Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2014

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Part 1 - Summary Details

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

CRDC Project Number: **DAQ1204**

Project Title: Management of mirids, stinkbugs and *Solenopsis* mealybug

Project Commencement Date: 01/07/11 **Project Completion Date:** 30/06/14

CRDC Program: Farming Systems

Part 2 – Contact Details

Administrator: Mrs Helen Kamel (Principal Coordinator, External Funding)
Organisation: **DAFF, Agri-Science Queensland**
Postal Address: PO Box 102, Toowoomba, QLD 4350
Ph: 07 46881246 **Fax:** 07 46881190 **E-mail:** helen.kamel@daff.qld.gov.au

Principal Researcher: **Dr Moazzem Khan (Senior Research Scientist (Entomology)**
Organisation: DAFF, Agri-Science Queensland
Postal Address: PO Box 102, Toowoomba, QLD 4350
Ph: 07 46881310 **Fax:** 07 46881199 **E-mail:** moazzem.khan@daff.qld.gov.au

Supervisor: **Dr Melina Miles** (Principal Entomologist)
Organisation: DAFF, Agri-Science Queensland
Postal Address: PO Box 102, Toowoomba, QLD 4350
Ph: 07 46881369 **Fax:** 07 46881199 **E-mail:** melina.miles@daff.qld.gov.au

Signature of Research Provider Representative: _____

Date Submitted: _____

Part 3 – Final Report

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

Background

1. Outline the background to the project.

Solenopsis mealybug is an emerging pest of cotton in Australia. Since their first outbreak in Emerald and the Burdekin in 2009, solenopsis mealybug have spread to the South Burnett, Darling Downs and St George regions with the potential to spread further and become an industry-wide pest. Overseas research documents the potential for Solenopsis to cause significant crop damage and describes the diffculties of effective management (Hodgson *et al*. 2008, Arif *et al*. 2009, Nagrare *et al*. 2009). The development of an effective management strategy for solenopsis mealybug will be underpinned by developing a comprehensive understanding of the pests ecology and biology, natural enemies and potential for chemical control within the context of the Australian cotton farming system.

Mirids are a regular pest of Bollgard® II cotton, requiring 2-3 sprays every season. However, pesticide use for mirid control can be problematic within an IPM program due to the disruption of natural enemy complexes and potential flaring of silverleaf whitefly and other secondary pests. The lack of selective or soft insecticide options means that effective monitoring and the judicious use of insecticides, guided by empirically derived thresholds is critical. The cotton industry has invested much effort into the development of thresholds for mirids, but recent evidence suggests that many growers and consultants do not use these thresholds (Whitehouse, 2006). It is important that we understand why adoption is not at expected levels and explore both the technical and social contributors in order to provide appropriate support to growers and consultants as they implement IPM. Researching some of the techincal aspects including monitoring (sample sizes at different precision levels), effects of temperature on mirid feeding, IPM fit management options, relationships between organisms responsible for boll rot and transmission by stinkbugs could further improve adopton of IPM in managing mirids and stinkbugs.

The aim of this report is to present the results of the studies conducted on solenopsis mealybugs, mirids and stinkbugs to provide data that can be used to inform the management of these pests within the context of IPM in cotton.

Objectives

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

This project includes research on three pests, mirids, stinkbugs and solenopsis mealybug. The project objectives are listed below:

1. Investigate solenopsis mealybug damage in Bollgard II cotton
2. Explore management options for mealybug
3. Develop improved monitoring techniques for mirids (sample size vs precision)
4. Determine temperature effects on mirid feeding
5. Investigate IPM-compatible management options for mirids and stinkbugs
6. Investigate cotton stainer damage in Bollgard II (develop threshold)
7. Investigate causal agents of boll rot and potential links with bug transmitted pathogens

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

MEALYBUG

Understanding solenosis mealybug damage in Bollgard® II cotton

Several experiments were conducted in the glasshouse and in the field to gain a better understanding of mealybug damage.

Glasshouse Experiments

Methodology
Two experiments were conducted to assess damage caused by different life stages of mealybug at various cotton growth stages.

Experiment 1
Four treatments (small and large nymphs, young adults and a control without mealybug) were used. Treatments were replicated 10 times within a Randomised Complete Block (RCB) design experiment on a 2 x 1.1m metal tray. Half of each tray was considered as a block. The control plants were placed in a separate tray to avoid mealybug cross-contamination. Ten mealybugs per plant were introduced onto the plants (Sicot 71BRF) at the first true leaf stage. The trial was terminated when all of the plants developed to the reached first square stage. Plant height was recorded at the commencement and completion of the experiment. Plants were checked regularly and date of 1st squaring was recorded for each plant. The number of mealybugs, squares and damage were recorded at termination of the trial. Damage was scored with a healthy plant having a score of 0 through to a dead plant having a damage score of 5 (Plate 1).

Data were subjected to analysis of variance (ANOVA) and means were separated by using Fisher's Least Significant Difference Test (LSD) at 5% level. For damage score and mealybug number control plants were not included in the analysis as all values were zero. Data on mealybug numbers were transformed using square root transformation before analysis.

Score 1

Score 2

Score 3

Score 4

Plate 1. Damage score used in the experiments to assess mealybug damage. Score 1- normal plant, standard inter node, leaves and tips; Score 2- plants showing sign of damage, top inter nodes shortening, some leaves on top curly; Score 3- plant clearly showing damage, top internodes short, most of the top leaves curly; Score 4- plant clearly showing damage, plant stunted, short internodes, leaves thick and curly.

Experiment 2

The experiment was conducted to assess damage caused by the establishment of mealybug at different crop stages. The stages assessed were 4-5 leaf, squaring, one week after flowering, four weeks after flowering and mature boll stage. Forty five mealybugs per plant (10 adults, 15 small and 20 large nymphs) were released onto 6 plants of each stage and allowed to feed and breed until harvest. Four plants of each stage without mealybug were used as controls. The plants were arranged in a Completely Randomised Design (RCD) for observation but separately with and without mealybugs. The plants were checked regularly to record damage symptoms. At the end of the trial the number of mealybug and harvestable bolls for each plant were recorded. Bolls were harvested individually.

The data were analysed using analysis of variance (ANOVA) in two steps- 1) analysis of treatments with mealybugs applied and 2) across experiments (including control plants) analysis.

Results

Experiment 1

Results showed that mealybug established as adults caused the most damage followed by large nymphs (Figure 1 and Table 1). The analysis (ANOVA) showed that there was a significant difference between adults and nymphs (either stage) for damage score ($F = 36.68$, $p < 0.001$), plant height ($F = 23.46$, $p < 0.001$), number of squares ($F = 4.25$, $p < 0.05$) and days to squaring ($F = 8.77$, $p < 0.001$). At termination, mealybug numbers were significantly higher ($F = 16.67$, $p < 0.05$) on the plants where mealybug established as adult than other mealybug stages. During the experimental period (6 weeks), plants with mealybug seeded with adults gave rise to second and third generations of progeny. There was therefore more mealybug on those plants than those inoculated with small and large nymphs which had only first and second generations of progeny and less resultant damage.

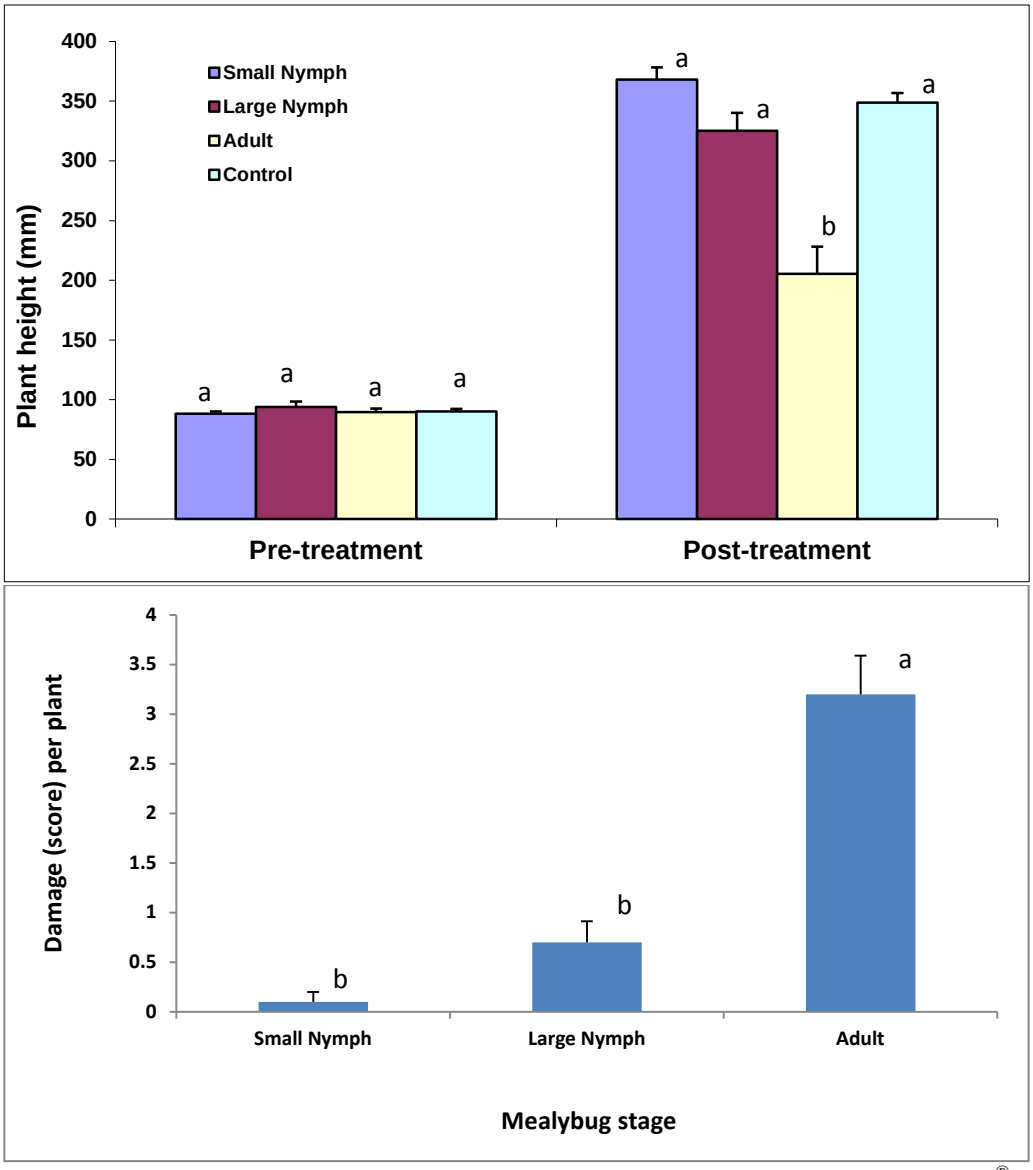


Figure 1. Damage caused by the establishment of different stages of mealybug to Bollgard® II cotton in the glasshouse. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

Table 1. Effects of establishment of different stages of mealybug on seedling cotton in the glasshouse. SE indicates standard error of means. The means with same letter within column are not significantly different ($p > 0.05$), Fisher's LSD test.

Treatments	Square no. ± SE	Days to square ± SE	Mealybug no. at the termination (square root) ± SE
Adult	0.5 ± 0.31 b	71.4 ± 3.38 a	18.58 ± 1.60 a
Large Nymph	1.9 ± 0.41 a	60.5 ± 1.06 b	10.92 ± 2.47 b
Small Nymph	2.2 ± 0.29 a	60.9 ± 0.80 b	3.57 ± 0.86 c
Control	1.7 ± 0.42 a	59.7 ± 0.90 b	-

Experiment 2

During the experiment mealybugs were found to congregate on the underside of leaves, initially on the leaf base (junction of the petiole and leaf blade) (Plate 2A). As the level of infestation progressed, mealybug spread to cover the entire leaf (Plate 2B). Once squares and bolls were produced the insects colonised the inside of the bracts (Plate 2C).

Plate 2. Mealybug population increase the leaf undersides over time - left to right.

Initial damage symptoms show up as brownish areas on the lower surface of the leaf base with a reddening on the upper surface (Plate 3A&B). When the population increased, the whole leaf turned yellow and brown (Plate 3C) and this was eventually shed by the plant.

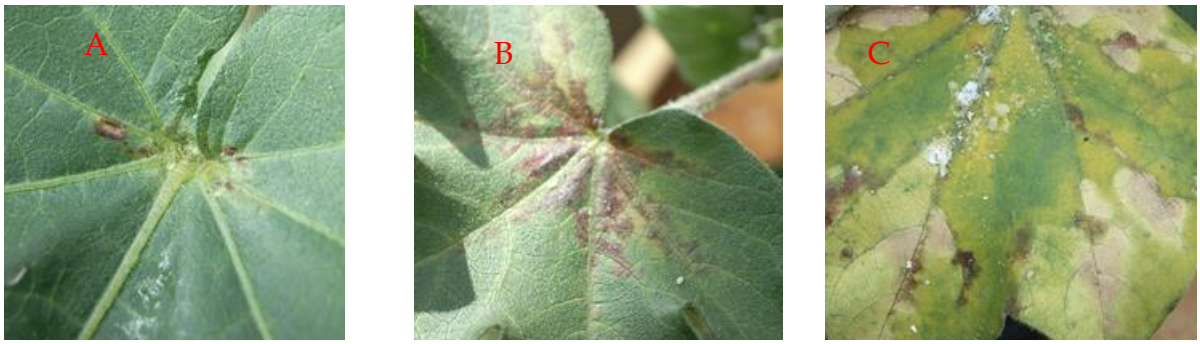


Plate 3. Progression of damage symptom on leaves from mealybug feeding over time.

Mealybug feeding inside the bracts caused these to become brown and papery (Plate 4A). Small squares and bolls also turned brown (Plate 4B) and dropped. When most of the leaves and some squares and bolls had dropped, mealybug moved to the top of the plant where they crowded on to the upper stem and tips (Plate 4C).

Plate 4. Damage symptom on squares, bolls and stems from mealybug feeding

Overall, the cotton plants used for this experiment grew poorly produced small number of fruits due to the experiment being conducted during winter and difficulties with maintaining warm enough temperatures in the glasshouse to ensure more vigorous growth. However, the analysis (ANOVA) established that mealybug introduced at the 4 – 5 leaf, squaring and 1 week after flowering stage plants produced significantly lower numbers of bolls ($F = 19.5$, $p < 0.001$) than other crop stages (Figure 2A). These plants suffered more damage than older plants as mealybug had a longer opportunity to feed and breed compared with later timed infestations. At harvest mealybug numbers were higher in these plants than on older plants. The introduction of mealybug to young 4 -5 leaf stage plants resulted in significant growth disruption and a failure to produce bolls. Yield (boll weight (g)/plant) was also significantly less ($F = 40.91$, $p < 0.001$) in these plants than older plants (Figure 2B). Compared to control plants (with no mealybug) mealybug established at mature and 4 weeks after flowering stage plants did not lose any yield whereas mealybug established at 1 week after flowering, squaring and 4 – 5 leaf stage yield loss was 65, 90 and 1005 respectively.

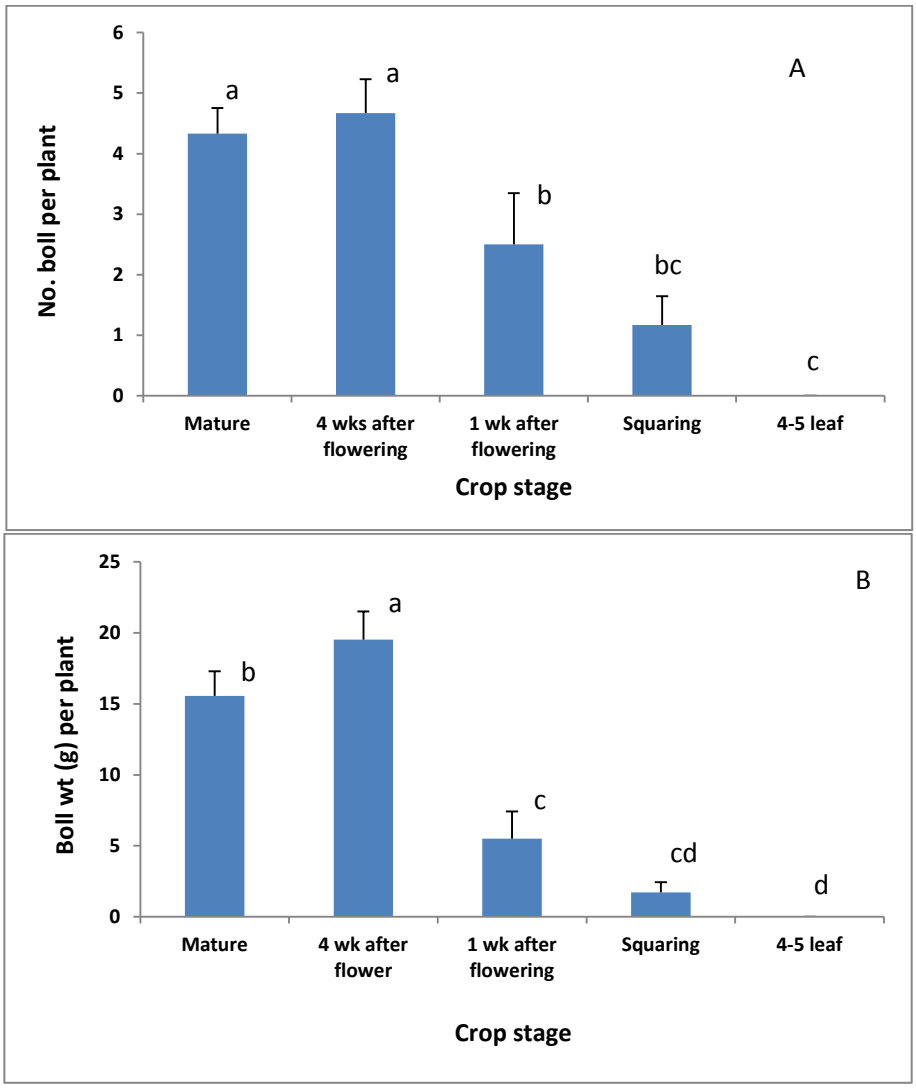


Figure 2. Number of boll (A) and boll weight (B) per plant infested with mealybug at different crop stages of Bollgard® II cotton in the glasshouse. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

Field Experiments

Experiments were conducted in Byee, Emerald and Condamine, Queensland.

Byee Experiments

Methodology

Only the 2011-12 season experiment could be completed as the 2012-13 experiment was abandoned due to severe flooding in January and there was no mealybug present at the experimental site during the 2013-14 season.

The 2011-12 experiment was conducted in dryland cotton (variety Sicot 71BRF). Canopy® oil (@ 1% v/v) and Supracide® (@ 1.4 L/ha) were used at different stages of cotton to manipulate mealybug numbers to attain an infestation gradient. Each of the chemicals was applied at squaring and boll setting stage in separate plots. Supracide® was also applied at both crop stages on the same plot. The treatments, including a control (without any chemical), were applied on 6 row x 30m plots with 3 replications each in a RCB design. The chemicals were applied using a ground rig sprayer (@ 100 – 110 L/ha). Assessments were made on 3 marked plants per plot at 7-15 day interval starting from 1st squaring stage. On each assessment date the number of mealybug (small and large nymphs, adults and adults with ovisacs), beneficials, squares, flowers and bolls were recorded. The planned harvest did not occur due to flooding and sunburn in between the last assessment and the harvest date. The plants in all treatments including control dropped almost all bolls at this time.

For both mealybug and fruit numbers square root transformation was used to normalise the data for analysis. The transformed data were analysed using a Repeated Measure Analysis.

Results

The experiment was affected initially by drought and then by rain and flooding. Immediately after flooding the cotton dropped almost all leaves and fruits and many of the trial plants died. Number of mealybug and fruits for each treatment are presented in Figure 3A and 3B respectively. For the last date, the fruit number refers to bolls from regrowth that never made it to harvest. The analysis showed no significant difference between treatments either for mealybug ($F = 1.14$, $p > 0.05$) or number of fruit ($F = 1.72$, $p > 0.05$). Therefore no attempt was made to further analyse the data.

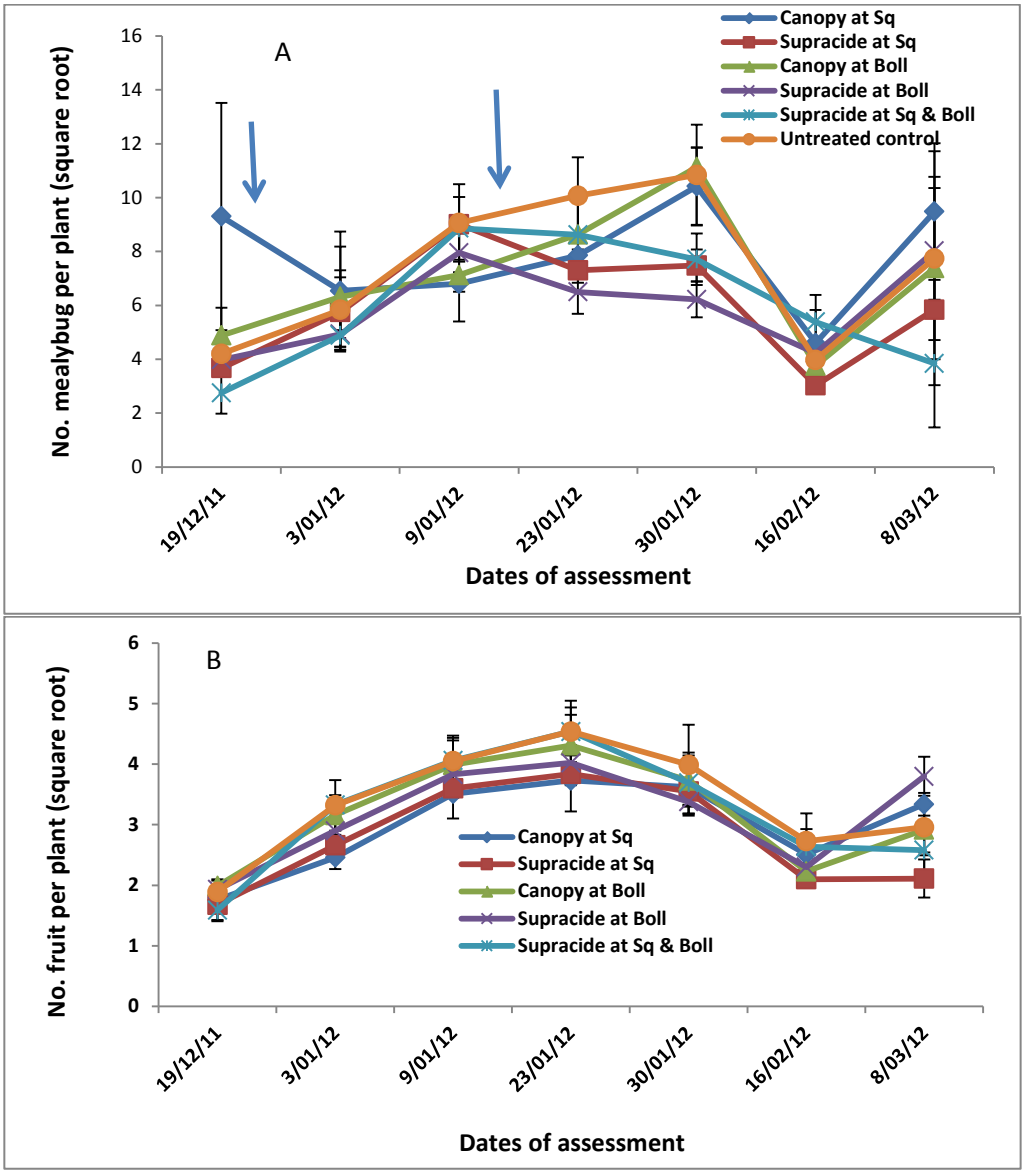


Figure 3. Number of mealybug (A) and fruit (B) per plant in a damage assessment trial in Byee. Vertical arrows indicate time of spray and error bars indicate standard error of means.

