVB populations during autumn, winter and spring.

Table 7. Percent parasitism of GVB by *Trichopoda* in Bollgard®II cotton in different regions. – indicate no GVB were found during survey.

Location	Month	Percent parasitism (no. collected)		
		2004-05	2005-06	2006-07
Dalby	November	-	-	-
	December	-	-	-
	January	-	-	-
	February	45.5 (11)	50.0 (4)	20.0 (5)
	March	-	25.0 (4)	66.7 (3)
	April	-	50.0 (4)	-
Macalister	November	-	-	No cotton due to drought
	December	0 (1)	-	
	January	0 (1)	0 (1)	
	February	45.5 (22)	54.6 (46)	
	March	42.9 (7)	38.5 (13)	
	April	-	-	
Byee	November	-	-	No cotton due to drought
	December	-	-	
	January	0 (1)	50.0 (2)	
	February	50 (2)	57.1 (7)	
	March	100 (1)	100.0 (1)	
	April	-	-	
Kingaroy	November	No cotton due to drought	-	-
	December		-	-
	January		80.0 (5)	-
	February		49.2 (59)	100.0 (1)
	March		50.0 (18)	0 (4)
	April		-	-

Evaluate plant volatile based attractant and GM sex pheromone

Evaluate magnet against mirids

The results showed that Magnet® failed to attract mirids significantly. Before treatment in unsprayed plots and in Magnet® sprayed plots mirid number was 2 and 1.5/metre respectively. Post treatment counts revealed that while in unsprayed plots mirid number remained the same, in Magnet® sprayed plots mirid number decreased to 1/m.

Evaluate GM sex pheromone

The results are given in Figure 35. During 2005-06 season, mirid adults caught in pheromone traps were synchronised to some extent with adult numbers recorded with suction or beat sheet samples.

During 2007-08 season synchrony was not very strong. Sex pheromones need to be further investigated before they can be recommended as a monitoring tool.

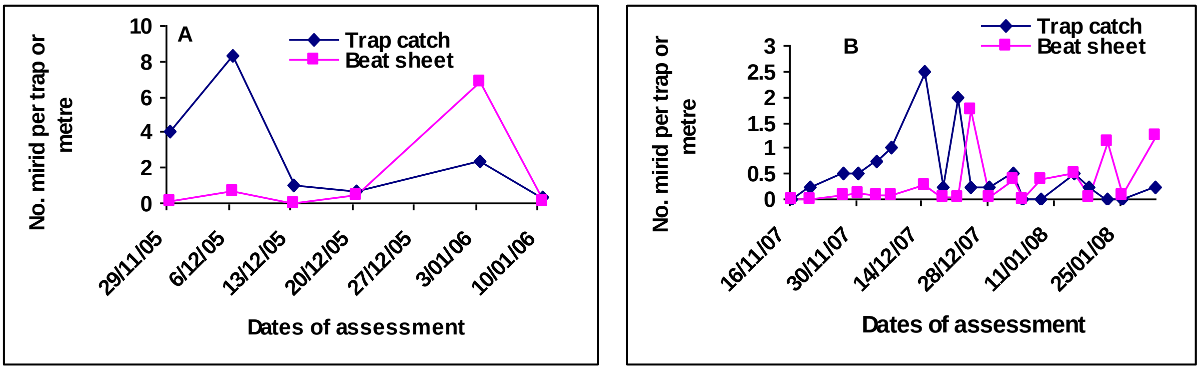


Figure 35. GM sex pheromone trap catch and mirid adult number in the field during 2005-06 (A) and 2007-08 (B) seasons.

Evaluate of kaolin based particle film technology against mirids and stinkbugs

Quadruple choice test

Immediately after release both mirids and GVB stayed on the top or side of the feeding arena. Mirids made their first selection within an hour of release whereas GVB took 3 hours to make their first selection. Unlike mirids, once GVB selected a treated boll, it remained there for some time, in one case for 3 days. The result showed mirids never selected SWP + PSO and SWP alone treated bolls and for GVB selection frequency to SWP + PSO treated bolls was 4% compared to 56% and 26% for control and PSO alone treated bolls respectively (Figure 36).