Emerald Experiments

Two experiments were conducted in Emerald one each at 2012-13 and 2013-14 season.

Experiment 1

Methodology

During 2012-13 an experiment was conducted through manipulation of the natural population similar to Byee as described above but using different chemicals. Movento® (spirotetramat) was used in one treatment and Supracide® (methidathion) plus Tokuthion® (prothiofos) in another treatment. Tokuthion® was applied 12 days after Supracide® to the same plots. The chemicals were applied first on 3rd January 2013 (about two 2 weeks after first flower) and finally on 16th January 2013 (about 4 weeks after first flower) with a gas-pressured hand boom sprayer at the rate of 107 L/ha, fitted with two overhead nozzles (DG Tee Jet 10015 vs flat fan) per row with 1.6 bar pressure and walking speed of 4 km/hr. The chemicals were applied on 8 rows x 10 m plots with three replications each. Two rows were applied at a time. Unsprayed plots were included as controls. Mealybug numbers and damage from five randomly selected plants per plot were recorded fortnightly from the day before the first spray (at 2 weeks after first flower) until boll open stage. Cotton was hand harvested from 5 x 1 m lengths per plot.

For mealybug numbers log (y + 0.1) transformation was used to normalise the data for analysis. The transformed data were analysed using Repeated Measure Analysis of Variance. Data on yield was subjected to analysis of variance (ANOVA).

Results

Mealybug was not detected in the field until 2 weeks into flowering and remained low (3 to 109 per plant); never reaching damaging levels even in the control plots (Figure 4). Repeated Measure Analysis showed that for mealybug numbers difference between treatments was not significant (F = 1.31, $p > 0.05$) and the interaction between days and treatment was also not significant (F = 0.26, $p >$ 0.05).

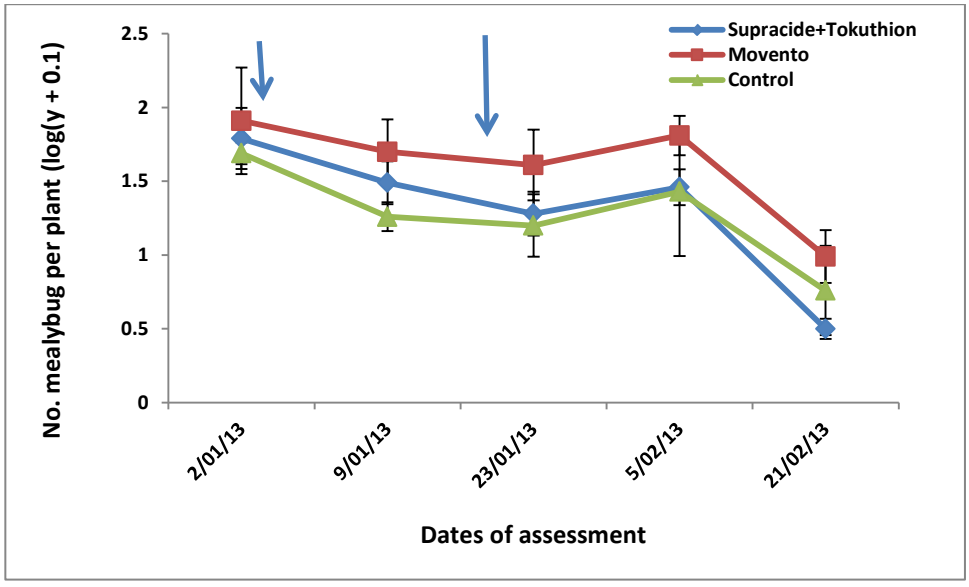


Figure 4. Number of mealybug per plant in a damage assessment trial in Emerald 2012-13. Error bars indicate standard error of means and vertical arrows indicate time of spray.

Yield was low, 6.3 to 6.6 bales per hectare (Figure 5). As with the low numbers of mealybugs the crop impacts were limited irrespective of the treatments. The ANOVA showed that there was no significant difference between treatments for yield (F = 1.71, $p > 0.05$).

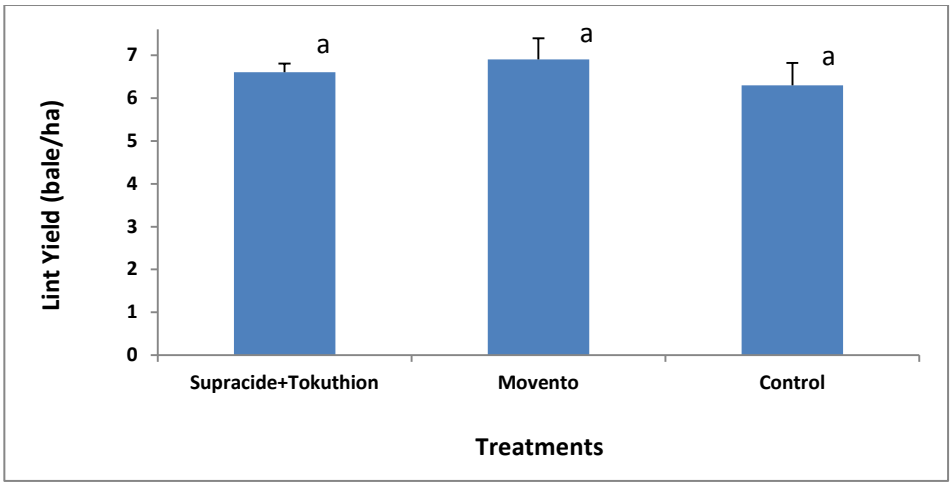


Figure 5. Lint yield (bale/ha) in a damage assessment trial in Emerald 2012-13. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different (p > 0.05), Fisher's LSD test.

Experiment 2

Methodology

During the 2013-14 season the planned field trial was modified to use single plant as a replication. This was necessary due to a very low overall mealybug population and the low numbers that were present were concentrated on scattered plants. There were 5 treatments were replicated 6 times together with a control (no insecticide applied). Treatments consisted of Clap® being sprayed at first flower and two 15 day intervals after that (peak flower and nearing cut out). The treatments were applied once mealybugs were detected in the field. The insecticides were applied using a knapsack sprayer to the point of runoff (250 L/ha). Plants were sampled regularly and the number of mealybug, beneficials, squares and bolls were recorded. Cotton was hand harvested for each plant.

The data was subjected to analysis of variance (ANOVA) and means were separated using Fisher's Least Significant Difference Test.

Results

Mealybug was first detected at flowering stage but was not widespread. Assessment was therefore conducted on individual plants. The results are summarised in Figure 6A for mealybug number per treatments and 6B for yield. The analysis showed that there was a significant difference between treatments for mealybug numbers ($F = 2.74$, $p < 0.05$). Fisher's Least Significant Test revealed that the application of Clap® at 15 day intervals had significantly less mealybug than control and the treatment that used Clap® spray at cut out stage. However, this did not translate into a yield difference. The analysis on the yield data showed that there was no significant difference between treatments ($F = 1.02$, $p > 0.05$). One of the reasons might be the overall mealybug number was low (Figure 6A). It was observed in the field and glasshouse that from flowering onwards only high numbers of mealybug (> 200/plant) are likely to cause plant damage whereas in this experiment mealybug numbers were only 86/plant (Figure 6A).

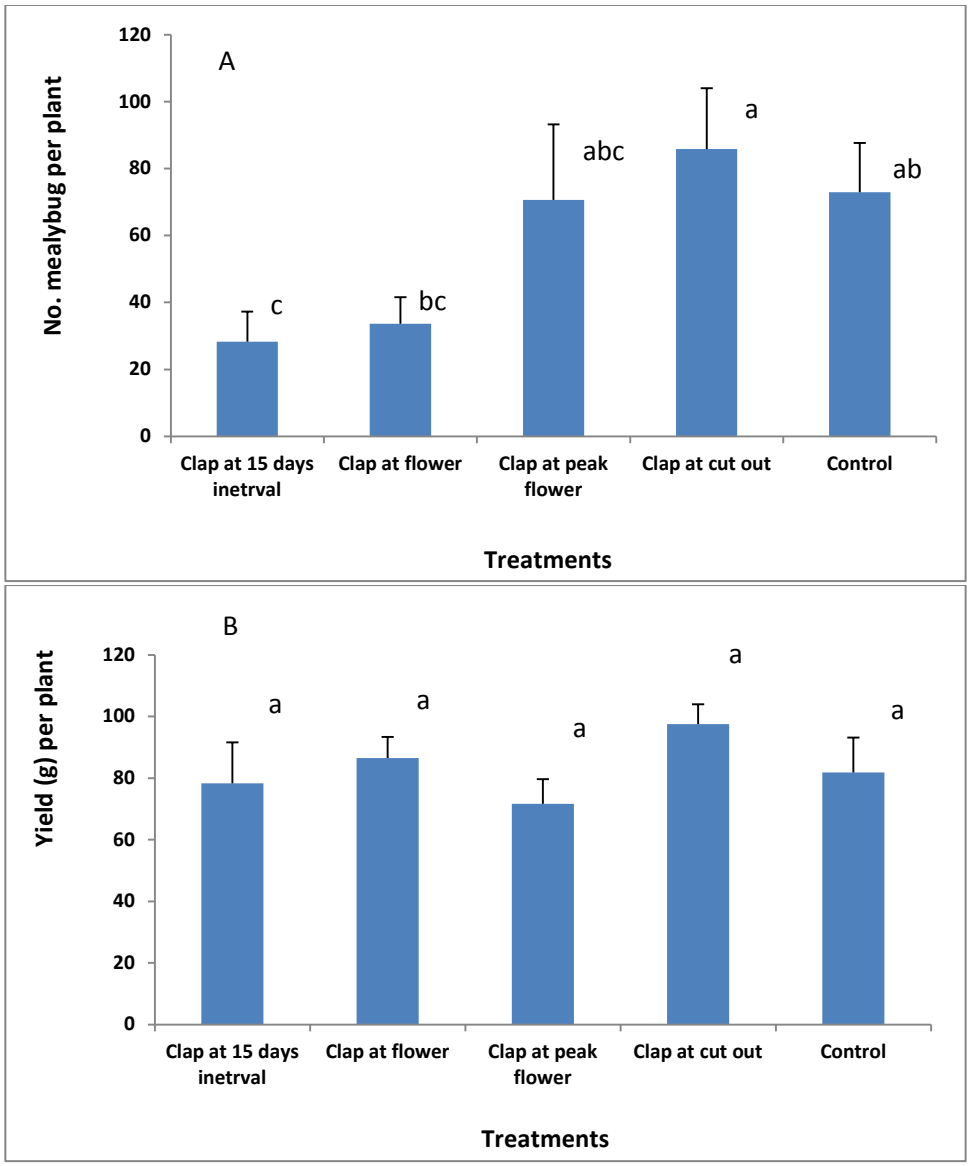


Figure 6. No. mealybug per plant (A) and yield (B) in damage assessment trial during 2013-14 season in Emerald. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

Condamine Experiment

Methodology

The experiment was conducted at peak flowering stage during the 2013-14 season. Actara® (thiamethoxam @ 400 g/ha) and Clap® (buprofezin @ 1.2 L/ha) were applied on 4 row x 10m plots to manipulate the natural mealybug population. Unsprayed plots served as controls. The treatments were replicated three times in a RCB design. The insecticides were applied twice with the second spray applied 10 days after the first application. A gas-pressured hand boom sprayer was used at the rate of 160 L/ha, fitted with two dropper and one overhead nozzle (DG Tee Jet 10015 vs flat fan) per row with 1.6 bar pressure and walking speed of 4 km/hr for the first spray and a knapsack sprayer (@ 200 L/ha) was used for the second spray. Mealybug and beneficial numbers were recorded from 5 marked plants per plot before spraying and at 7-15 day intervals after spraying. Plants were assessed before spraying and again at harvest to record the number of bolls. 3 x 1m strips per plot were harvested by hand to determine yield.

For mealybug numbers log (y + 0.1) transformation was used to normalise the data for analysis. The transformed data were analysed using Repeated Measure Analysis of Variance. If interactions were identified data was also subjected to REML testing of fixed effects for differences between treatments within each day. Data on yield was subjected to analysis of variance (ANOVA).

Results

The incidence of mealybug was reported for the first time in the experimental field from this area at peak flowering stage. The cotton was suffering from drought as the grower did not have water to irrigate and in the subsequent weeks the crop suffered severely which affected the experiment's outcomes.

The results are summarised in Figure 7. The analysis showed that mealybug numbers were significantly different between treatments (F = 9.95, d.f. = 4, P = 0.03) (Figure 7A). The analysis also showed that the interaction between treatments and days of assessment was significant (*F* = 5.14, d.f. = 18, *P* = 0.003). REML testing of fixed effects revealed that in the last two assessments mealybug numbers were significantly different between treatments (*F* = 8.25, d.f. = 12.8, *P* = 0.005 and *F* = 19.45, d.f. = 12.8, *P* = <0.001). However, this difference did not have effect on yield (Figure 7B). The analysis on the yield data showed that there was no significant difference between treatments (*F* = 0.34, *P* = 0.729).This was perhaps due to the fact that the crop was suffering severely from drought which masked any mealybug impacts across the treatments.

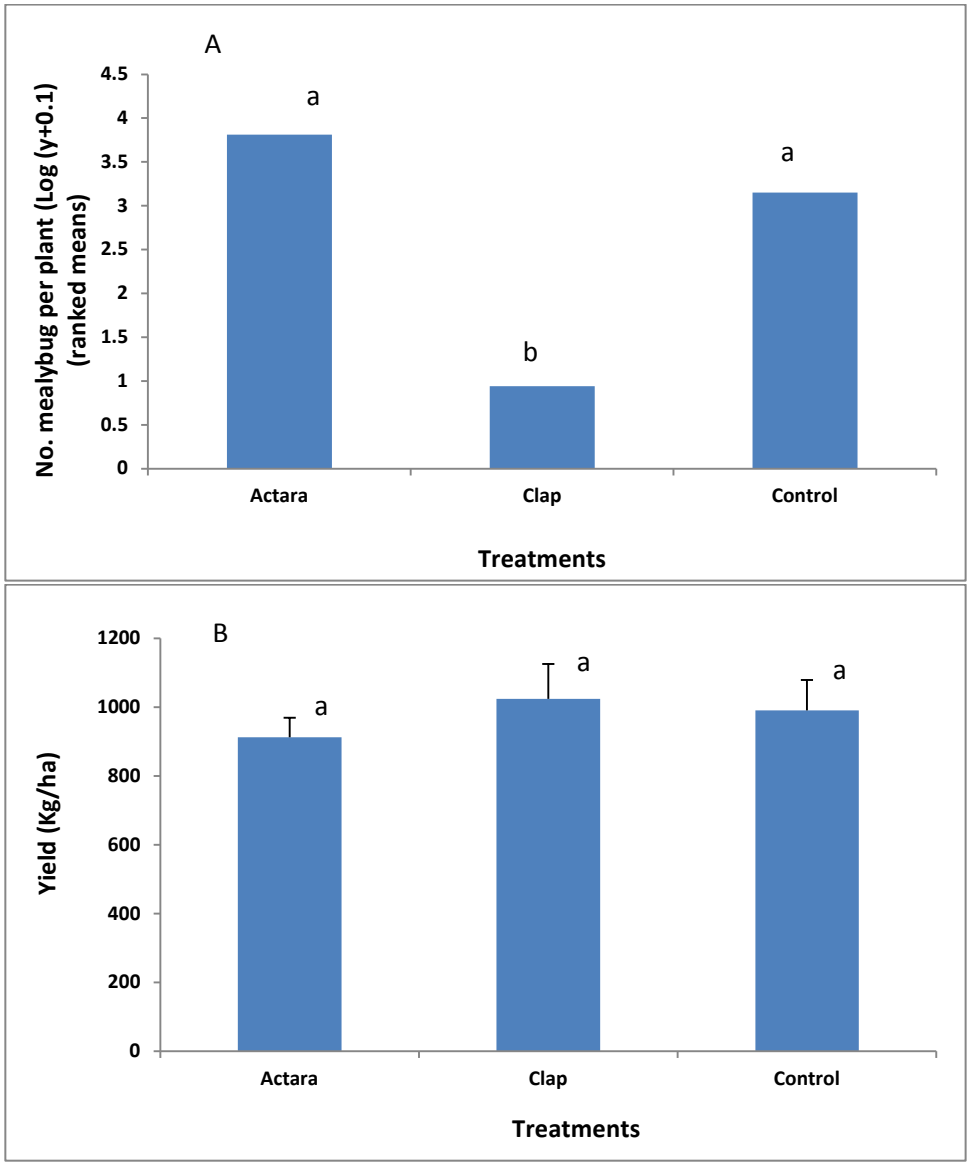


Figure 7. No. mealybug per plant (A) and yield in damage assessment experiment in Condamine during 2013-14 season. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different (p > 0.05), Fisher's LSD test.

Mealybug damage in St George

Methodology

An assessment was also made in a mealybug infested field in St George (cotton variety Sicot 74BRF). No treatments were applied in this trial but a yield assessment was made from plants with different levels of mealybug infestation. Solenopsis mealybug was first detected when the crop was close to defoliation. The field was also sprayed three times throughout the season with Abamectin (@ 600 mL/ha) and fipronil (@ 63 mL/ha), fipronil (@ 40 mL/ha) plus salt and pix and pyriproxyfen (@ 500 mL/ha) to control mites, mirids and whitefly. These insecticides are unlikely to have a direct effect on solenopsis mealybug. However, they will have an adverse impact on those beneficials that can keep solenopsis mealybug populations under control.

Mealybug damage in this field ranged from no damage (no mealybug present) to severe damage (dead plants). Five levels of damage were categorised-

- Severe damage - dead plants, no leaves, little or no remaining bolls.
- High damage - plants dying, top 8/9 nodal leaves and bolls dropped and few remaining bolls.
- Medium damage - plants were normal with tip and 1st and 2nd nodal leaves yellowing, most top bolls dropped.
- Low damage - plants were normal some top bolls dropped.
- No damage – there was no evidence of mealybug on plants.

In each damage category, 5 x 1m areas were hand harvested to determine yield.

The data was subjected to Analysis of Variance (ANOVA) and means were separated using Fisher's Least Significance Difference Test.

Results

The highest lint yield achieved in the field experiment was 6 bales per hectare in the Solenopsis mealybug free areas of the crop (Figure 8). This low yield may be attributed to the field having one less irrigation than required. The analysis showed that for lint yield there was significant difference between different damage level (F = 63.19, P = 0.000). Fisher's Least Significant difference test revealed that lint yield in severely damaged cotton was significantly lower than other damage level followed by high and medium damaged cotton Figure 8). However, there was no significant difference between a low level of damage and the control and low and medium level of damage (Figure 8). When compared to the control, lint yield was 16.7, 35, 66.7 and 93.3% lower for low, medium, high and severe levels of damage respectively.

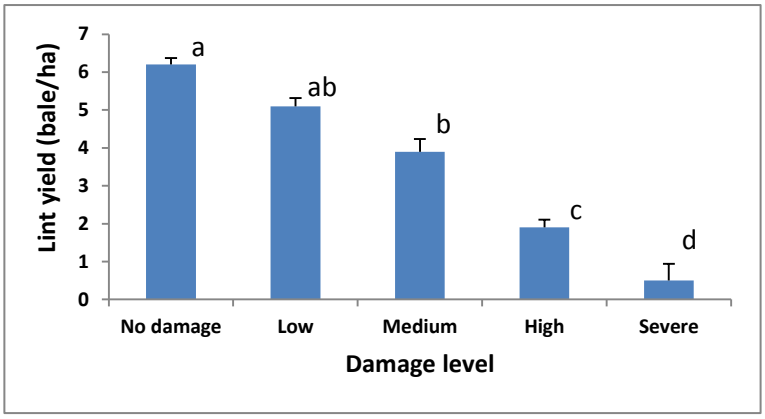


Figure 8. Lint yield (bale/ha) for different damage levels in the field, St George. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different (p > 0.05), Fisher's LSD test.

Mealybug population dynamics and their key beneficials on Bollgard® cotton

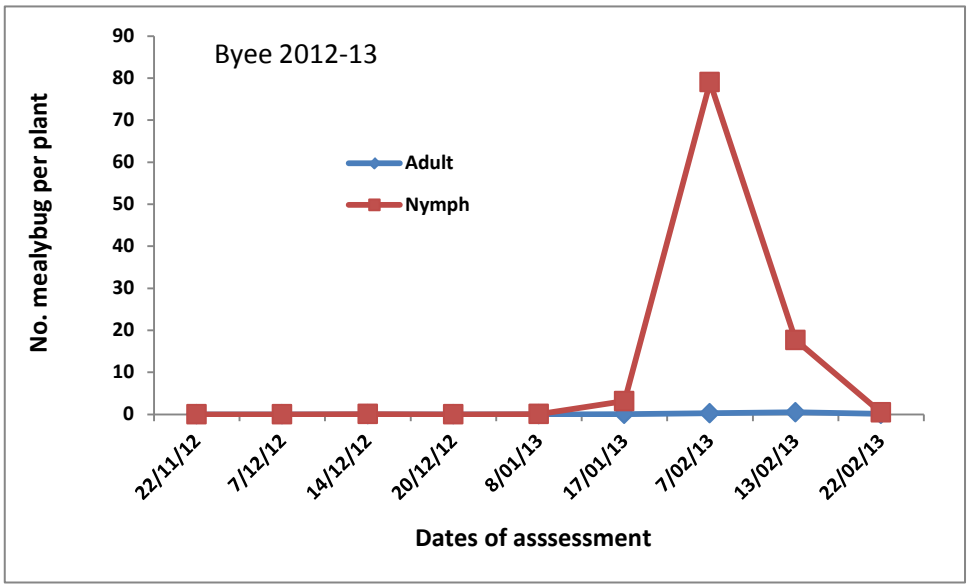
Methodology

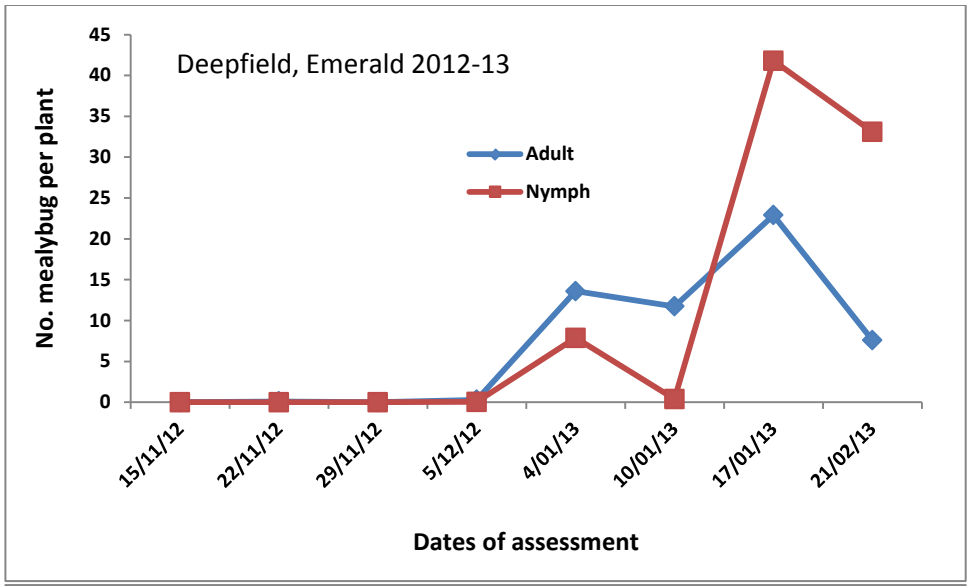
To understand mealybug seasonal abundance and the relationship between mealybug and its key beneficials, cotton was regularly sampled in Byee and in Emerald during 2012-13 and 2013-14. One field in Byee and three fields (Deepfield, Killara and Kerry Downs during 2012-13 and Ryan 1 & 2 and Walters during 2013-14) in Emerald were visually sampled weekly starting from seedling stage. In both locations fields with a previous history of mealybug were selected and at each field, from seedling to flowering stage 30 plants and from flowering and onward 20 plants from a 2-5 hectare area were sampled. Plants were randomly selected by walking across the field and mealybug and beneficials numbers were recorded.

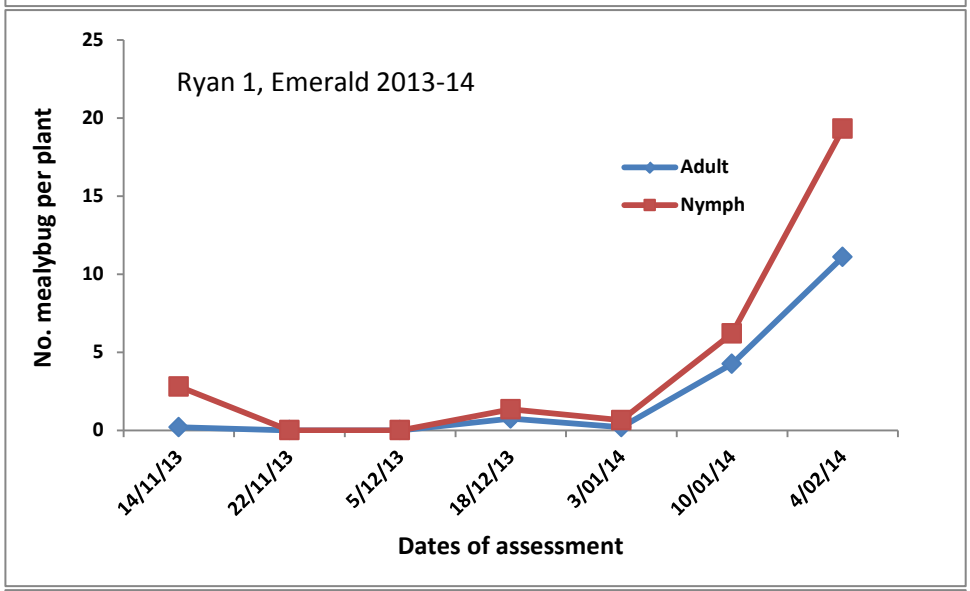
Square root transformation was used to normalise the data for working out correlation between mealybug and their main natural enemies using Pearson Correlation function.

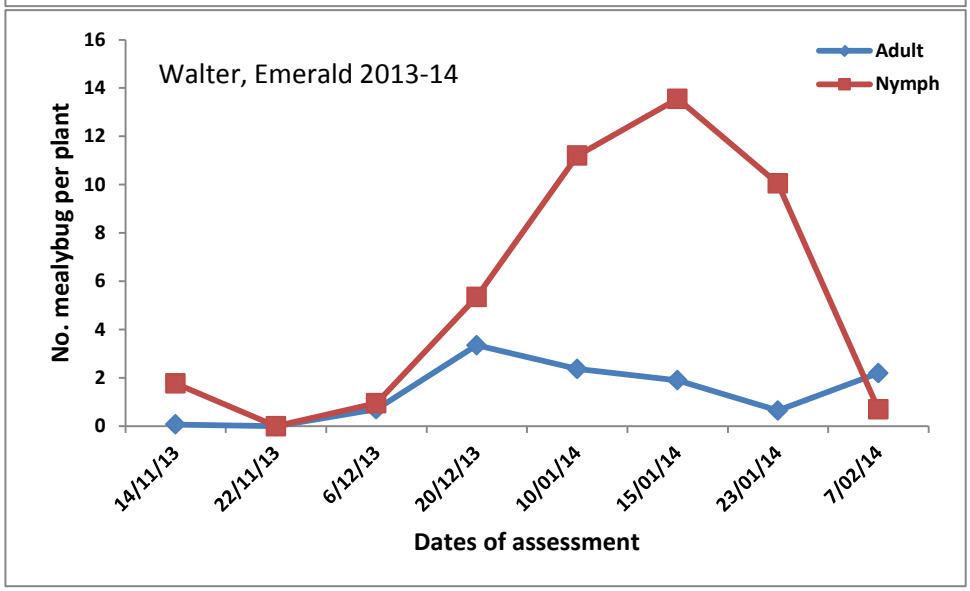
Results

Overall mealybug numbers were very low in the trial fields particularly at Kerry Downs and Killara in Emerald during 2012-13 where population never exceeded 1 per plant therefore the results for these fields are not presented here. The seasonal abundance data from other sites are summarised in Figure 9. The results showed that mealybug was not found until flowering stage except Ryan1 and Walter in Emerald during the 2013-14 season. At Ryan 1 and Walter mealybug were found at the squaring stages. In all sites dominant stage was nymph. The result also showed that whenever mealybug established in the trial sites they grew slowly and reached peak once at close to cut out stage but never developed hot spots. One of the reasons might be that the starting population in the experimental fields was very low. Another reason could be that natural enemies such as lacewings, lady beetles and spiders kept the mealybug populations in check.




Deepfield, Emerald 2012-13
No. mealybug per plant
45
40
35
30
25
20
15
10
5
0
Adult
Nymph
15/11/12
22/11/12
29/11/12
5/12/12
4/01/13
10/01/13
17/01/13
21/02/13
Dates of assessment


Ryan 1, Emerald 2013-14
No. mealybug per plant
25
20
15
10
5
0
Adult
Nymph
14/11/13
22/11/13
5/12/13
18/12/13
3/01/14
10/01/14
4/02/14
Dates of assessment


Walter, Emerald 2013-14
No. mealybug per plant
16
14
12
10
8
6
4
2
0
Adult
Nymph
14/11/13
22/11/13
6/12/13
20/12/13
10/01/14
15/01/14
23/01/14
7/02/14
Dates of assessment

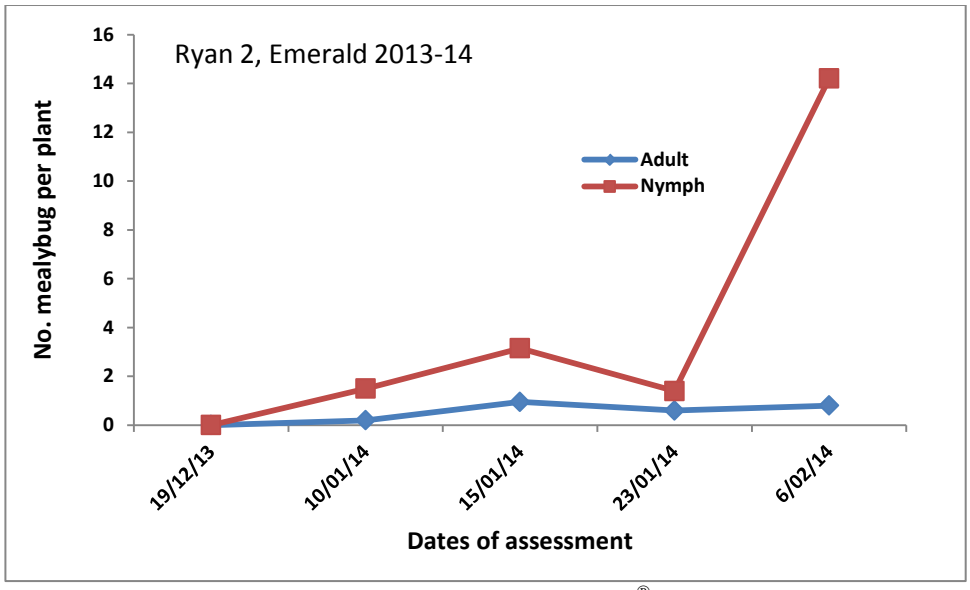
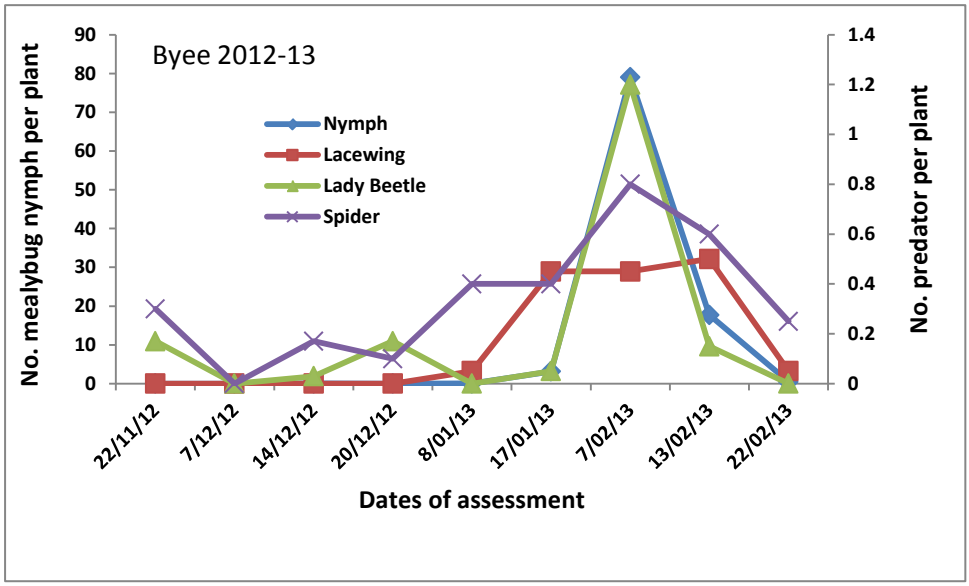
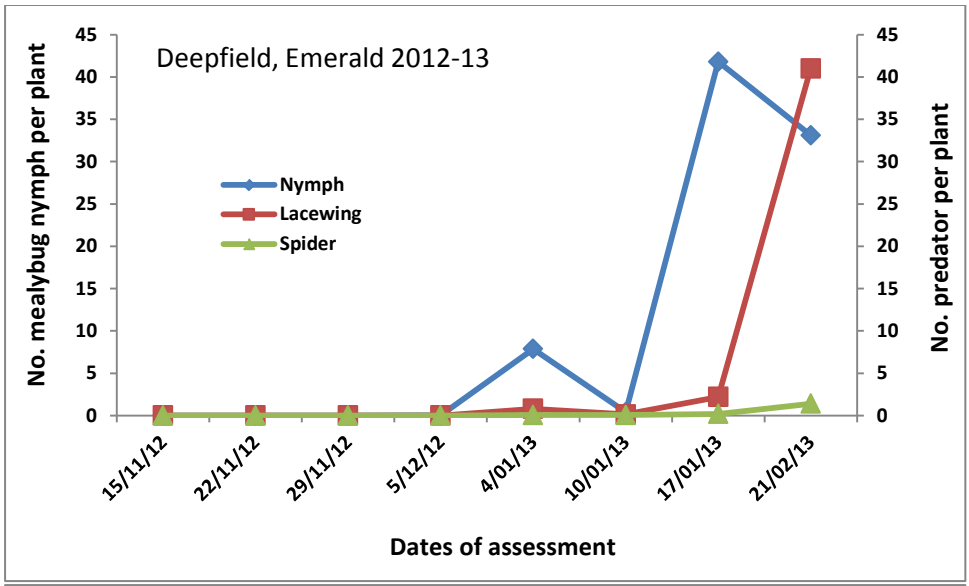


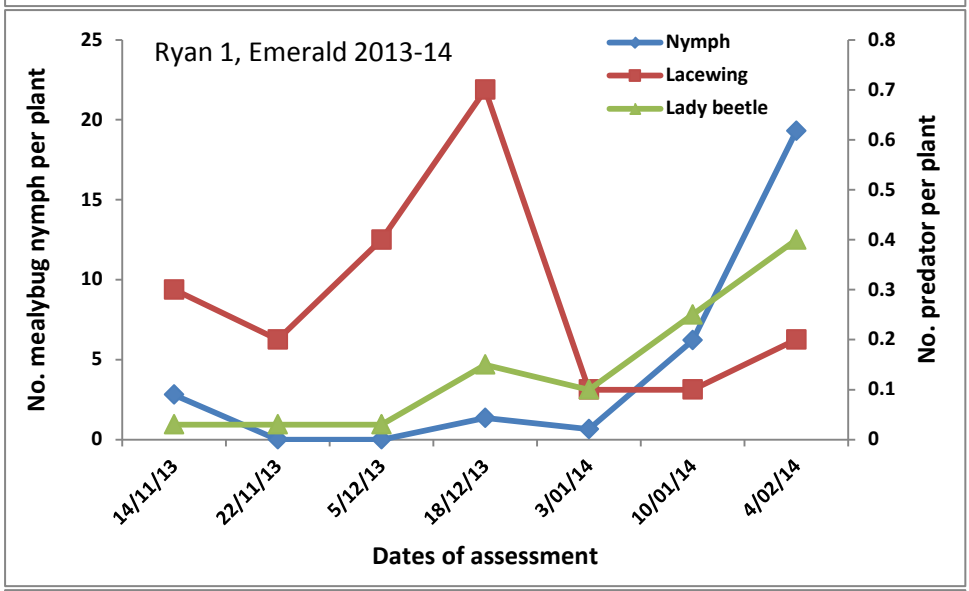
Figure 9. Seasonal abundance of mealybug in Bollgard® II cotton

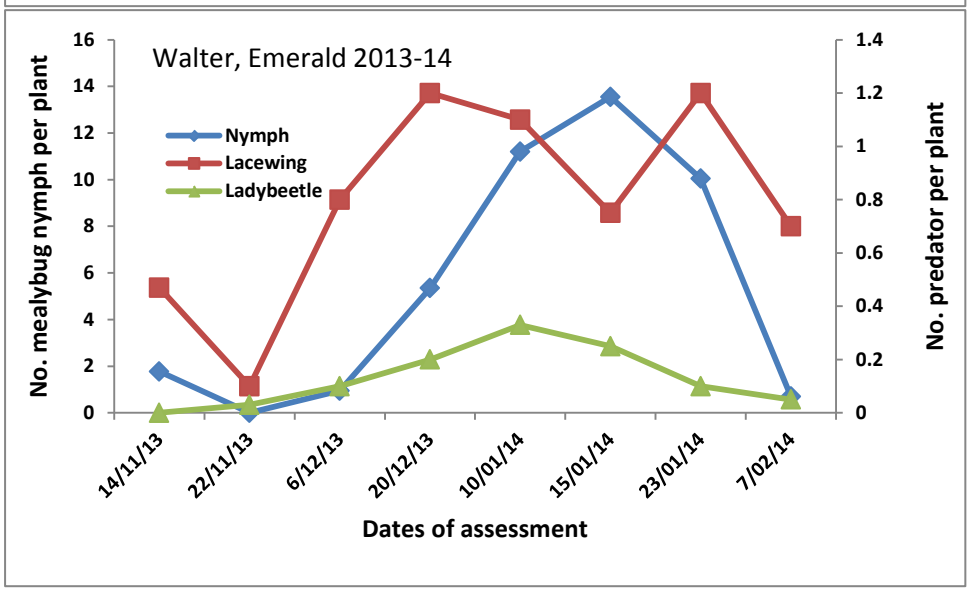
As mealybug nymphs were the dominant life stage observed in these fields the relationship between mealybug nymphs and their main predators such as lacewings and lady beetles (Cryptolaemus montrouzieri and other species) are presented in Figure 10 and Table 2. The analysis also showed a significant ($p < 0.05$) positive relationship between mealybug nymphs and main predators except for lacewing in Ryan 1, Emerald during 2013-14 where relationship was negative but was not significant ($p > 0.05$). As a result mealybug numbers were not reduced completely and later both predators and mealybug populations progressed slowly.

This seasonal abundance data suggest that mealybug can establish on the cotton at any crop stage and the severity of infestation (hot spots) depends on the starting population size along with other factors such as natural enemies and weather parameters. However, to pinpoint key factors that regulate mealybug population a detailed study on these aspects is needed.




Deepfield, Emerald 2012-13
Nymph
Lacewing
Spider
No. mealybug nymph per plant
No. predator per plant
0
5
10
15
20
25
30
35
40
45
15/11/12
22/11/12
29/11/12
5/12/12
4/01/13
10/01/13
17/01/13
21/02/13
Dates of assessment


Ryan 1, Emerald 2013-14
Nymph
Lacewing
Lady beetle
No. mealybug nymph per plant
No. predator per plant
0
5
10
15
20
25
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
14/11/13
22/11/13
5/12/13
18/12/13
3/01/14
10/01/14
4/02/14
Dates of assessment


Walter, Emerald 2013-14
Nymph
Lacewing
Ladybeetle
No. mealybug nymph per plant
No. predator per plant
0
2
4
6
8
10
12
14
16
0
0.2
0.4
0.6
0.8
1
1.2
1.4
14/11/13
22/11/13
6/12/13
20/12/13
10/01/14
15/01/14
23/01/14
7/02/14
Dates of assessment

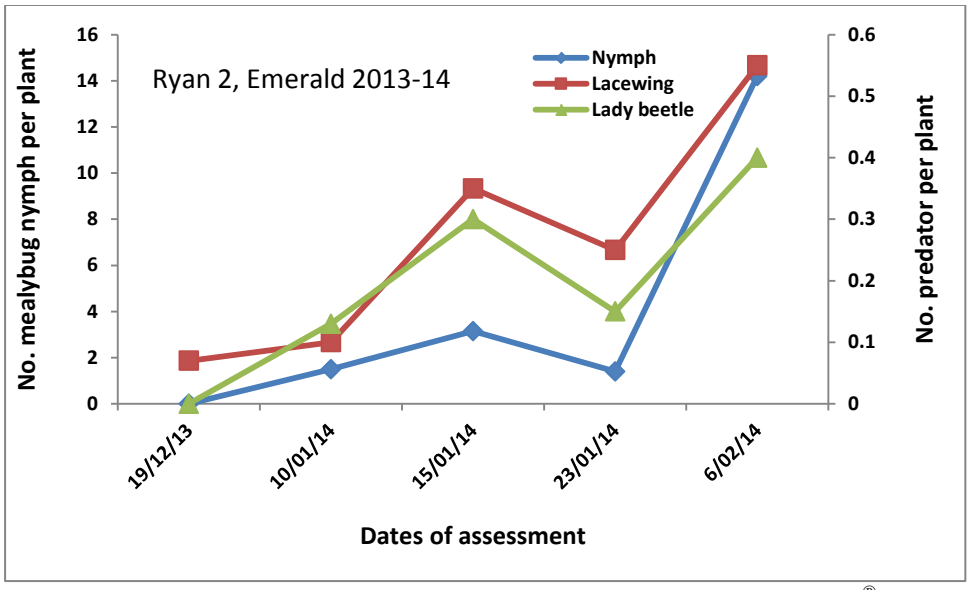


Figure 10. Relationships between mealybug and its predators in Bollgard® II cotton

Table 2. Pearson correlation between mealybug nymph and its main predators (square root transformed) in Bollgard® II cotton

Location	Lacewing	P-Value	Ladybeetle	P-Value	Spider	P-value
Byee 2012-13	0.772	0.015	0.843	0.004	0.717	0.030
Deepfield, Emerald 2012-13	0.743	0.035	-	-	0.818	0.013
Ryan 1, Emerald 2013-14	-0.250	0.588	0.874	0.010	-	-
Walter, Emerald 2013-14	0.730	0.040	0.730	0.040	-	-
Ryan 2, Emerald 2013-14	0.908	0.033	0.877	0.045	-	-

Mealybug parasitoid identification

Methodology

Brown coloured mummified solenopsis mealybugs were discovered on solenopsis infested cotton plants at Byee in the same field described above for the population dynamics study in the second week of April 2012. The mummies were brought to the laboratory and reared out to adults in small transparent plastic jars in a controlled temperature room (25±1°C, 60±5% RH and 14:10h light-dark cycle). The insects were preserved in 70% alcohol and sent to Dr John La Salle, CSIRO, Canberra, for identification.