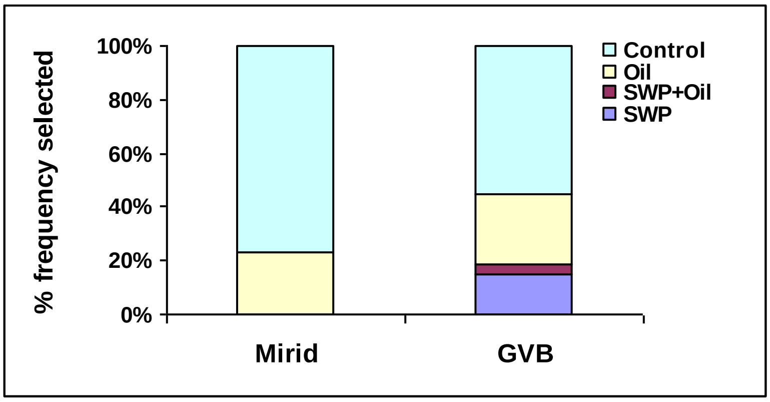


Figure 36. Frequency of selection of treated bolls by mirids and GVB

Field Trial

The majority of mirids were GM and among the stinkbugs, GVB and green stinkbugs (GSB) (*Plautia affinis*) were the predominant species. Other stinkbugs present included harlequin bugs and CSB. The effect of kaolin on mirid and stinkbug populations is summarised in Figure 37. The result showed that after two sprays, the kaolin coating successfully repelled the mirid population. The effect was more pronounced when SWP was mixed with PSO (Figure 37A).

Stinkbugs moved to the cotton after the second week of January when plants already had two coatings of kaolin. Two more sprays made plants virtually unrecognisable to stinkbugs. On average unsprayed control plots attracted 11 times more stinkbugs than (SWP + PSO) treated plots (Figure 37B).

In (SWP + PSO) treated plots, both mirid and stinkbug populations were as low as in chemical treated plots.

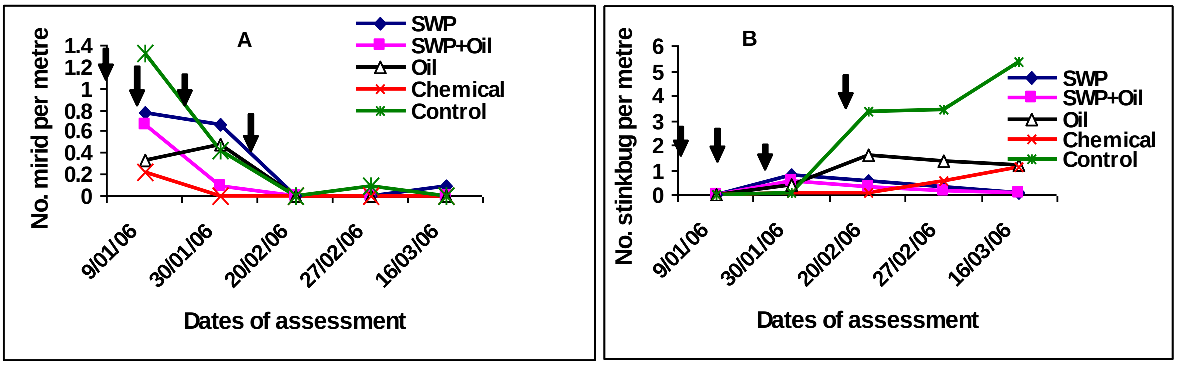


Figure 37. Effect of kaolin on mirids (A) and stinkbugs (B). Arrows indicate time of spray.

Boll damage and yield for each treatment are presented in Figure 38. Figure 38A shows that boll damage in (SWP + PSO) treated plots was significantly lower than in PSO alone, chemical and untreated control plots. Similarly yield in (SWP + PSO) treated plots was significantly higher than in SWP and PSO alone and untreated control plots (Figure 38B).