In addition to morphological identification, species was confirmed by PCR analysis conducted using parasitoid specific primers for *Aenasius bambawalei*, AenF1 (GTTTCTCACATAATTTGTAG) and AenR1 (CCTCGGAGGATAAAAAGAC) developed by Ashfaq *et al*. 2010.

Results

On two occasions, 17 mummified mealybugs (Plate 5) were collected for parasitic emergence. Of these, 9 (52.9%) emerged as adults. The parasitoid was identified as *A. bambawalei* by John La Salle in consultation with Dr John Noyes (Natural History Museum, UK and an expert on Encyrtids) and Dr Mohammad Hayat (Aligarh Muslim University, India and the author of this species).

This species can be separated from the other species of *Aenasius* in India by its cylindrical antennal scape, distinctly broader frontal vertex and presence of a hyaline streak adjacent to the postmarginal and stigmal veins of the fore wing. Both male and female parasitoids collected from Byee agree with these characters (Plate 6). The Byee specimens however were slightly darker and had a greater infuscation on the fore wing than the published specimen. This may be due to geographical variation.

Confirmation of the species was also established by PCR analysis. A single PCR band of 744bp was observed for each wasp sample (Plate 7). This result is consistent with published results for *A. bambawalei* and thus confirms that the parasitic wasp discovered at Byee is *A. bambawalei*.

Plate 5. Mummified mealybug on cotton square parasitised by *A. bambawalei* in Bollgard® II cotton at Byee.

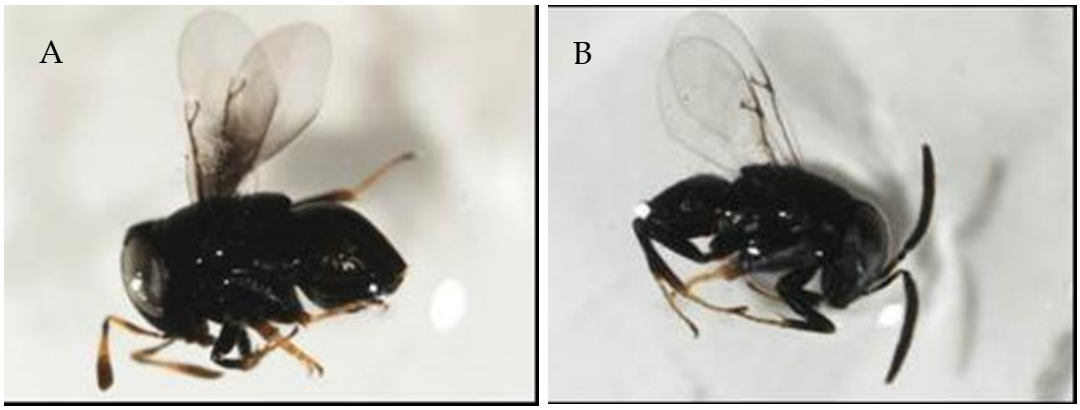


Plate 6. Adult *A. bambawalei* female (A) and male (B) showing streak adjacent to the post marginal and stigmal veins on fore wings and differential antennal type for female and male.

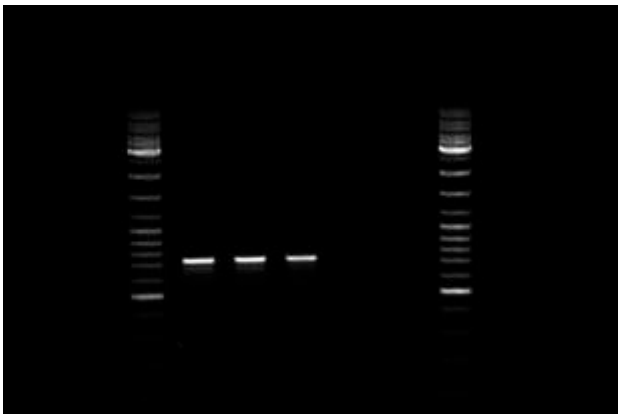


Plate 7. PCR detection of a 744bp fragment from three individual wasps (lanes 2,3,4) using *A. bambawalei* specific primers AenF1 and AenR1.

Level of parasitism

Methodology

Following its first record in Byee regular sampling was conducted to determine the level of parasitism on 30 plants until harvest in June in the same field described above for the population dynamics study. Mummified mealybugs were transported back to the laboratory and reared out to adults for species confirmation.

The level of parasitism was also determined in St George in March 2014 in Bollgard® II cotton (variety Sicot 74BRF). Mealybug was first reported in this field in February 2014 at the post-cut out stage. The field was sampled once in early March. Survey was conducted on 21 spots, 5 plants per spot thus 105 plants with a low to medium mealybug population in a 2 hectare area. The proportion of parasitism was calculated from total number of adults and large nymphs since the parasitoid do not parasitise small nymphs.

Byee data was transformed into log transformation before analysis. Transformed data was subjected to Pearson Correlation analysis to determine the relationship between mealybug and parasitism.

Results

The level of parasitism in Byee during the survey period was very low with the highest percentage being 5% (Figure 11 and Table 3). As would be expected with this low parasitism level correlation analysis showed that the relationship between mealybug number and parasitism was positive and was not significant (Pearson Correlation = 0.252, P-Value = 0.482) suggesting parasitoid did not have significant impact on mealybug population during observation period. The low level of parasitism in Byee was perhaps due to a chemical sprays used to control mealybug (Shield and Supracide) along with other factors such as weather parameters which was not included in the observation. On the contrary, the percentage of parasitism in one field in St George was quite high reached up to 64% despite the field being sprayed with abamectin, fipronil and pyriproxyfen.

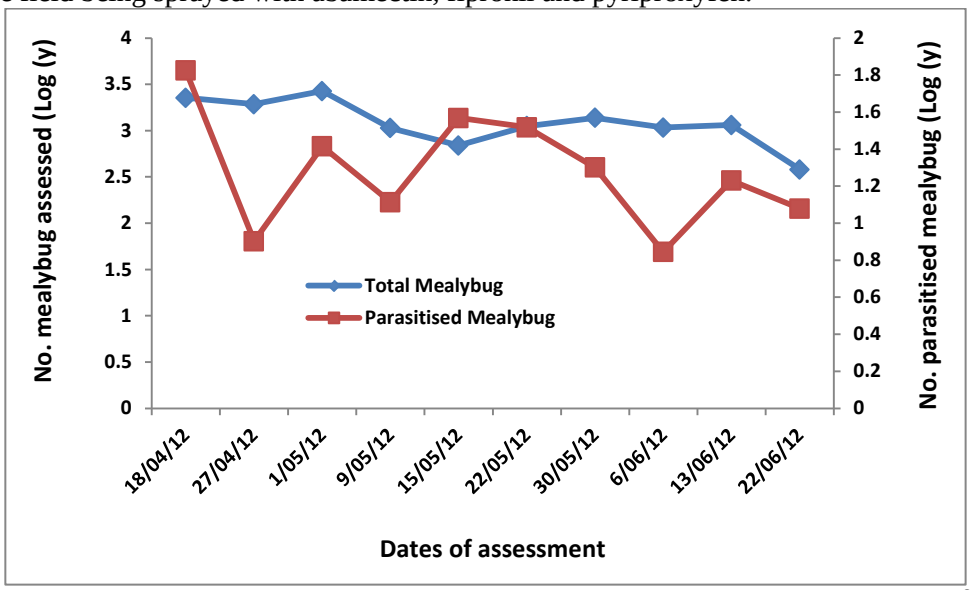


Figure 11. Relationship between mealybug and its parasitoid (*A. bambawalei*) in Bollgard® cotton in Byee during 2011-12 season

Table 3. Level of parasitism of mealybug in Byee during 2011-12 season

Dates of assessment	% parasitism	No. of mealybug assessed
3rd Week April 2012	2.96	2262
4th Week April 2012	0.42	1927
1st Week May 2012	0.97	2670
2nd Week May 2012	1.22	1068
3rd Week May 2012	5.36	690
4th Week May 2012	2.95	1120
1st Week June 2012	1.45	1318

2nd Week June 2012	1.48	1151
3rd Week June 2012	3.16	380

Sampling solenopsis mealybug in Bollgard® cotton

Distribution pattern of mealybug in the field

Methodology

A distribution pattern of mealybug was determined using the data collected for the population dynamics study as mentioned in the previous section. One field in Byee during 2012-13 and three fields (Deepfield, Killara and Kerry Downs during 2012-13 and Ryan 1 & 2 and Walter during 2013-14) in Emerald were visually sampled weekly starting from seedling stage. In both locations fields with a previous history of mealybug were selected and at each field 30 plants at early stage and later 20 plants from a 2-5 hectare area were sampled. Plants were randomly selected by walking across the field and mealybug and beneficials numbers were recorded. To determine the mealybug distribution pattern in the field Taylor's Power Law (TPL): $s^2 = am^b$ was used, where s^2 is sample variance, m is sample mean and a and b are fitted parameters. The parameters a and b were estimated using linear regression of log transformed data, $\log_{10}s^2 = a + b^* \log_{10}m$, where b is the Taylor's index of aggregation (if b = 1 the distribution is random, if b < 1 the distribution is uniform and if b > 1 the distribution is clumped).

Results

At all experimental sites, except Kerry Downs in Emerald (2012-13 season), Taylor's Index of Aggregation (TIA) was close to 2 (Table 4) indicating that the distribution pattern of mealybug in the field was highly clumped. At Kerry Downs mealybug numbers were very low, less than 0.2 per plant which may explain why distribution pattern was different (random).

Table 4. Taylor's Index of Aggregation for mealybug at different sites in Bollgard® cotton

Location	Taylor Index of Aggregation
Byee 2012-13	1.8
Deepfield, Emerald 2012-13	1.6
Killara, Emerald 2012-13	1.7
Kerry Downs, Emerald 2012-13	1.0
Ryan 1, Emerald 2013-14	2.0
Ryan 2, Emerald 2013-14	2.1
Walter, Emerald 2013-14	1.7
Condamine 2013-14	1.8

Distribution of mealybug within plant

Methodology

An experiment was conducted in Emerald during 2012-13 to determine mealybug distribution within the plant at seedling, squaring, flowering and at peak flowering stages with the objective of developing a sampling protocol. Thirty seedlings and 10 plants at later growth stages were randomly selected throughout the field and checked thoroughly for mealybug. Numbers were recorded from whole plants in relation to node number (counted from the top) and plant structures (leaf, stem, squares and bolls). For leaves mealybug numbers were recorded as being on the upper or lower leaf surfaces. For squares and bolls numbers were recorded as inside the bract or on the outside of the squares and bolls.

An experiment was also conducted in Condamine during 2013-14 at the peak flowering stage for distribution of mealybug within the plant at different population levels to determine if distribution patterns vary with population densities. The population levels were high (> 300/plant), medium (100 – 300/plant) and low (< 100/plant). Mealybug numbers were recorded in a similar way to the Emerald trial as described above.

Data was converted to per cent mealybug found on each node or plant structure. The percentage was calculated as number of mealybug found on each node or plant structure compared to the total density of mealybug found on the plant at each crop stage.

Results

The results are summarised in Figure 12 for crop stage and in Figure 13 for population level.

Results showed that for each crop stage except during peak flowering that most mealybug were distributed on the top 10 nodes. At squaring and flowering stages, the 10 top nodes accounted for 88 and 74 per cent population respectively. At the vegetative stage, 100% of the population was all over the canopy (≈on the top 5 nodes) and at peak flowering stage mealybug was equally distributed throughout the plant (Figure 12A).

The results also showed that in relation to plant structure, for crop stage most of the mealybug were distributed on squares and bolls followed by leaves except during the early vegetative stage (Figure 12B). On seedlings, mealybug was equally distributed on stems and tips (40% each) with the remainder occurring on the leaves. The distribution in relation to aspects of plant structure for leaf population 95– 100% were on the underside and among the square and boll population 84 - 100% were inside the bracts

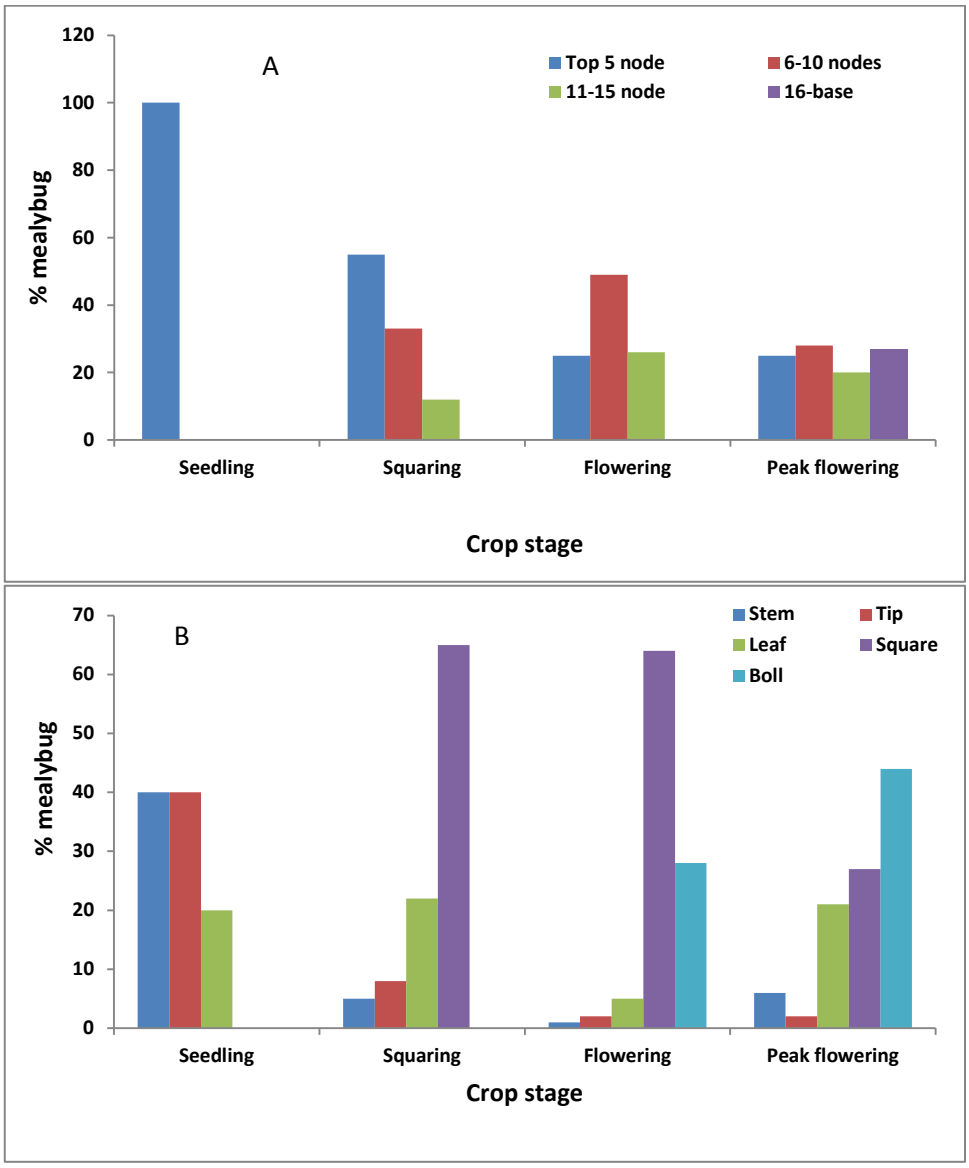


Figure 12. Mealybug distribution in relation to node number (A) and in relation to plant structure (B) at different crop stage in Bollgard® cotton, Emerald 2012-13.

Similar to crop stage irrespective of population densities most of the mealybug were distributed on the top 10 nodes (Figure 13A). For high, medium and low population levels top 10 nodes were accounted for 97, 89 and 91% of the mealybug populations respectively.

In relation to plant structures, for high and medium population levels most of the mealybugs were distributed on squares and bolls followed by leaves whilst for low population densities most of the population resided on squares followed by leaf and stem (Figure 13B). Among the leaf population 81 – 93% was on the underside and among the square and boll population 67 - 99% were inside the bracts.

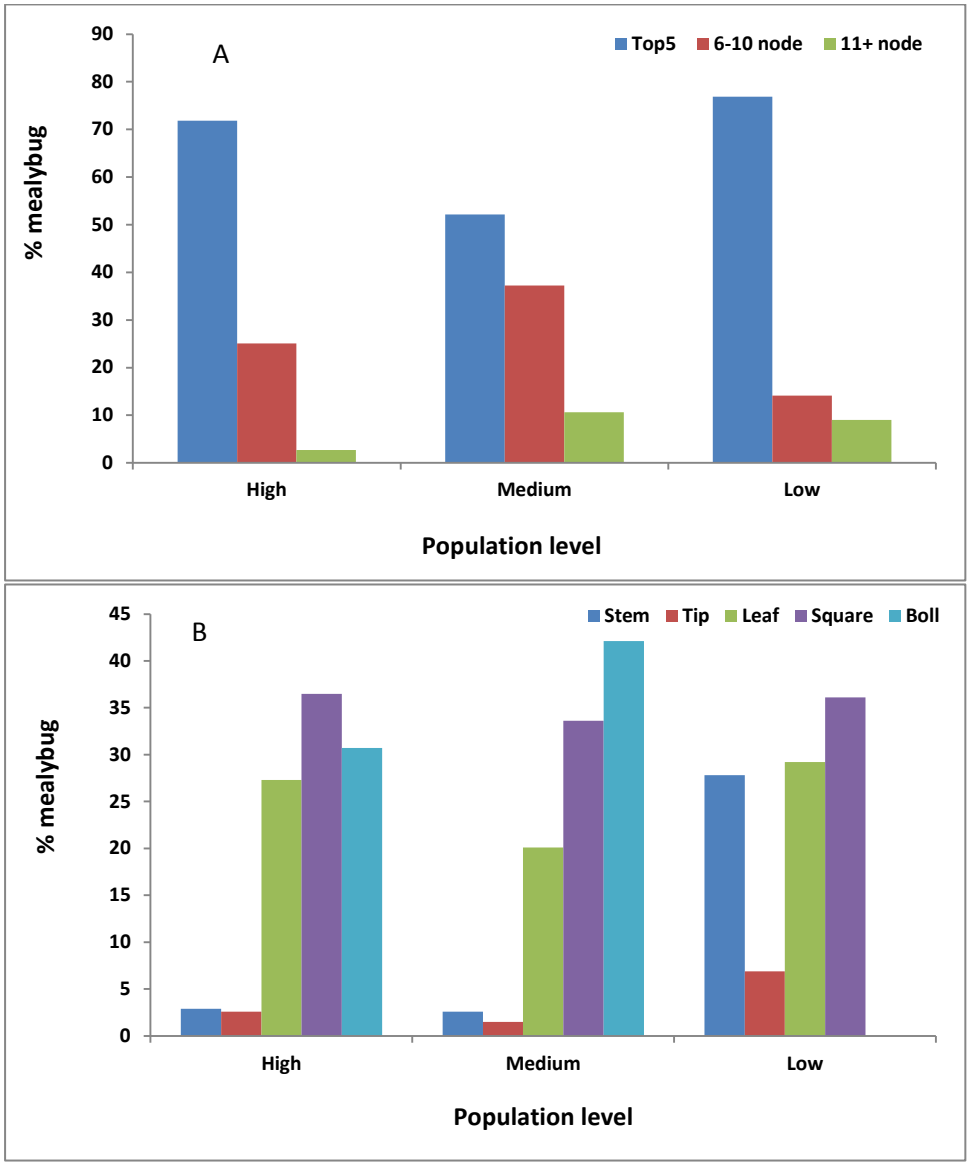


Figure 13. Mealybug distribution in relation to node number (A) and in relation to plant structure (B) at different population level in Bollgard® cotton, Condamine 2013-14.

These results suggest that for population assessment the underside of the leaf and inside the bract of squares and bolls of the top 10 nodes may need to look for mealybug on an individual plant.

Survey of overwintering hosts of mealybug

Methodology

Surveys were conducted on available vegetation (weeds) on cotton farms with a previous history of mealybug in Byee and Emerald to identify possible alternative hosts of mealybug. In Byee surveys were conducted at one site in 2011 (August – September) and 2012 (June – September) and in Emerald at three sites in 2012 (April – October) only. This was due to the host plants not growing in

large areas and their ephemeral growth habit. Therefore, surveys on the same host plants were not possible throughout the survey period or in replication due to their lack of availability.

At Byee in 2012, stagger weed (*Stachys arvensis*) was found to be the most common overwintering host. Quantitative data was therefore taken from 5 spots (1 plant per spot) on each sampling occasion from these weeds. Mealybug numbers were recorded together with their location on the plant.

Results

A wide variety of plants (covering 13 families) in and around cotton fields were found to be overwintering hosts for mealybug (Table 5). Among them, the most common hosts were Parthenium and red pigweed in Emerald and stagger weed and rasp weed in Byee. Quantitative data on stagger weed showed mealybug overwinter both as adults and nymphs (Figure 14). Results also showed that during the winter months mealybug move into the soil and reside on the root zone of the hosts (Figure 15).

Table 5. Lists of overwintering hosts of mealybug in Byee and in Emerald

Common Name	Scientific Name	Family	Location
Parthenium	*Parthenium hysterophorus*	Asteraceae	Emerald
Fleabane	*Conyza spp.*	Asteraceae	Emerald & Byee
Green Amaranth	*Amaranthus viridis*	Amaranthaceae	Emerald
Wild Mustard	*Sinapsis arvensis*	Brassicaceae	Emerald
African Turnip	*Sisymbrium thellungii*	Brassicaceae	Emerald
Turnip Weed	*Rapistrum rugosum*	Brassicaceae	Emerald & Byee
Wild Turnip	*Brassica tournefortii*	Brassicaceae	Emerald & byee
Wild Radish	*Raphanus raphanistrum*	Brassucaceae	Emerald & Byee
Common Peppergrass	*Lepidium africanum*	Brassicaceae	Emerald
Saltbush	*Atriplex muelleri*	Chenopodiaceae	Emerald
Bindweed	*Convolvulus erubescens*	Convolvulaceae	Byee
Caustic Creeper	*Chamaesyce drummondii*	Euphorbiaceae	Emerald
Rasp Weed	*Haloragis aspera*	Haloragaceae	Byee
Stagger Weed	*Stachys aevensis*	Lamiaceae	Byee
Marshmallow	*Malva parviflora*	Malvaceae	Byee & Emerald
Sida	*Sida cardifolia*	Malvaceae	Emerald
Sensitive Weed	*Neptunia gracilis*	Mimosaceae	Byee & Emerald
Purple-topped Chloris	*Chloris inflate*	Poaceae	Byee
Slender knotweed	*Persicaria decipiens*	Polygonaceae	Emerald
Red Pigweed	*Portulaca oleracea*	Polygonaceae	Byee & Emerald
Fierce thornapple	*Datura ferox*	Solanaceae	Byee & Emerald
Wild Gooseberry	*Physalis minima*	Solanaceae	Byee & Emerald

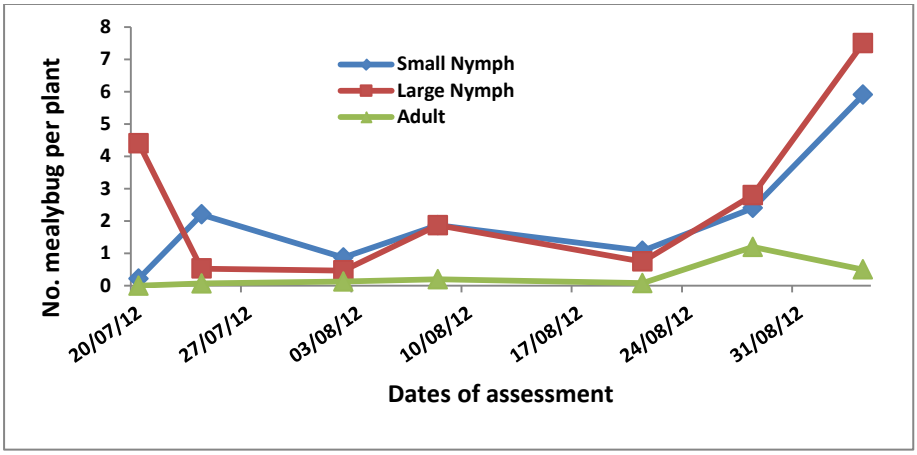


Figure 14. Number of mealybug on stagger weed during winter months in Byee

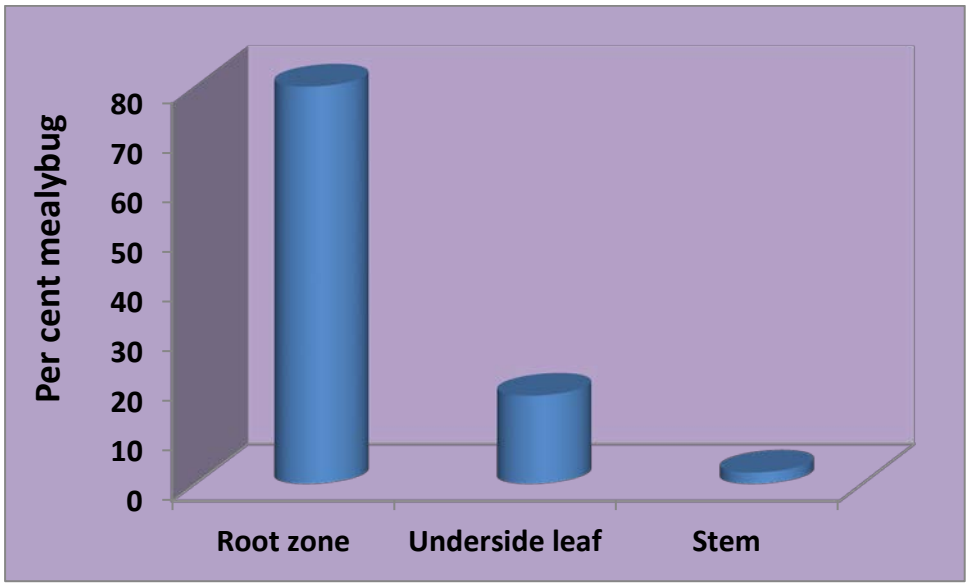


Figure 15. Per cent mealybug on different plant parts on overwintering host (stagger weed) in Byee.

Pre-season operation and establishment of mealybug on seedling cotton

Methodology

The experiment was conducted in a field that had an outbreak of mealybug in the 2010-2011 season in Byee. Three treatments were tested with each treatment containing a combination of tillage operations. The treatments included:

1) Mulching, deep cultivation/pupae busting, light cultivation, 2 herbicide applications (Roundup & Zulu and Gramoxone) + Cruiser-treated seed
2) Mulching, deep cultivation/pupae busting + Cruiser-treated seed
3) Mulching, deep cultivation/pupae busting + untreated seed

The paddock was mulched on 18 May 2011 following harvest. Pupae busting was conducted on 6 August. Herbicide was applied twice to Treatment 1. A cotton crop was planted on 24 October. All treatments were assessed 4 times from cotton emergence to flowering on 22 December 2011.

Data were transformed into square root transformation to normalise the data for analysis. The transformed data were analysed using Repeated Measure Analysis of Variance. If interactions were found significant data was then subjected to REML testing of fixed effects for differences between treatments within each day. Data on cumulative number of mealybugs were subject to analysis of variance (ANOVA).

Results

The results are summarised in Figure 16. The results showed that there was significant difference between treatments for all dates of assessment ($F = 7.85$, $P = 0.007$; $F = 37.02$, $P = < 0.001$; and $F = 13.34$, $P = <0.001$ respectively for Dates 1, 2 and 3) except last date where difference was not significant ($F = 1.67$, $P = 0.216$) (Figure 16A). The analysis also showed that treatment 1 plots had a significantly lower mealybug population at all dates than treatment 3. The analysis on cumulative mealybug number also showed significant difference between treatments ($F = 25.02$, $P = <0.001$) (Figure 16B). Results suggest that keeping fields clean from harvest to planting, as well as using treated seed, may delay early establishment of mealybug on cotton therefore may delay development of hot spots.

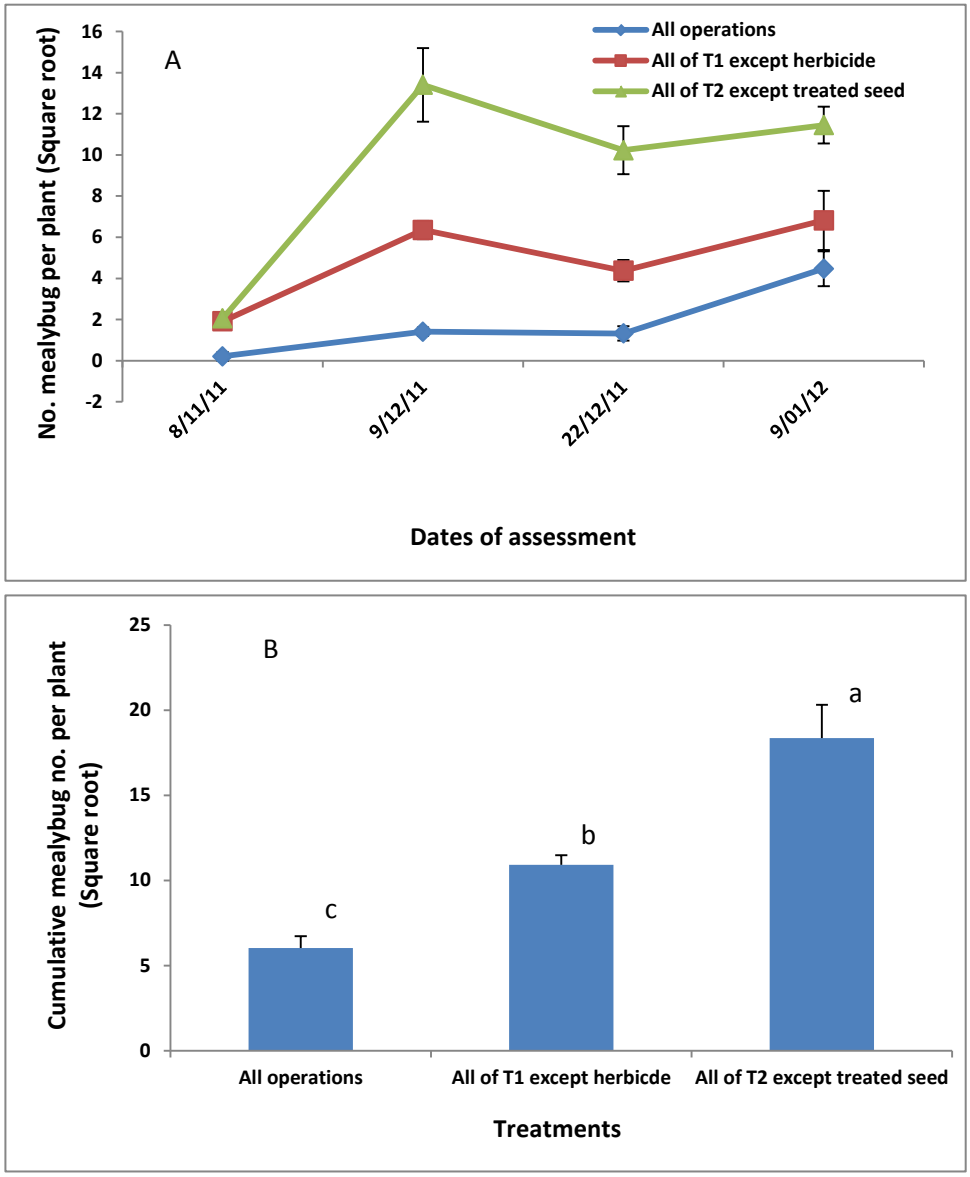


Figure 16. Effect of off-season operations on the establishment of solenopsis mealybug on early stage cotton. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

Factor affecting mealybug establishment on cotton

Volunteer cotton and mealybug establishment on cotton

Methodology

Observations were made in Emerald during 2012-13 to understand if volunteer cotton in a field contributes to mealybug populations within the crop. Observations were made at 3 to 4 leaf stage in four fields on volunteer plants in a row of cotton, plants adjacent to volunteers and plants at least 5 metres away from volunteers. In each field 10 volunteer plants were randomly selected which were at least 50m from each other. All plants were checked thoroughly for mealybug.

Results

Mealybug were found on volunteer cotton in all fields and on plants adjacent to volunteer cotton in two of the fields (Table 6). There was no mealybug in any of the fields on plants 5 metres away from volunteer cotton. This result suggests that volunteer cotton may be a major contributor to initial infestation of mealybug in the field. More detailed studies are needed to confirm this finding.

Table 6. Effects of volunteer cotton on the establishment of mealybug in cotton crops. SE indicates standard error of means.

	No. mealybug ± SE			
Treatments	Killara Field 1	Killara Field 2	Kerry Downs Field 1	Kerry Downs Field 2
Volunteer	43.0 ± 32.8	8.7 ± 8.7	11.6 ± 7.42	64.2 ± 26.3
Plant adjacent to volunteer	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.77
5m away from volunteer	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Effect of at-planting chemical application on mealybug establishment

Methodology

The experiment was conducted in Emerald in a field with a previous history of mealybug over two seasons, 2012-13 and 2013-14. Three treatments, Lorsban® (@ 500 mL/ha), Confidor® (@ 1 L/ha) and an untreated control were applied to 8 rows x 50m during 2012-13 of Sicot 71BRF Dynasty® treated and to 16 rows x 300m during 2013-14 of 74BRF Dynasty® treated cotton with 3 replications in a RCB design. The chemicals were applied as an infurrow treatment by the growers during planting. Cotton was planted in the 1st and 3rd week of October in 2012-13 and 2013-14 respectively. Assessment of mealybug abundance was made at 7-12 day intervals starting from two true leaf stage and continuing until first flowering stage. During the 2012-13 season 7 plants per plot were sampled and during 2013-14 season initially 30 plants and later 10 plants per plot were sampled. The plants were selected randomly.

Results

During the 2012-13 season mealybug numbers were very low (Figure 17) and there was no mealybug during 2013-14 season. Therefore, results are presented for the 2012-13 season only. Mealybug (adult) were first detected at 6 weeks after planting in both Lorsban® and untreated plots. In Confidor® treated plots mealybug (adult) was first detected at 9 weeks after planting. However analysis on pooled data for all dates revealed that there was no significant difference ($p > 0.05$) between treatments.

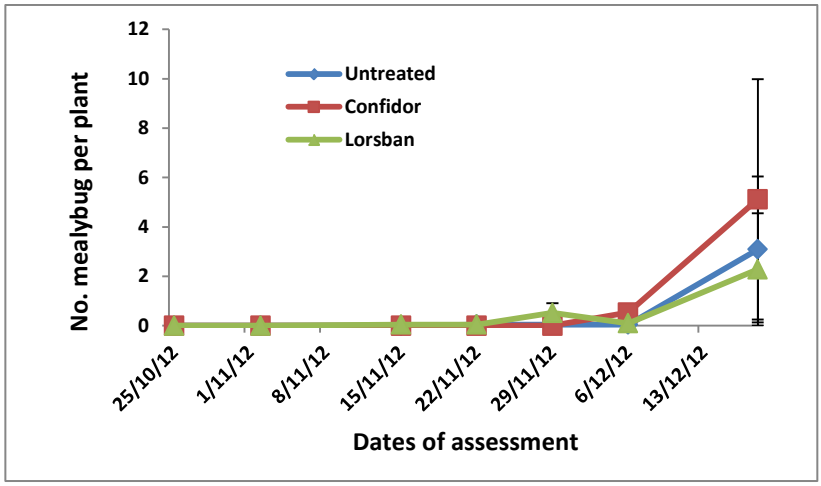


Figure 17. Number of mealybug in at-planting chemical treated plots. Error bars indicate standard error of means.

Effect of seed treatments on the establishment of mealybug

Evaluation was made using glasshouse bioassay and experiment in the field.

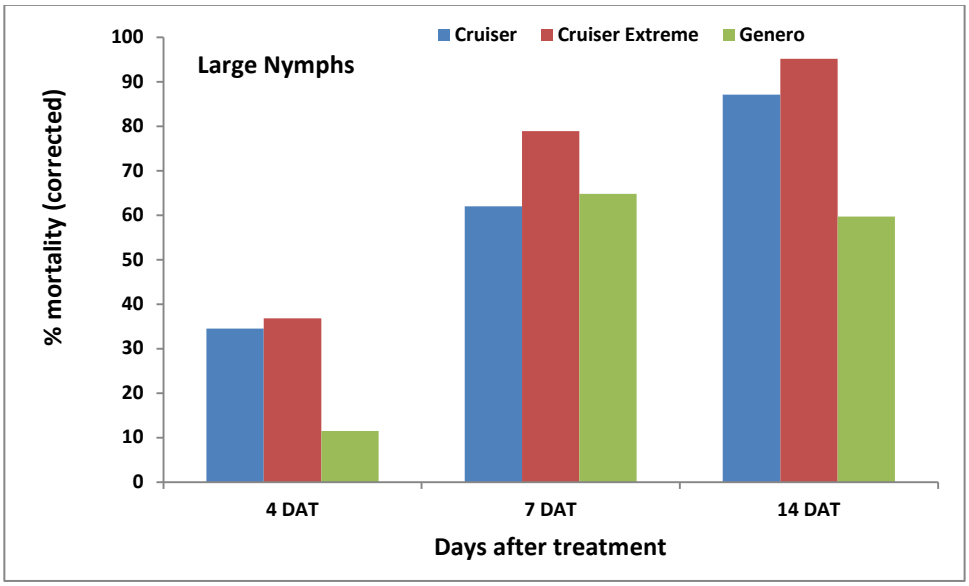
Glasshouse Bioassay

Methodology

Three different seed treatments (Cruiser®, Cruiser Extreme® and Genero®) were tested against large nymphs (Expt. 1) and adults (Expt. 2) separately. A treatment with untreated seeds served as a control. An experiment to evaluate the residual effectiveness of these seed treatments against mealybug was also conducted (Expt. 3). In Experiments 1 and 2 the treatments were replicated 6 times and with 5 replications for Experiment 3. Sicot 74BRF treated seeds, were grown in 55mm plastic pots (1 plant per pot) in each trial. In Experiments 1 and 2, 10 insects per plant were released at cotyledon stage. For experiment 3, 10 insects per plant were released on 7, 14 and 30 day old plants and at 1st squaring. Upon emergence individual pots was placed inside a translucent 750mL round plastic tumbler (except 30 day old and squaring plants for Experiment 3). Upon release of insects the plant was covered with another tumbler and the two tumblers were sealed and secured with parafilm to contain the insects. Assessments were made at 3, 7 and 14 days after insect release (DAT) for Experiments 1 and 2 and at 7 DAT for Experiment 3. For Experiments 1 and 2, mortality was corrected using Abbott (1925) formula. In addition to ANOVA, data from Experiment 3 was subjected to General Linear Model (GLM) analysis to determine the interaction between treatments and plant age.

Results

For both large nymphs and adults Cruiser Extreme® was found to be most effective followed by Cruiser® (Figure 18). Mortality was higher for large nymphs than adults irrespective of treatments. At 14 DAT corrected mortality (using Abbott formula) was 95, 79 and 37% for large nymphs and 67, 54 and 26% for adults when treated with Cruiser Extreme®, Cruiser®, and Genero® respectively. Analysis showed that mortality was significantly higher for Cruiser Extreme® ($p < 0.05$) but there was no significant difference ($p > 0.05$) between Cruiser Extreme® and Cruiser® for either large nymphs or adults.



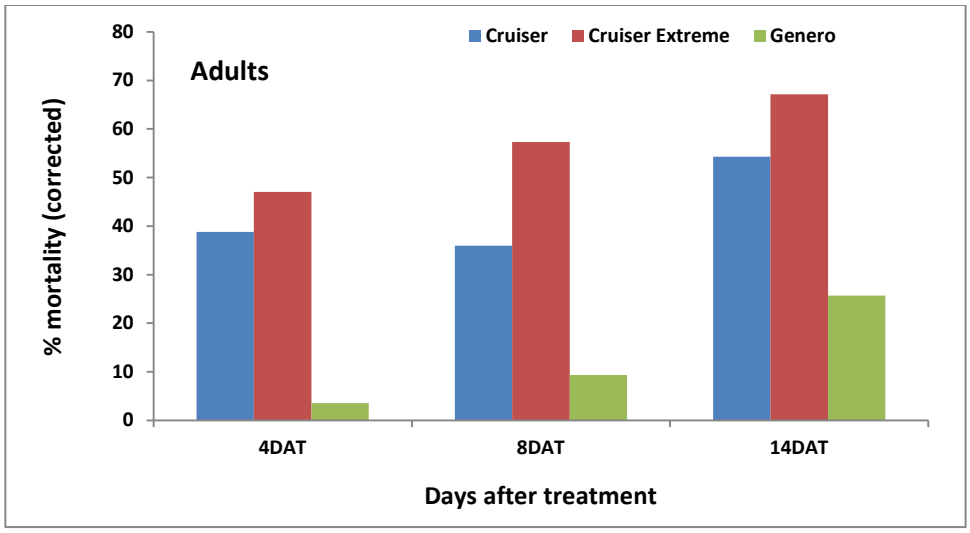


Figure 18. Per cent mortality (corrected using Abbott formula) for different seed treatments against mealybug (large nymphs and adults) in the glasshouse

The results for residual effectiveness of seed treatments are presented in Figure 19 and show that effectiveness of seed treatments diminished as the plant grew. Analysis showed that there was a significant difference ($p < 0.05$) between treatments up to 30 days after planting but for squaring plants the difference was not significant ($p > 0.05$). The GLM analysis revealed that there was significant interaction ($p < 0.05$) between treatments and plant age. However, while Genero® protected plants for up to 14 days after planting, Cruiser® and Cruiser Extreme® protected plants up to 30 days after planting.

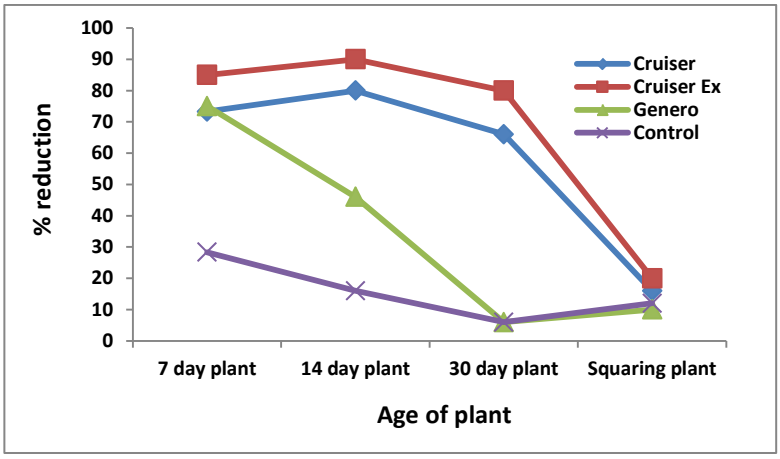


Figure 19. Residual effectiveness of seed treatments against mealybug in the glasshouse

Field Experiment

Methodology

The experiment was conducted in Byee during the 2012-13 season. Treatments consisted of four Sicot 74BRF insecticide seed treatments (Amparo®, Cruiser®, Cruiser Extreme® and Genero®) and Dynasty® treated seeds as a control. The treatments were replicated 3 times in a RCB design. The cotton was planted on 19th October in 8 rows x 25m plots. Assessments were made weekly on 10 plants per plot, selected randomly, to record mealybug numbers starting from 2 true leaf stage and continuing until first flower.