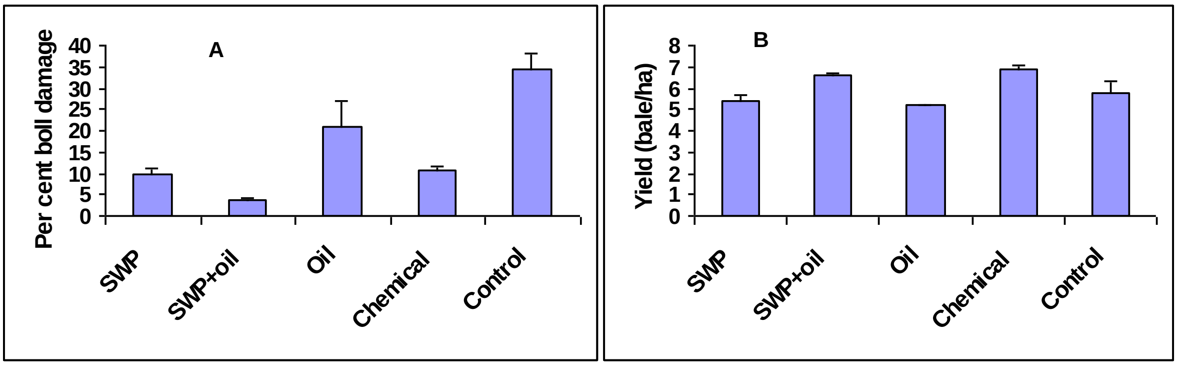


Figure 38. Percentage boll damage (A) and yield (bale/ha) (B) in kaolin treated plots.

This is the first reported use of kaolin in Australian cotton. The effectiveness of kaolin increased many fold when mixed with PSO. It is thought the kaolin makes plants visually unrecognizable to mirids and stinkbugs and also plants lose suitability as a host. Mirid and stinkbug movement and feeding might reduce as kaolin particles attach to the insect body. Considering the fact that this technology doesn't kill insects directly, as do synthetic chemicals, it may serve as an IPM tool for sucking pest management in Bollgard®II cotton. However, further research is needed to conduct more detailed studies to determine the impact of kaolin products on beneficial arthropods, to determine its impact on crop growth and fibre quality and to study its effects on other sucking pests such as aphids and whitefly.

Pale cotton stainer damage

Pale cotton stainer caused damage similar to that caused by mirids and GVB to the bolls. Their feeding caused warty growth inside boll walls, brown coloured lint and seed (Plate 2). The results on CSB damage to different age of bolls are summarised in Figure 39. The results showed that damage varied with boll age. Younger bolls (15 days old) incurred significantly ($P<0.05$) more damage than mature (>25 days old) and open bolls. Mature bolls also incurred significantly ($P<0.05$) more damage than open bolls. For younger bolls most of the damage was category 2 and 3 while for mature bolls most of the damage was category 1 and 2 and for open it was category 1. For all age groups, damage increased with the insect number except for mature bolls where 5 insects per boll caused less damage than 3 insects per boll. One of the reasons might be some insect died immediately after release.

2a **2b** **2c**

Plate 2. Damage caused by pale cotton stainer. 2a warty growth inside boll wall; 2b damage to seed; 2c lint damage

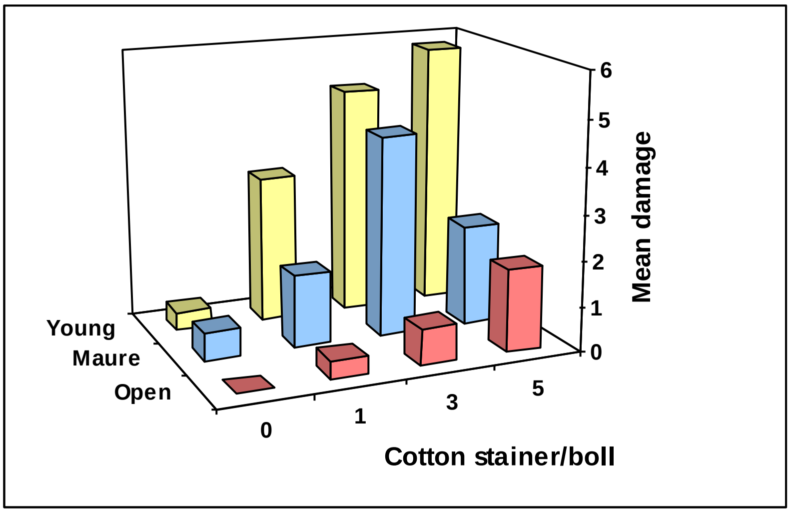


Figure 39. Pale cotton stainer damage to different age of bolls.

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.