A similar experiment was also conducted in Byee during 2013-14 but without Amparo®. The Sicot 74BRF cotton was planted in the first week of November in 8 row x 20m plots.

Results

There was no mealybug during the 2013-14 season, therefore only the results from the 2012-13 experiment are presented.

Mealybugs were detected on two occasions in the first and fourth week of November in Amparo™, Genero® and untreated plots, however the overall population was too low (less than 0.1 per plant) to draw any conclusions (Figure 20). Analysis on pooled data for all dates also showed no significant difference ($p > 0.05$) between treatments.

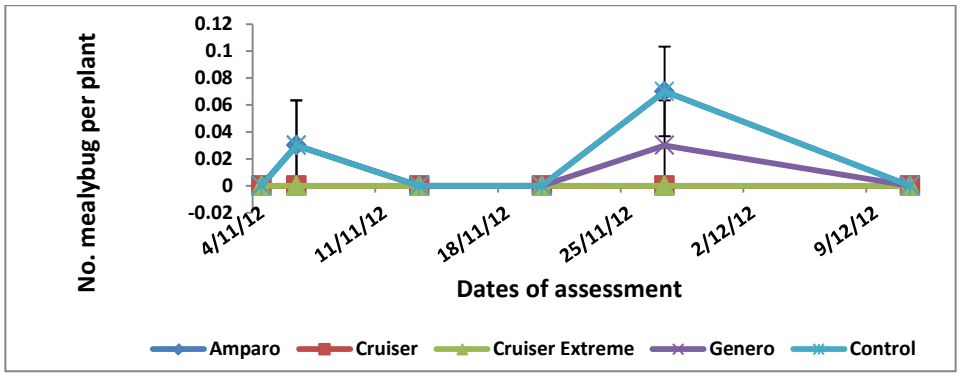


Figure 20. Effect of seed treatments against mealybug in the field. Error bars indicate standard error of means.

Evaluation of insecticides against mealybug

Field Experiments

Methodology

Five experiments were conducted using different insecticides. These occurred in

- Byee during 2011-12 at cut out stage,
- Emerald during 2011-12 at 2 to 3 weeks before defoliation
- Emerald, during 2012-13 at cut out stage
- Emerald during 2013-14 at cut out stage
- Condamine during 2013-14 at peak flowering stage.

Treatment details for each experiment are given in Table 7. The treatments were replicated 3 times each in a RCB design. Insecticides were applied on 4 row x 10m plots using a gas-pressured hand boom sprayer at a rate of 107 L/ha, fitted with two overhead nozzles (DG Tee Jet 10015 vs flat fan) per row with 1.6 bar pressure and walking speed of 4 km/hr for Byee during 2011-12 and in Emerald during 2011-12 and 2012-13 seasons. In Emerald and in Condamine during 2013-14 seasons insecticides were applied with same gas-pressured hand boom sprayer but at a rate of 160 L/ha and fitted with two dropper and one overhead nozzle per row to obtain better coverage. In Condamine insecticides were sprayed twice with the second spray applied 9 days after the first spray using a knapsack sprayer (@ 200 L/ha).

Mealybug and beneficials were assessed visually on 3 marked plants per plot during 2011-12 in Byee and Emerald and 2012-13 in Emerald and 5 marked plants during 2013-14 in Emerald and Condamine. Beneficials were also assessed using a beat sheet, 2 x 1m per plot, during 2011-12 and 2012-13 seasons. Assessments were made on the day before spraying and then at 5, 14 and 20 days after treatment (DAT) in Byee (2011-12) and in Emerald at 4, 8 and 14 DAT during 2011-12 season. In Emerald assessments were made at 6 and 20 DAT during 2012-13 and at 4, 7 and 14 DAT during 2013-14 seasons. In Condamine assessments were made at 8, 15 and 22 days after the first spray.

Data was transformed into square root transformation before analysis except Condamine 2013-14. Condamine data was transformed into log +1transformation. Transformed data were analysed using Repeated Measure Analysis of Variance. For Condamine data as interaction between insecticides and

days was significant and therefore data was further subjected to REML testing of fixed effects for differences between treatments within each day.

Table 7. Treatments applied against mealybug in different seasons

Treatment	Formulation (g/L)	Rate (g or mL/ha)
Byee 2011-12		
Shield® + Maxx	Clothianidin 200 g/L + Maxx	250 mL + 2% (v/v)
Lorsban®	Chlorpyrifos 500 g/L	500 mL
Transform®	Sulfloxaflor 240 g/L	400 mL
Supracide®	Methidathion 400 g/L	1400 mL
Movento®	Spirotetramat 240 g/L	400 mL
Tokuthion®	Prothiofos 500 g/L	350 mL
Talstar®	Bifenthrin 100 g/L	600 mL
Untreated control	Control	_
Emerald 2011-12		
Movento®	Spirotetramat 240 g/L	400 mL
Canopy®	Paraffinic oil ($_n$C27) 792 g/L	2% (v/v)
Shield® + Maxx	Clothianidin 200 g/L + Maxx	250 mL + 2% (v/v)
Rogor®	Dimethoate 400 g/L	500 mL
Bulldock Duo	Beta-cyfluthrin 25 g/L	600 mL
Untreated control	Control	_
Emerald 2012-13		
Clap®	Buprofezin 440 g/L	1200 mL
Pegasus®	Diafenthiuron 500 g/L	800 mL
Shield® + Maxx	Clothianidin 200 g/L + Maxx	250 mL + 2% (v/v)
Pirimor®	Primicarb 500 g/kg	750 g
Tokuthion®	Prothiofos 500 g/L	350 mL
Transform®	Sulfloxaflor 240 g/L	400 mL
Untreated control	Control	_
Emerald 2013-14		
Actara®	Thiamethoxam 250 g/kg	200 g
Affirm®	Emamectin benzoate 17 g/L	700 mL
Larvin®	Thiodicarb 375 g/L	1000 mL
Confidor®	Imidacloprid 200 g/L	250 mL
Untreated control	Control	-
Condamine 2013-14		
Actara®	Thiamethoxam 250 g/kg	200 g
Clap®	Buprofezin 440 g/L	1200 mL
Untreated control	Control	-

Results

Byee 2011-12

Pre-spray mealybug number was 213 to 342 per plant. Of them 97% were nymphs. As there was no significant difference between treatments for either mealybug adult or nymph results are presented for adult and nymph together in Figure 21. The repeated measure analysis of variance revealed that there was no significant difference between insecticides ($F = 2.67$, $P = 0.053$). The analysis also showed that there was no significant interaction between insecticides and days ($F = 1.08$, $P = 0.403$).

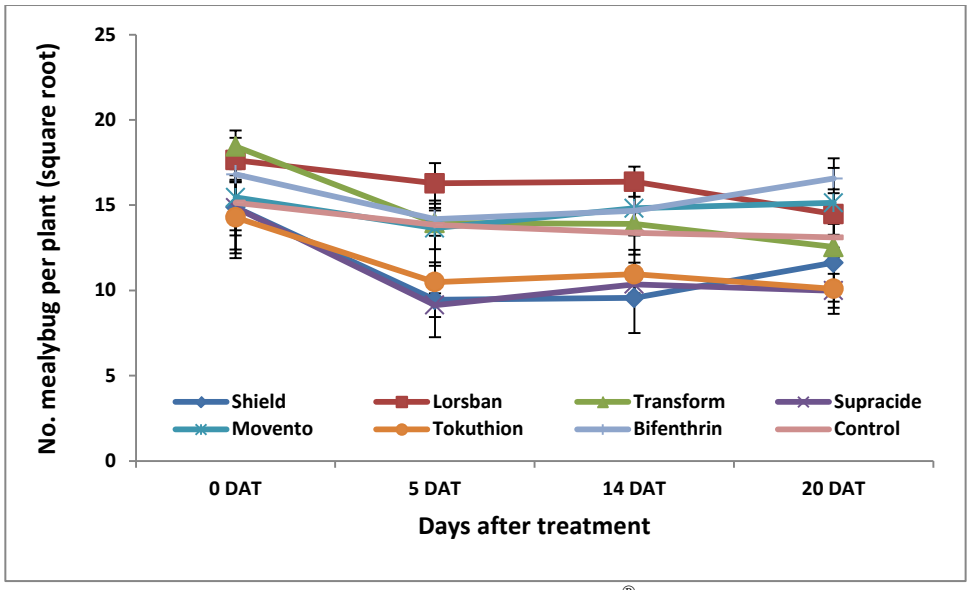
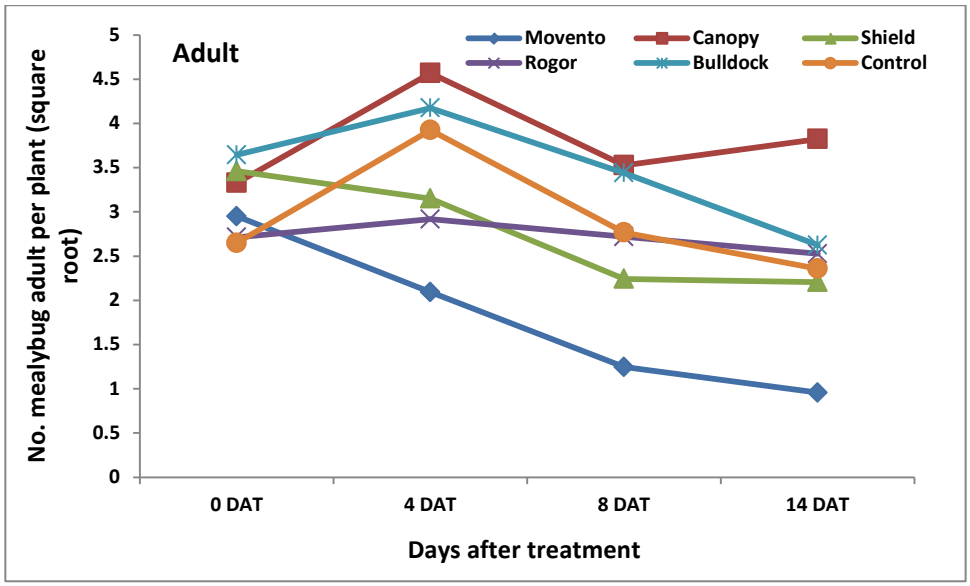


Figure 21. Effect of insecticides against mealybug in Bollgard® cotton at cut out stage in Byee during 2011-12 season. Error bars indicate standard error of means.

Emerald 2011-12

Pre-spray mealybug numbers ranged between 55 to 89 per plant. Of them 85% were nymphs. The results are summarised for adult and nymph separately in Figure 22. Repeated measure analysis showed that while for adult there was significant difference between treatments (F = 4.91, P = 0.016) for nymphs difference was not significant (F = 2.17, P = 0.114). After the spray, nymph population reduced sharply across the treatments. One of the reasons might be as cotton was finishing quickly top leaves and squares were dropped off where most of the nymphs were harboured.



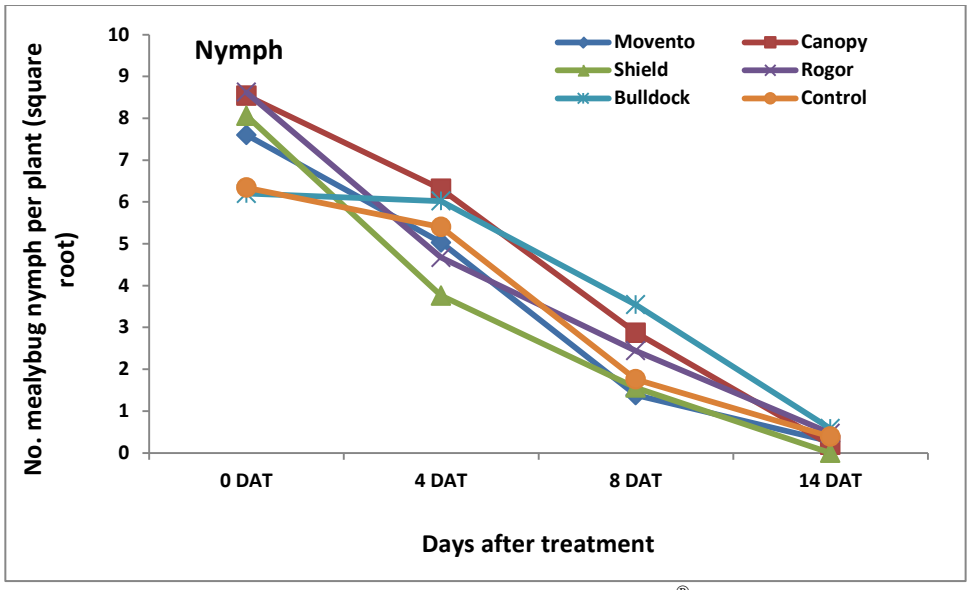


Figure 22. Effect of insecticides against mealybug in Bollgard® cotton at 2 to 3 weeks before defoliation in Emerald during 2011-12 season.

Analysis on pooled data for all dates together revealed that for adults Movento® reduced population significantly than control, bulldock and canopy (Table 8).

Table 8. Insecticides means for all dates together against mealybug in Bollgard® cotton at 2 to 3 weeks before defoliation in Emearld during 2011-12 season.

Treatments	Adult	Nymph
Movento	1.81a	5.25a
Canopy	3.81c	5.91a
Shield	2.77ab	4.50a
Rogor	2.72ab	5.25a
Bulldock	3.47bc	4.67a
Control	2.93bc	4.46a

Means within column followed by same letter are not significantly different (p > 0.05), Fisher's LSD Test

Emerald 2012-13

Pre-spray numbers of mealybugs ranged between 65 to 149 per plant. Of them 72% were nymphs. The results for adults and nymphs together are summarised in Figure 23. The repeated measure analysis revealed that there was no significant difference between treatments (F = 0.4, P = 0.865).

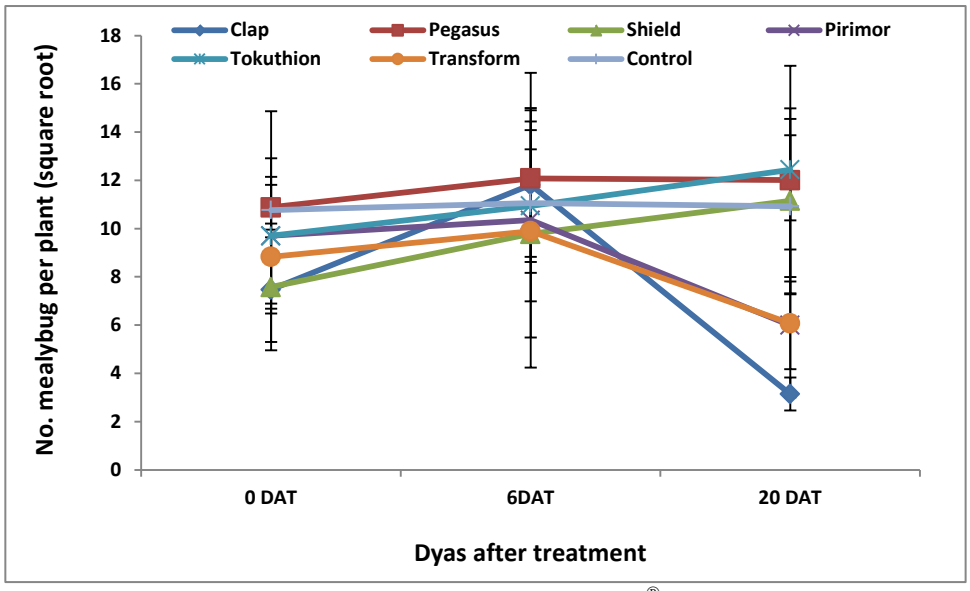


Figure 23. Effect of insecticides against mealybug in Bollgard® cotton at cut out stage in Emerald during 2012-13 season. Error bars indicate standard error of means.

Emerald 2013-14

In the experimental plot pre-spray mealybug numbers ranged between 476 to 1188 per plant. Of them 68% were nymphs. The results showed that the test insecticides had no effect on mealybug and the repeated measure analysis also revealed that the difference between treatments and the interaction between days and insecticides was not significant (F = 0.21, P = 0.926 and F = 1.01, P = 0.466 respectively) (Figure 24).

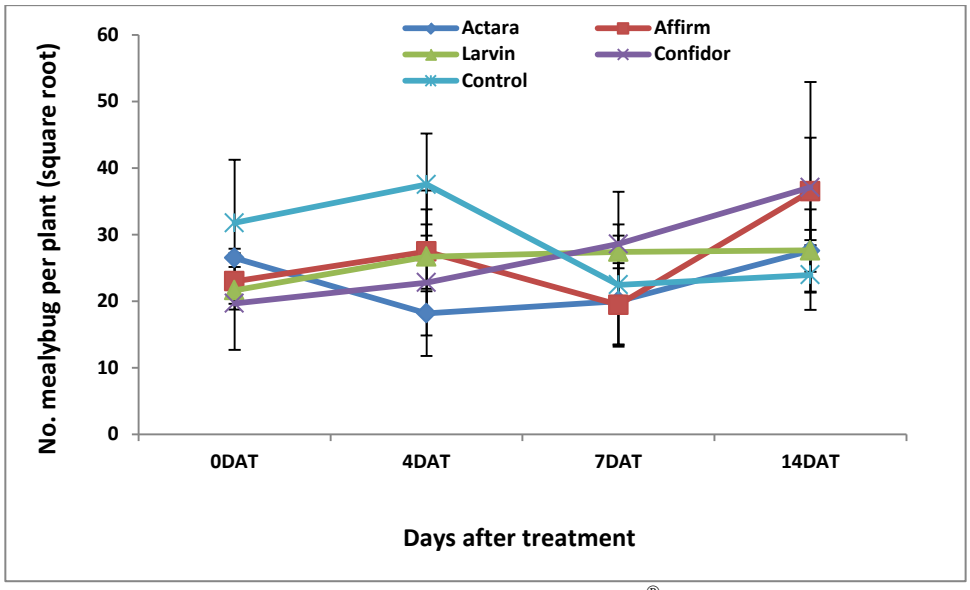
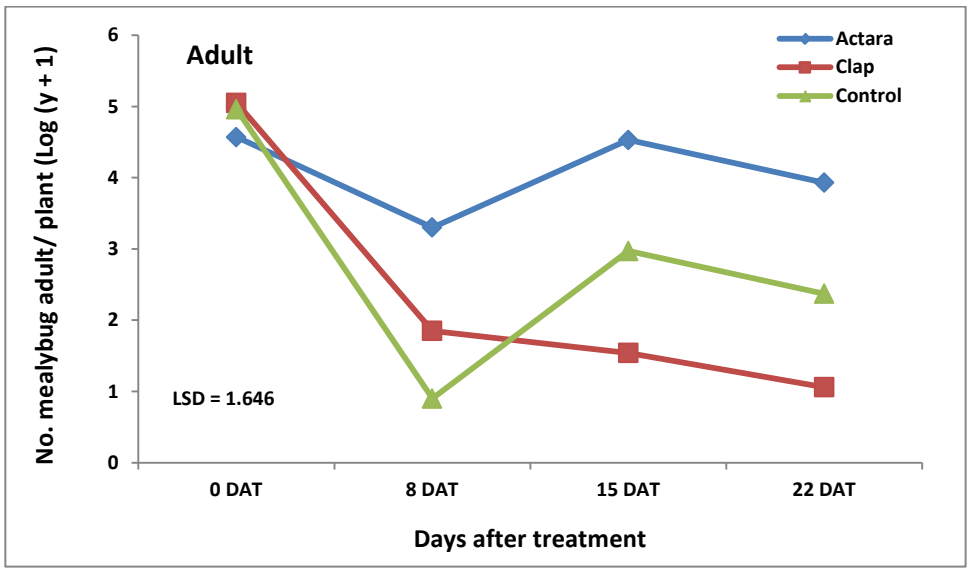


Figure 24. Effect of insecticides against mealybug in Bollgard® cotton at cut out stage in Emerald during 2013-14 season. Error bars indicate standard error of means.

Condamine 2013-14

Pre-spray mealybug numbers ranged from 135 to 210 per plant. Of them 81% were nymphs. As the effect of test insecticides are different for different mealybug life stage results are presented separately for each stage (Figure 25). The repeated measure analysis showed that there was significant difference between treatments for adult (F = 9.18, P = 0.032) and small nymph (F = 30.49, P = 0.004). However for large nymphs the difference was not significant (F = 6.1, P = 0.061).



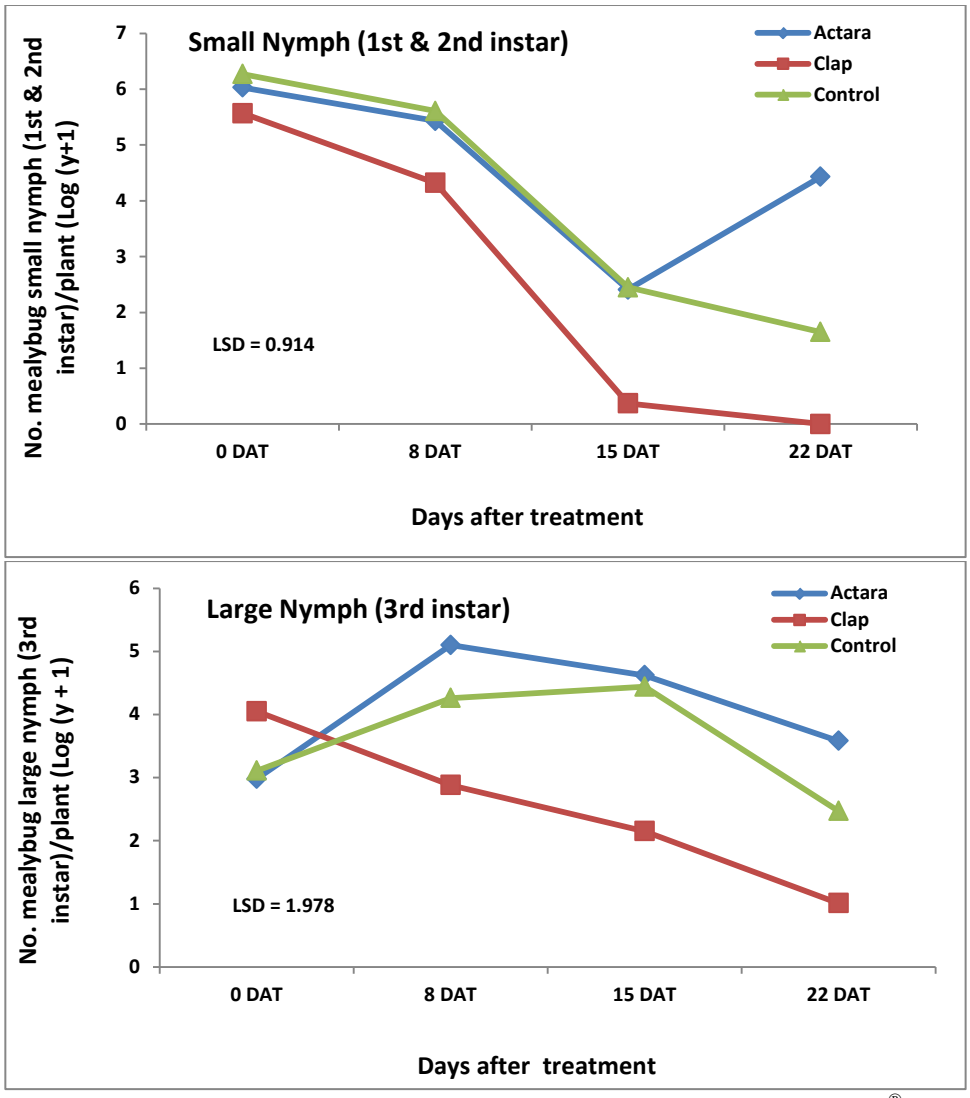


Figure 25. Effect of insecticides against different stages of mealybug in Bollgard® cotton at peak flowering stage in Condamine during 2013-14 season.

Analysis on pooled data for all dates together revealed that effect of Clap® was significantly different from Actara® for all stages and for small nymphs difference was significant from control (Table 9). This suggests that Clap® can be used in managing mealybug, however, further large field trials is needed to confirm this finding.

Table 9. Insecticides means for all dates together against mealybug in Bollgard® cotton at 2 to 3 weeks before defoliation in Emerald during 2011-12 season.

Treatments	Adult (square root)	Small Nymph (1st & 2nd instar) (square root)	Large Nymph (3rd instar) (square root)
Actara	3.92a	4.09a	4.44a
Clap	1.48b	1.56b	2.02b
Control	2.08b	3.23a	3.72ab

Means within column followed by same letter are not significantly different (p > 0.05), Fisher's LSD Test

Glasshouse Experiment

Methodology

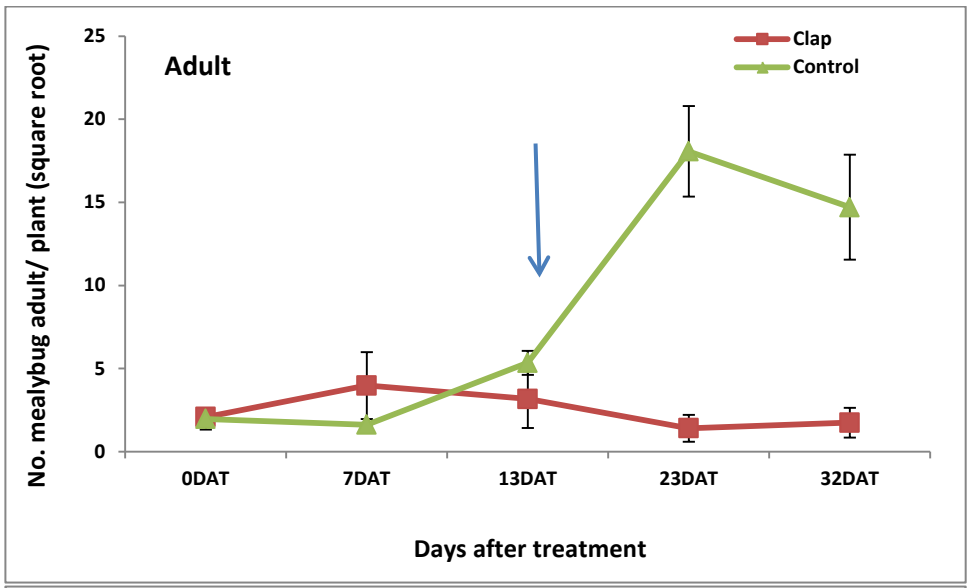
A glasshouse experiment was conducted using Clap® (@ 1 L/ha) along with an untreated control against a well-established mealybug population (>1600 per plant). Insecticide was applied twice with a second application made 16 days after the first spray on individual plants using a knapsack sprayer (@ 200 L/ha). The treatments were replicated 3 times. Pre-treatment counts were made one day

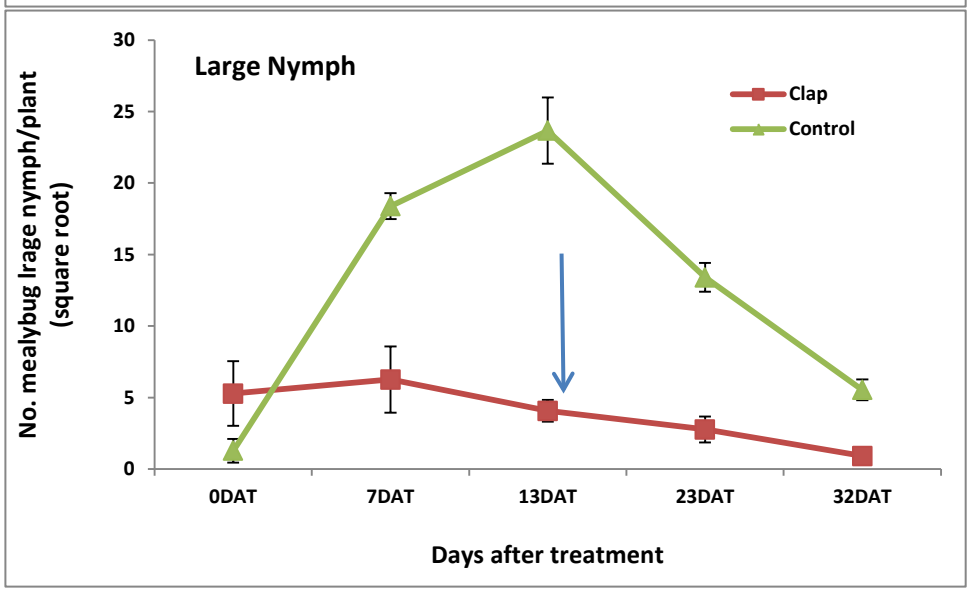
before spray application and post treatment counts were made at 7, 13, 23 and 32 days after the first spray.

The data was subject to a square root transformation and analysed using Repeated Measure Analysis of Variance. As interaction between insecticides and days was significant data was further subjected to REML testing of fixed effects for differences between treatments within each day.

Results

Pre-spray mealybug numbers ranged between 1641 to 1695 per plant. Of them 99% were nymphs. The results for each mealybug life stage are presented in Figure 26. The results showed that Clap® reduced the mealybug population steadily irrespective of mealybug life-stage. The repeated measure analysis showed that (excepting adults) that the difference between treatments were significant (F = 35.98, P = 0.003 for small nymph; F = 72.68, P = < 0.0001 for large nymph and F = 56.01, P = 0.001 for total mealybug). The analysis also revealed that Clap® reduced mealybug population significantly more than the control for small nymphs at 23 and 23 DAT and for large nymphs and total mealybug at 13, 23 and 32 DAT.





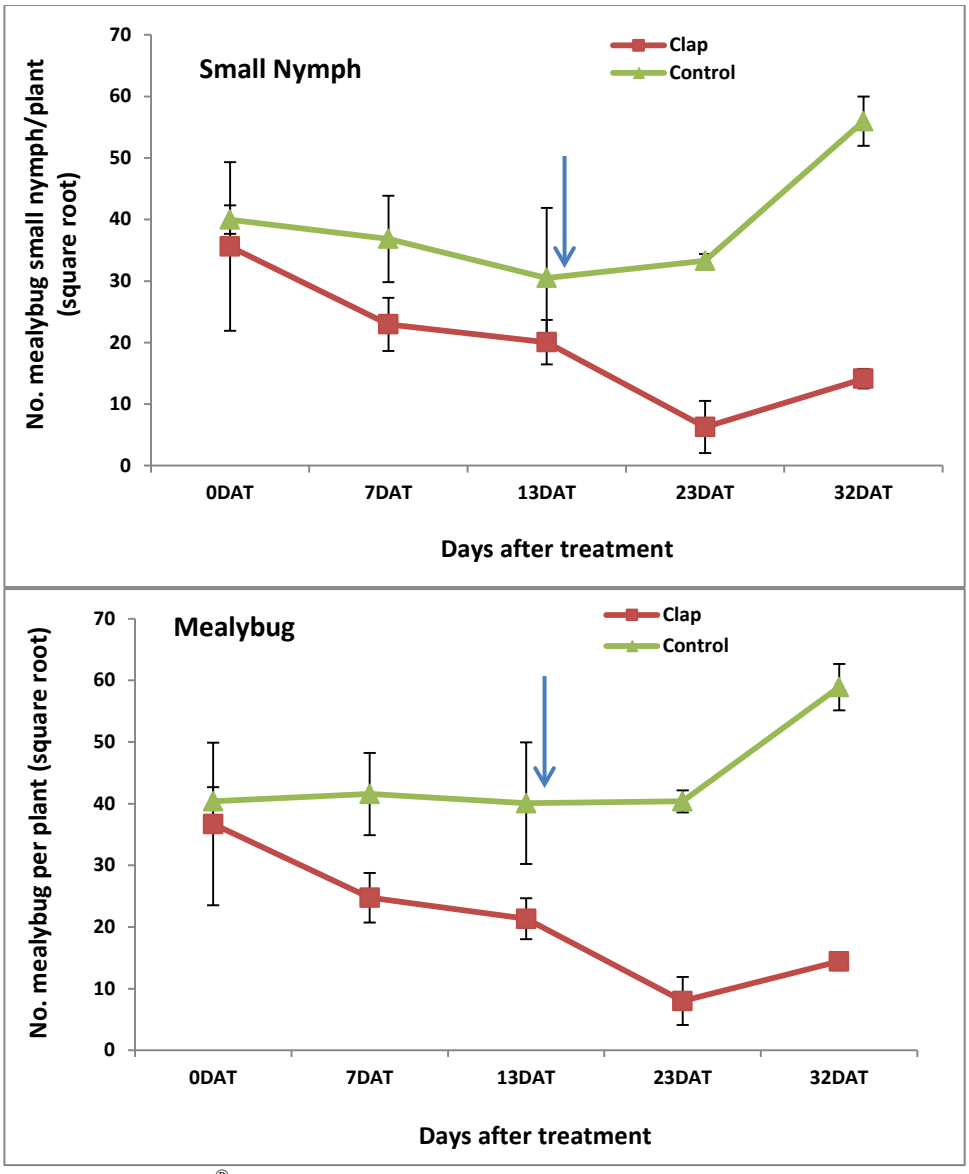


Figure 26. Effect of Clap® against mealybug in the glasshouse. Vertical arrows indicate time of 2nd spray and error bars indicate standard error of means.

Analysis on pooled data for all dates together revealed that effect of Clap® was significantly different from biopesticide and control for all stages except adult (Table 10). This result support field result that Clap® can be used in managing mealybug.

Table 10. Clap® and other treatments mean for all dates together against mealybug in the glasshouse

Treatments	Adult (square root)	Small Nymph (1st & 2nd instar) (square root)	Large Nymph (3rd instar) (square root)	Total mealybug (square root)
Clap	2.48a	19.8a	19.27a	21.01a
Control	8.35a	39.31b	62.24b	44.28b

Means within column followed by same letter are not significantly different ($p > 0.05$), Fisher's LSD Test

Conclusions

Understanding mealybug damage in Bollgard® II cotton

Impact of mealybug establishment at different life and crop stages were examined. All life stages of mealybug can cause damage. In our experiment mealybug establishment on the cotton plants as adults caused more damage compared with infestations of juvenile life stages. One of the reasons was due to higher numbers of mealybug that developed when adults were used to infest plants as reproduction

was immediate. Our experiment showed that the early mealybug established on the cotton the more damage they caused.

The results on damage assessment in the field were not conclusive. The relationships between mealybug number and damage in the experiments were very poor. This was perhaps due to the fact that mealybugs were not detected until late in the season at all experimental sites. In Emerald during 2013-14 season mealybugs were detected earlier but never reached a high enough number to cause significant damage. In some instances the crops suffered from drought (Condamine 2013-14) and flood (Byee 2011-12).

Mealybug population dynamics and their key beneficials
In the field mealybug population are usually characterised by high numbers of developing nymphs. Severe infestation (hot spots) may depend on the starting population size along with other factors such as natural enemies and weather parameters.

Sampling mealybug in Bollgard® cotton
Mealybug distribution patterns in the field were found to be highly clumped and their distribution within the plant canopy varied with crop stages. At seedling stage mealybugs are mainly found on tip and stem whereas at squaring and flowering mostly on squares and at peak flowering they are found mostly on bolls followed by squares. In case of leaf they live mostly underside of the leaf and in case of squares and bolls they live mostly inside bracts.

Overwintering strategy of mealybug
Mealybugs overwinter both as adults and nymphs on a wide variety of weed hosts in and around cotton fields. During winter months they move into the soil and live on the root zone of the hosts. Keeping fields clean after harvest through until next planting may reduce mealybug establishment in cotton.

Factors affecting mealybug establishment on cotton
Volunteer cotton can play an important role in allowing the carryover of mealybug in cotton fields between seasons. Therefore, controlling volunteers will assist with mealybug management. Seed treatments, particularly Cruiser Extreme®, can protect cotton plants for up to 30 days from infesting mealybug.

Evaluation of insecticides against mealybug
Among the test insecticides Clap® was found to be most effective followed by Supracide®, Movento® and Transform®. However, for Clap® further testing is required particularly to devise the most effective application strategy for this IGR product.

MIRIDS

Expt. 1. Sample size for monitoring mirids at different precision levels

Methodology
The experiment was conducted over three seasons in irrigated Bollgard® II cotton. There were 3 sites in each year during 2011-12, 2012-13 and one site during 2013-14 on the Darling Downs and in the South Burnett. Details of these sites are given in Table 11.

Table 11. Description of trial sites

Season	Trial site	Variety	Plot size (ha)
2011-12	Tarcoola, Macalister, (Neville Walton)	Sicot 71BRF	7
	Gebur, Jandowae (Simon Donaldson)	Sicot 71BRF	10
	Mayfield, Nandi (Glen /Shaun Fresser)	Sicot 71BRF	10
2012-13	Tarcoola, Macalister, (Neville Walton)	Sicot 71BRF	7

	Byee (Mike Stuart)	Sicot 71BRF	7
	Kingaroy Research Station	Sicot 71BRF	1.6
2013-14	Kingaroy Research Station	Sicot 71BRF	1.6

Except at Kingaroy Research Station, fields were divided into 40 quadrants, each quadrant of 100 x 25 m for 10 hectare field and 100 x 17 m 7 hectare field. At the Kingaroy Research Station the field was divided into 20 quadrants, each quadrant of 40 x 20 m. The quadrants were sampled weekly using a beat sheet, 1m per quadrant starting from 5-6 leaf stage. After mid-January in the 2012-13 season, heavy rain and flooding prevented sampling for 3 weeks at Macalister and Kingaroy and for 4 weeks at Byee. Mirid numbers were recorded as adults, small (1st to 3rd instar) and large (4th & 5th instar) nymphs.

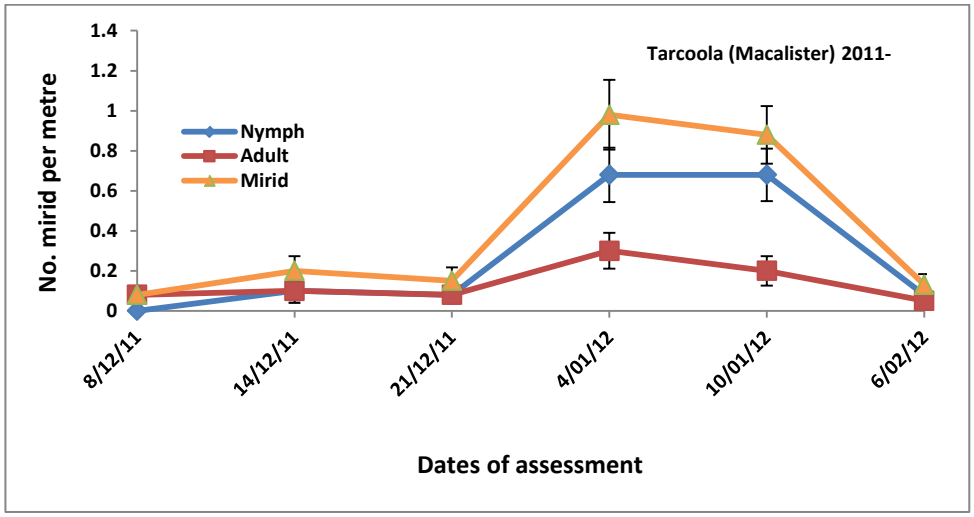
The distribution pattern for all sites during the pre and post flowering growth phases were determined using Taylor's Power Law (TPL): $s^2 = am^b$, where s^2 is sample variance, m is sample mean and a and b are fitted parameters. The parameters a and b were estimated using linear regression of log transformed data, $\log_{10}s^2 = a + b^* \log_{10}m$, where b is the Taylor's index of aggregation (if b = 1 the distribution is random, if b < 1 the distribution is uniform and if b > 1 the distribution is clumped). Since the data fit negative binomial distribution, optimal sample size at different precision levels (0.2, 0.3, 0.4 and 0.5) for negative binomial distribution was determined for all sites and crop stages using the following model:

$$n = ((1/\bar{e}) + (1/k))/D^2$$

For the above model, n is the required sample size, $\bar{e}$ is the sample mean, k is the dispersion parameter and D is the desired level of precision. The dispersion parameter is $k = \bar{e}^2/(s^2 - \bar{e})$, where s^2 is the sample variance. Sample sizes for different precision levels were then plotted against mirid density to calculate the required sample size for mirid numbers.

Results

Overall mirid numbers were low with the threshold being reached only twice at Byee in the third week of January during 2012-13. Threshold was only reached at Nandi in the first week of February during 2011-12. The highest mirid number reached in Macalister was 1.7/m during 2012-13. In Jandowae the highest number was 1.1/m during 2011-12 and at KRS the highest number was 2.3/m during 2013-14. Population changes over the years at different sites are given in Figures 27 for 2011-12 and in 28 for 2012-13. Except at KRS, all fields were sprayed once threshold was reached and mirid populations did not recover post application.



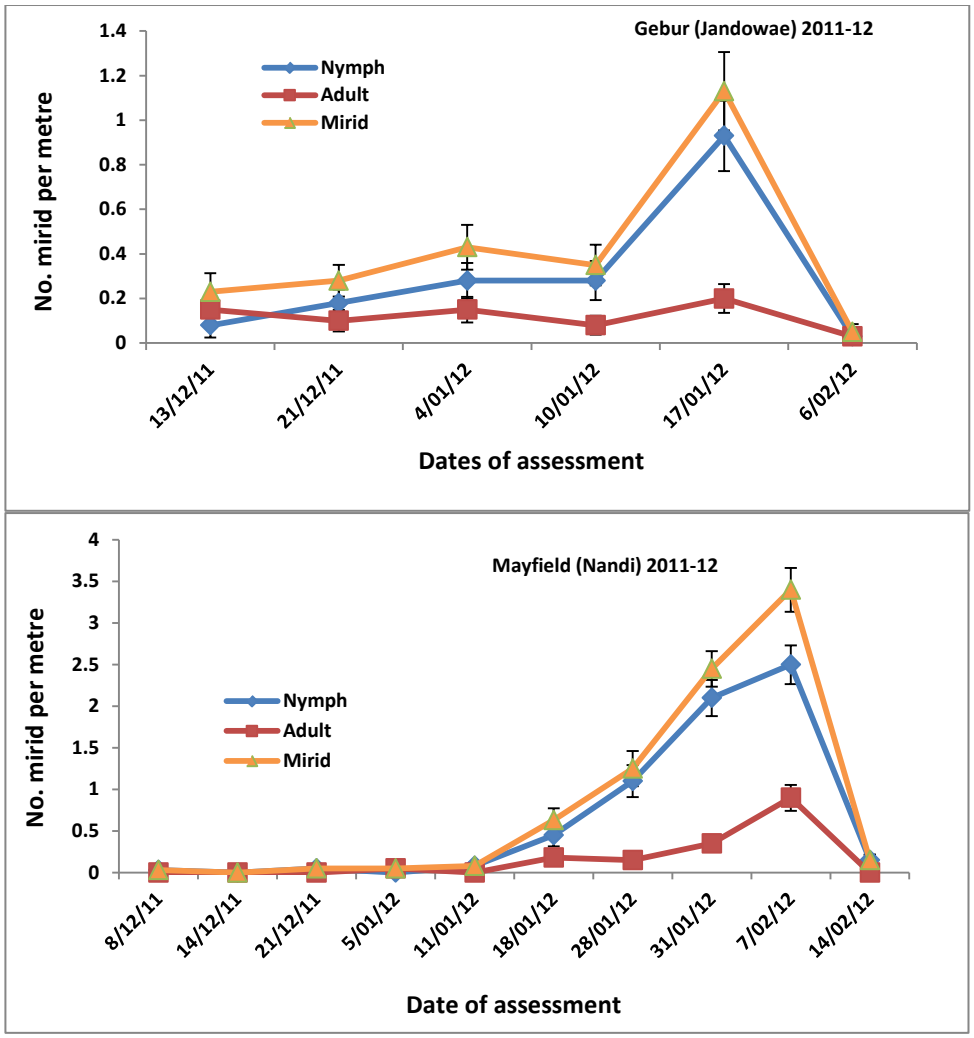
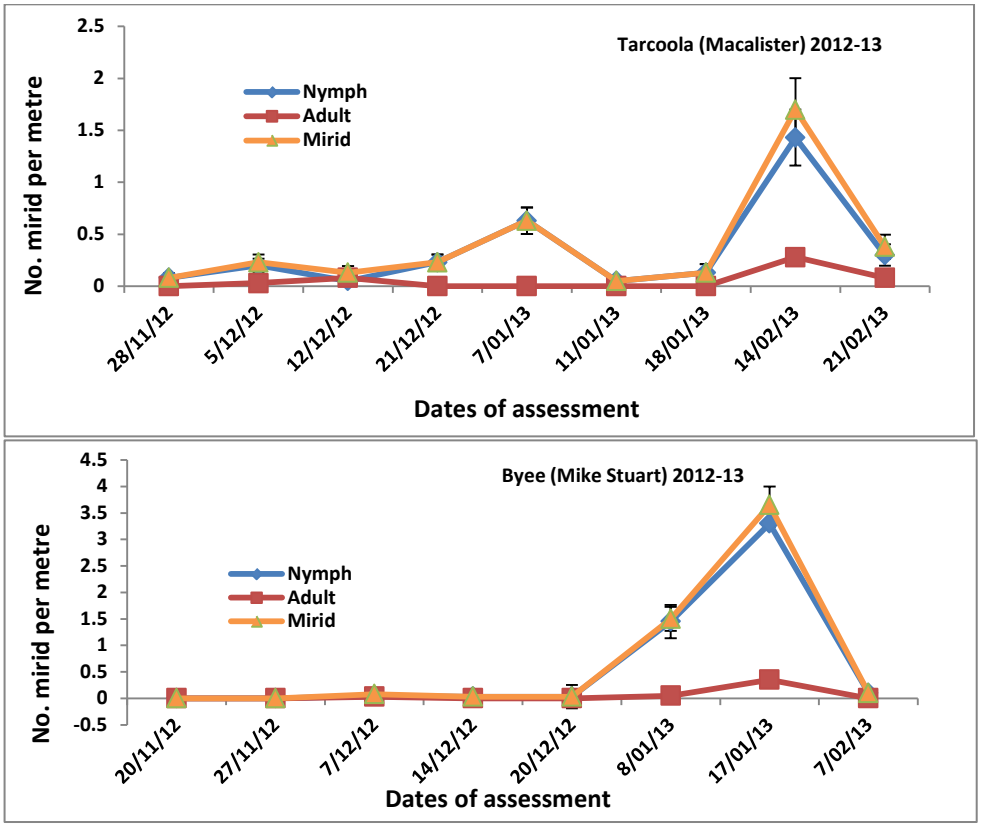


Figure 27. Seasonal changes in mirid populations during 2011-12 seasons at different sites. Error bars indicate standard error of mean.