Through this project I was able to determine stage wise action thresholds for mirids, to understand mirid damage in Bollgard®II cotton including identification of the critical damaging stage, to identify environmentally friendly management tools for mirids and stinkbugs, and to compare damage between GM and BM and their species composition on different hosts. Through this project I was also able to improve our understanding of pale cotton stainer damage, and to identify chemicals that can be used to control pale cotton stainer. All these outputs will substantially contribute to the planned outcomes. For example stage wise action thresholds and critical damage stage will clearly improve grower and consultant decision processes, thereby facilitating more judicious and timely application of chemicals which will ultimately increase the cotton industry's economic profile. Environmentally friendly tools such as salt mixture will allow chemical use for mirids and stinkbugs to be reduced by 33-50%. This will also reduce the cost of some insecticide sprays substantially. There will be follow on effects from this management approach. As reduced rate chemical plus salt is comparatively softer than broad-spectrum chemicals, it will encourage higher survival of beneficials and reduce the likelihood of secondary pest outbreaks by whitefly, aphids and mites.

Recent surveys across the industry conducted by Dr Mary Whitehouse revealed that while a substantial portion of growers and consultants are using salt mixtures to control mirids and stink bugs, the action thresholds used are highly variable. Knowledge of pale cotton stainer damage and chemical control options substantially helped growers and consultants effectively manage this pest during the 2007-08 outbreak.

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.

A new tool, kaolin film technology has been identified. However, more research is needed to develop this technology as a viable IPM option for mirids and stinkbug management.

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 outputs from this project, such as the critical damage stage and action threshold for mirids, will improve grower and consultant decision-making processes. This information will increase their confidence and better enable them to make decisions about when to apply chemical control and how many mirids can be tolerated without suffering economic loss. The information on salt mixture will also help them to choose more selective chemical management options to avoid flaring whitefly, aphids and mites in the later part of the crop.

Overall conclusions of the project and take home massages are outlined below.

- Mirid damage to Bollgard®II varies with crop stage.
- Plants compensate for damage at squaring stage (from seedling to 60% plants at first flower) and do not necessarily suffer yield loss. However, if percent fruit loss exceeds 30%, plants may fail to fully compensate for losses.
- Early boll stage (from 60% plants at first flower to 60% bolls at 20 days old) is the critical stage for mirid damage. Mirid population usually peak at this stage in Bollgard®II and cause significant fruit loss which can contribute to significant yield loss.
- At late boll stage (from 60% boll at 20 days old to cut out) the mirid populations decline and bolls generally do not incur further damage.
- All stages of mirids cause damage to bolls. Fourth and 5th instar nymphs and adult males and females cause significantly more damage than other stages. Third instar nymphs cause 30% and 1st and 2nd instar nymphs cause 25% of the damage caused by 4th instar nymphs.
- Bolls aged up to 20 days incur significant damage compared to older (25 days old and over) bolls and damage to older bolls does not contribute towards yield loss.
- The action thresholds for irrigated cotton are as follows:
  Squaring stage - 4 mirids/m and/or 65% fruit retention
  Early boll stage - 3 mirids/m and/or 65% fruit retention
  Late boll stage - mirids do not cause significant damage at this stage
  The action thresholds for dryland cotton are as follows:
  Since dryland cotton performance is dependent on water availability (which may be very unreliable), the action threshold is 3 mirids/m for both squaring and early boll stage along with 65% fruit loss.
  Estimates for the action threshold are based on beat sheet sampling.
- Damage cause by green and brown mirids varies with crop stage. While green mirids cause significantly more square loss, brown mirids cause significantly more boll damage. Brown

mirids move to Bollgard®II 4 to 8 weeks later than green mirid, around the first week of January. In Bollgard®II >90% are green mirid.

- When salt (NaCl) is mixed with reduced rates of some chemicals, targeted pest mortality is increased by by 20-40%. Five to 10 g of salt per litre of water produces maximum affect.
- Salt mixed with 1/3 rate of Regent®, ½ rate of dimethoate, ½ rate of Steward® and Steward® full rate alone are highly effective against mirid. Calypso® full rate and Calypso® plus Pulse® are moderate to highly effective. Intrepid® full rate and Intrepid® ½ rates plus salt are moderately effective. Intruder® and BAS 320 are not effective against mirids.
- When salt is mixed with reduced rate of some chemicals, the overall impact on beneficials is reduced. However, low rate of Regent® plus salt impacts highly on damsel bugs and Steward® full rate impacts moderately on damsel bugs and spiders.
- Decis® full rate, ½ rate and ½ rate plus salt, and Steward® ½ rate plus salt are highly effective against pale cotton stainer. Regent® full rate, ½ rate and ½ rate plus salt are moderate to highly effective against pale cotton stainer. On the other hand Steward® full rate is low to moderately effective against this pest.

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 action threshold for mirids needs to be verified using large scale trials involving growers and consultants. This activity will also boost user confidence, a factor that seems to be hampering the more universal adoption of the developed action thresholds.

It is planned to publish the information generated from this project into cotton grower magazine and placed on Cotton CRC website. Throughout the project Dr Khan has been proactive in communicating his research outputs to the broader industry. He already has taken the initiative to update the action threshold information into the 2008-09 Cotton Pest Management Guide. The project outcomes will be further disseminated through grower meetings, field days, CCA meetings, farm walks and conferences. The principal researcher will also write scientific journal articles on research outcomes.

In the field situation, the relationship between mirid number and fruit loss is not always obvious, making grower and consultant decision-making process more challenging. When chemical control of mirids is applied, there is often a delay in the recovery of fruit retention. This issue needs to be factored into plant based monitoring. Further research is thus needed to refine plant based thresholds and monitoring procedures for mirids. If opportunity presents itself, further research is needed to study pale cotton stainer damage, thresholds and control options. Research on more selective management options for mirids and stinkbugs needs to be continued.

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

1. Khan, M., Gregg, P. and Mensah, R. (2008). Effect of temperature on the biology of *Creontiades dilutus* (Stål) (Heteroptera: Miridae). *Australian Journal of Entomology* (in press).
2. Khan, M., Quade, A. and Boshammer, M. (2008). Insecticides for use against pale cotton stainer bug. *The Australian Cottongrower* 29(2): 41 – 42.
3. Wilson, L., Khan, M. and Farrell, T. (2008). Pale Cotton Stainers, *Dysdercus sidae*. Pest Profile. Australian Cotton Catchment Communities CRC. www.cottoncrc.org.au
4. Khan, M. and Quade, A. (2208). Biology and pictorial identification of mirids. *The Australian Cottongrower* 29(1): 24 – 27.
5. Wilson, L. J., Fitt, G. P., Deutscher, S., Khan, M. and Pyke, B. A. (2007). Cotton Pests. In: Pests of Field Crops and Pastures: Identification and Control (Bailey, P. T. ed). CSIRO Publishing, Melbourne, Victoria. Pp. 63 – 119.

6. Khan, M., Quade, A. and Murray, D. (2007). Damage assessment and action threshold for mirids, *Creontiades spp.* in Bollgard II cotton in Australia. Proceedings of the Second International Lygus Bug Symposium, Asilomar Conference Center, Pacific Grove, CA USA.
7. Khan, M., Quade, A. and Murray, D. (2007). Reduced rate of chemical plus additive- an eefctive IPM tool for managing mirids, *Creontiades spp.* in Australian cotton. Proceedings of the Second International Lygus Bug Symposium, Asilomar Conference Center, Pacific Grove, CA USA.
8. Khan, M. and Quade, A. (2006). Kaolin cons cotton suckers. *The Australian Cottongrower* 27(6): 16 – 20.
9. Khan, M., Quade, A. and Murray, D. (2006). Mirid damage assessment in Bollgard II- critical damage stage and action thresholds at different stages in irrigated and raingrown cotton. Proceedings of the 13th Australian Cotton Conference, Gold Coast.
10. Khan, M., Quade, A. and Murray, D. (2006). Mirid management- effect of salt rate when mixed with reduced rate of chemical. Proceedings of the 13th Australian Cotton Conference, Gold Coast.
11. Khan, M. and Murray, A. (2005). Predator puts bite on green mirid eggs. *The Australian cottongrower* 26(2): 18
12. Khan, M., Kelly, D., Hickman, M., Mensah, R., Brier, H. and Wilson, L. (2004). Mirid ecology in Australian cotton. Australian Cotton CRC Review 14. Australian Cotton Co-operative Research Centre, Technology Resource Centre Press, Narrabri, NSW Australia
13. Khan, M., Kelly, D., Hickman, M., Mensah, R., Brier, H. and Wilson, L. (2004). Mirid management in Australian cotton. Australian Cotton CRC Review 15. Australian Cotton Co-operative Research Centre, Technology Resource Centre Press, Narrabri, NSW Australia
14. Khan, M. and Murray, D. (2004). Salt mixture- an IPM option for managing sucking pest in cotton. Twenty Second International Congress in Entomology, Brisbane.

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

Yes, website address is www.cottoncrc.org.au

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.

The project on 'improved understanding of the damage, ecology and management of mirids and stinkbugs in Bollgard®II' was originally planned for 3 years, from 1 July 2004 to 30 June 2007; later the project was extended up to 30 June 2008 with limited funding and objectives.

The main objectives of this project were to understand mirid damage and ecology in Bollgard®II, to develop action thresholds and to investigate selective management options for mirids and stinkbugs. This project also aimed to provide the cotton industry with improved management guidelines for mirids and stinkbugs that are compatible with the adoption of IPM approaches and Bollgard®II.

The information generated through this project includes:

Both green (*Creontiades dilutus*) and brown (*Creontiades pacificaus*) mirids are causing damage to Bollgard®II cotton. While green mirids move to cotton at seedling stage, brown mirids move in around early boll formation stage. In Bollgard®II abundance of brown mirids is greater in mixed cropping areas with soybean and mungbean than in cotton monoculture areas. In monoculture Bollgard®II farming systems, >95% of the mirid population is green mirid. This contrasts with mixed cropping areas where the green mirid population is around 80% of the mirid population. Green mirids cause significantly more square loss than brown mirid, whereas brown mirids cause significantly more boll damage than green mirids.

All stages of mirids cause damage to bolls. Fourth and 5th instar nymphs and adult males and females cause significantly more damage than other stages. Third instar nymphs cause 33% and 1st and 2nd instar cause 25% of the damage cause by 4th instar nymphs. Bolls aged up to 20 days old incur significantly more damage compared to older (25 days old and over) bolls. Older bolls incur negligible damage.

Yield loss due to mirid feeding varies with crop stage. Damage at squaring stage (from seedling to 60% plants at first flower) fully recover later in the season provided plants do not suffer from any other stress such as water stress. However, at this stage if mirid feeding causes >30% fruit loss, plants fail to recover fully. Early boll stage (from 60% plants at first flower to 60% bolls reached 20 days old) is the critical stage for mirid damage. At this stage the mirid population has usually reached its peak and caused maximum damage (fruit loss) which will contribute to significant yield loss. At late boll stage (from 60% boll reached 20 days to cut out) the mirid population usually declines and bolls do not incur any significant damage.

Action thresholds for mirids in irrigated Bollgard®II at squaring and early boll stages are proposed as 4 and 3/metre respectively and/or 65% fruit retention. At late boll stage mirids cause negligible damage and therefore do not warrant control. Since dryland cotton performance is dependent on moisture availability, which can be very unreliable, the action threshold proposed for both squaring and early boll stage is 3/m and/or 65% fruit retention. Assessment for action threshold is based on beat sheet sampling.

Salt mixtures increase mortality by 20 to 40% compared to reduced rates of chemical alone. Five to 10 g of salt per litre of water produces maximum effect. Salt mixed with reduced rates of fipronil (Regent®), dimethoate (Rogor®) and indoxacarb (Steward®) are quite effective against mirids. Low rate of indoxacarb and fipronil plus salt are also quite effective against pale cotton stainer as are deltamethrin (Decis®) full and half rate.

Knowledge of mirid damage to Bollgard®II will increase the cotton industry's confidence in mirid management decision-making processes. Crop stage wise action thresholds will facilitate judicious and timely application of chemicals. The Australian Cotton industry is now better informed about mirid management and lead to more timely and appropriate chemical selection. These developments will ultimately increase cotton industry's economic, environmental and social profile. Use of salt mixture as a mirid management option will reduce chemical rates and the impact of chemicals on benefcials. Such decisions will reduce the likelihood of flaring other pests such as whitefly, aphids and mites. The reduced use of broad-spectrum chemical will reduce the environmental foot print of growing cotton.

Further information

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