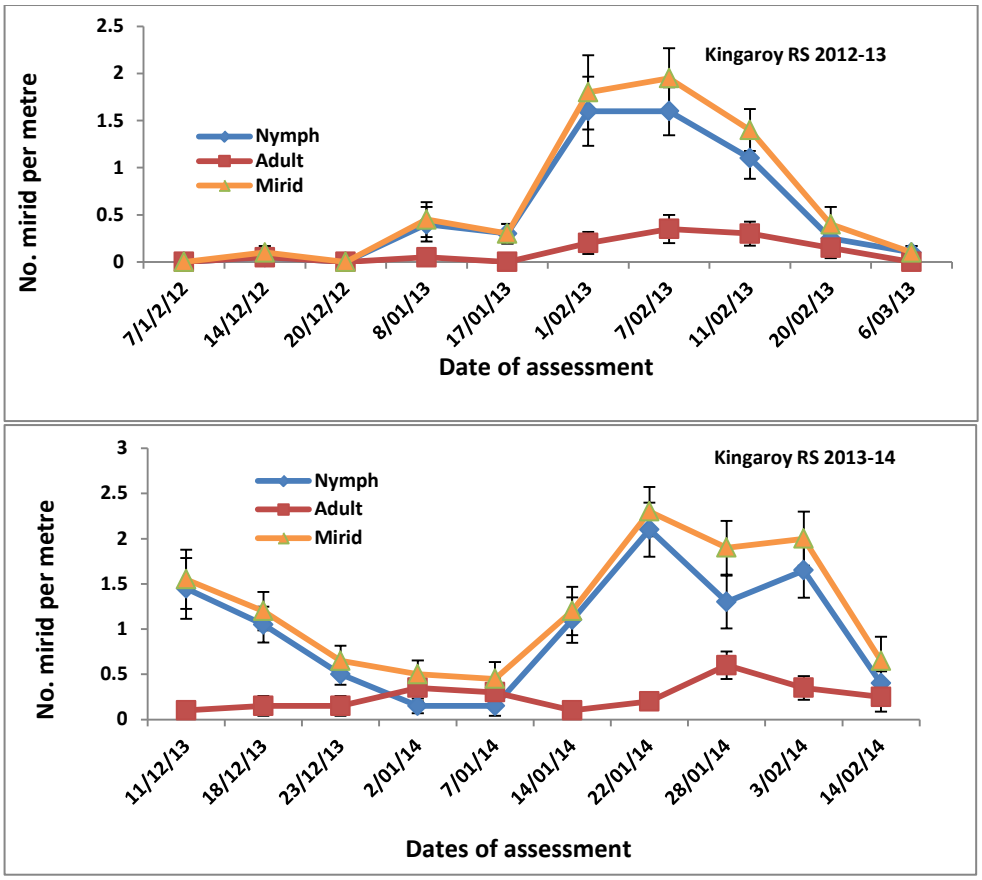


Figure 28. Seasonal changes in mirid populations during 2012-13 seasons at different sites. Error bars indicate standard error of mean.

The Taylor's Index of Aggregation (TIA) for different sites at squaring and flowering and onward stage are given in Table 12. Since mirid number was low and 80% of the population was nymphs TIA was calculated for nymphs and adults together. The TIA indicated that the distribution pattern of mirids in the fields out of 14 times seven times was clumped, three times was close to random and four times was uniform.

Table 12. Taylor's index of aggregation at different sites

Season	Trial Site	Squaring stage	Flowering stage
2011-12	Tarcoola, Macalister	1.17	1.10
	Gebur, Jandowae	0.74	1.03
	Mayfield, Nandi	1.07	0.86
2012-13	Tarcoola, Macalister	1.01	1.10
	Byee	0.50	0.98
	Kingaroy RS	0.03	1.06
2013-14	Kingaroy RS	0.95	0.15

Sample sizes at different precision levels were calculated using three years of pooled squaring and flowering data from the different sites. These were then plotted against mirid density to calculate the required sample size for different mirid population levels (Figure 29 and Table 13).

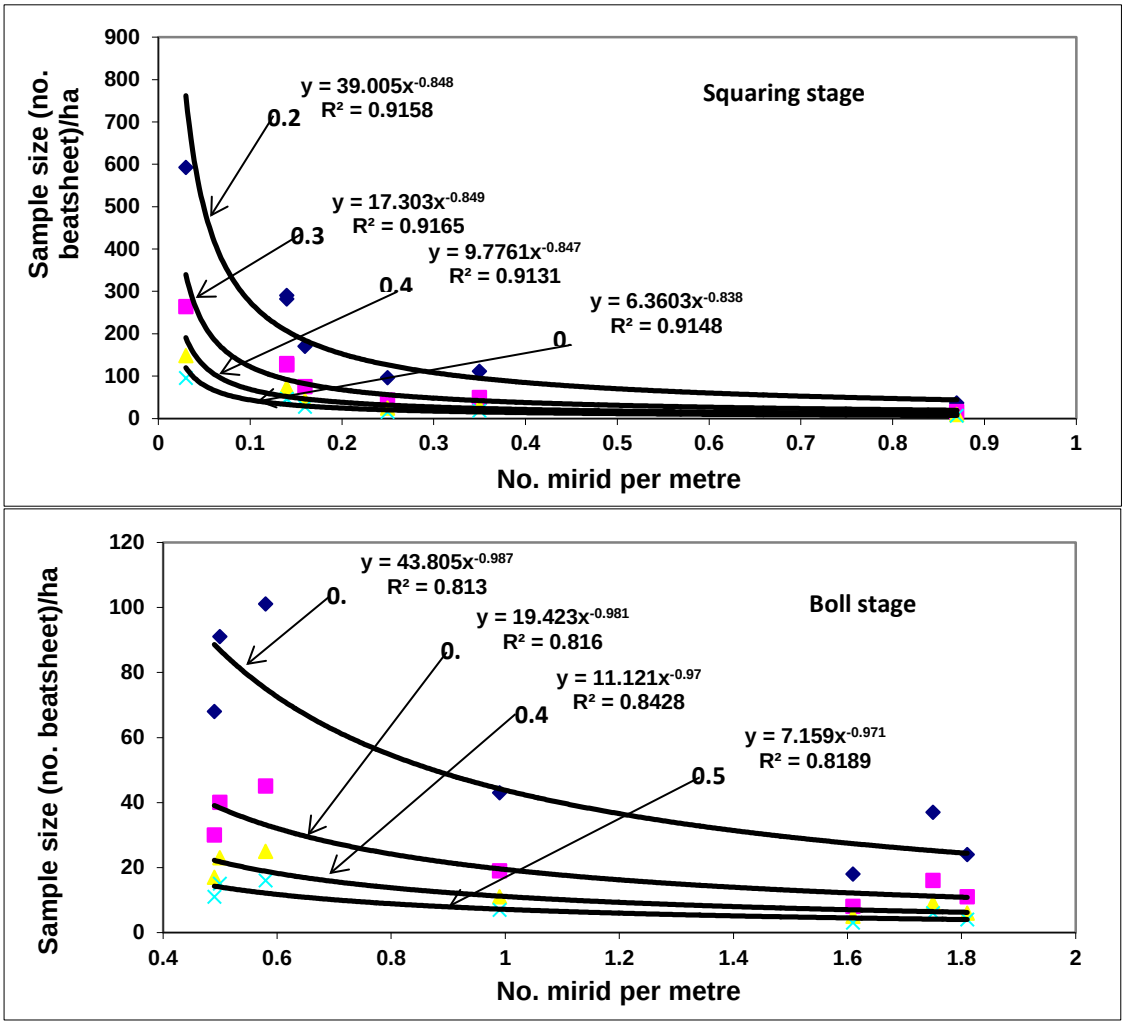


Figure 29. Relationship between mirid density and sample size ($n = ((1/\bar{e}) + (1/k))/D^2$) for different levels of precision. Each dot point indicates sample size for each site at each precision level.

Table 13. Required (calculated from the described model) sample size at different precision levels for different mirid densities

		Sample size at different precision level			
Crop Stage	Mirid No	**0.2**	**0.3**	**0.4**	**0.5**
Squaring Stage	1	39	17	10	6
	2	22	10	5	4
	3	15	7	4	3
	4	12	5	3	2
Flowering Stage	1	44	19	11	7
	2	22	10	6	4
	3	15	7	4	3
	4	11	5	3	2

Expt. 2. Temperature effect on mirid feeding

Methodology

The experiment was conducted in the laboratory in a controlled temperature cubicle using a water-jacketed multiple gradient temperature cabinet. Five different temperatures, 18, 23, 27, 32 and 36°C were generated to conduct the trial. Adult mirids were originally collected from a lucerne crop in Gatton and reared on green beans in a controlled temperature room. Fourth instar nymphs were used to assess mirid feeding behaviour on 12-14 day old bolls. The cotton bolls were taken from plants grown in a glasshouse. Boll age was determined by tagging at flowering. Each treatment was

replicated 4 times with 2 mirids per replication thus 8 mirids per temperature regime were used. The mirids were allowed to feed for 3 days since 4th instar nymphs take about 3 days to develop. Damage was assessed as black spots on bolls, warts inside bolls and lint damage. For lint damage, an assessment of each lock was made for spots of brown coloured lint, ¼, ½, ¾ and full lock damage. For ease of analysis a damage score was assigned: no damage = 0, a spot or two of brown coloured lint = 1, ¼ lock damage = 2, ½ lock damage = 3, ¾ lock damage = 4 and full lock damage = 5.

Results

Results showed that at 27°C and 32°C mirids caused significantly (F = 12.34, $p < 0.01$ for black spot; F = 6.84, $p < 0.01$ for wart and F = 4.21, $p < 0.05$) more damage than at higher and lower temperatures (Figure 30). The relationship between mirid feeding and temperature was curvilinear, similar to the relationship between temperature and mirid developments. Mirid feeding increased as temperature increased until an optimum temperature range where feeding was maximised. Thereafter, mirid feeding decreased. The least damage was found at 18°C and 36°C. When fit with a third order polynomial model, the r-square values increased substantially for all damage parameters compared to the linear relationship (Figure 31). The r-square values for the third order polynomial were 0.81, 0.80 and 0.57 for black spots, warts and lint damage respectively. The calculated optimum feeding temperature range was 30 - 31ºC.

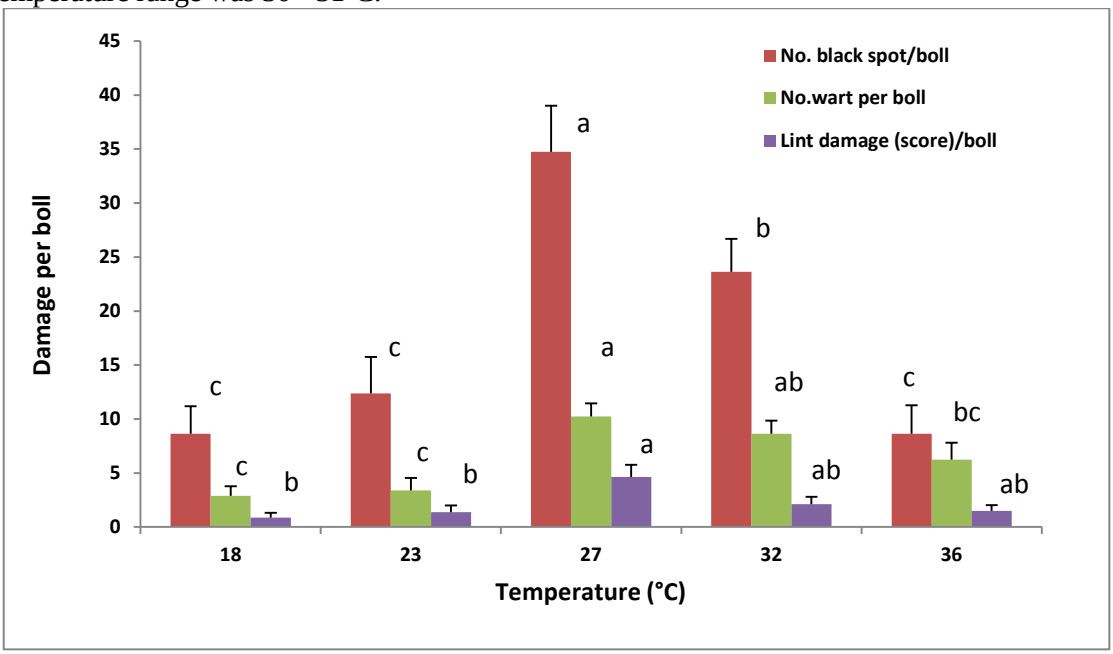
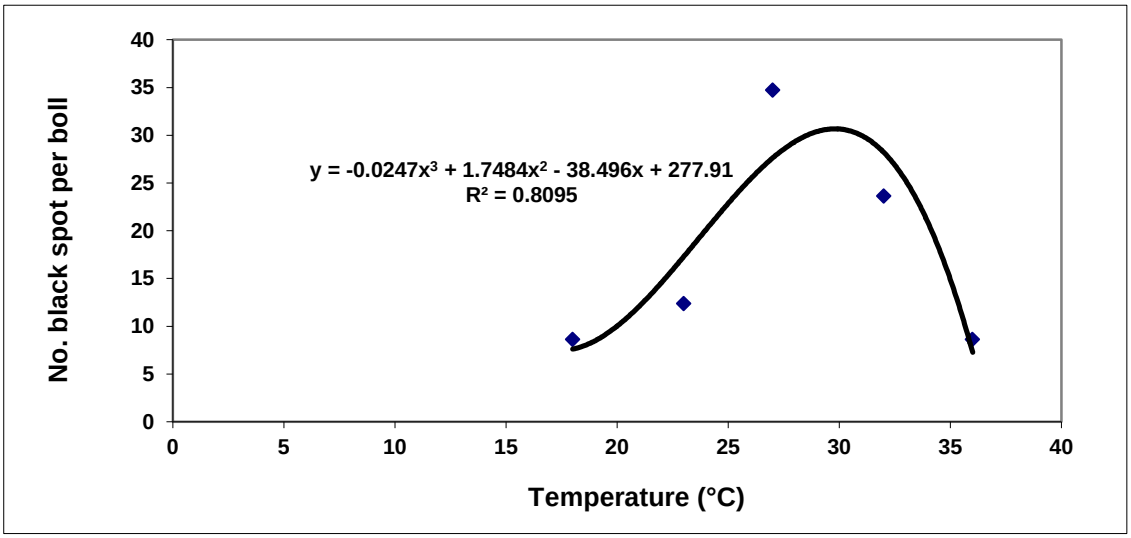


Figure 30. Mirid (4 instar nymphs) feeding at different temperatures (°C) in the laboratory. Error bars indicate standard error of mean. The means with same letter in the bars of each damage type are not significantly different (p > 0.05), Fisher's LSD test.



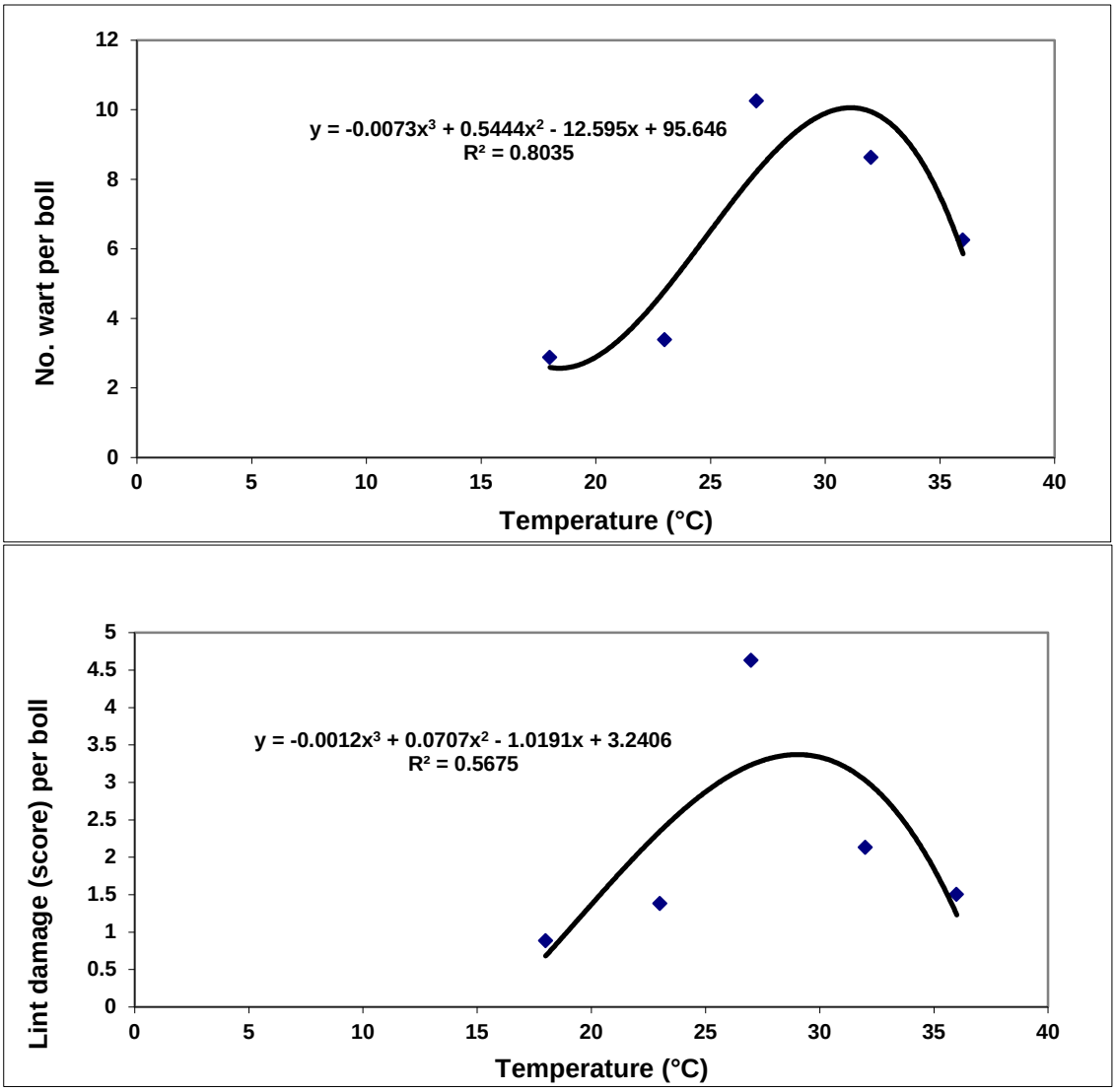


Figure 31. Relationship between temperature and damage parameters

Expt. 3. Kaolin based particle film technology against mirids and stinkbugs

Methodology

The experiment was conducted in a farmer's field near Dalby during the 2011-12 season. Two rates of kaolin, 40 and 60 g/L of water, were applied on 8 x 20m plots at three crop stages separately (squaring, flowering and 4 weeks after flowering). In addition, a third treatment of 60 g/L was applied at all three stages and at cut out. The treatments, including a control (without kaolin) were replicated 3 times in a RCB design. Kaolin was applied with a knapsack sprayer at the rate of 250 L/ha. Insect pests and beneficials were assessed weekly using a beat sheet, 1 x 3m per plot. At cut out the field was infested with whitefly and an assessment was made on 5 leaves per plot from the top 5-6 nodes (counting from first unfolded top leaf). The leaves were brought back to the laboratory and assessed under microscope to record whitefly nymphs and species composition (silver leaf vs greenhouse whitefly). No attempt was made to count whitefly adult. Cotton was harvested by hand 1 x 3m per plot.

In addition to ANOVA, data was also subjected to General Linear Model analysis to determine the interaction between treatments and date.

Results

Results are summarised in Figure 32. Overall, mirid numbers were low (less than 1 per metre) until peak flowering when mirid numbers reached around 2 per metre (Figure 32). There were no stinkbugs present in the field. Among the predators lady beetles and spiders were the most dominant. The analysis showed that there were no significant differences between treatments for mirids (F = 0.17, $p > 0.05$), lady beetles (F = 0.47, $p > 0.05$), spiders (F = 0.10, $p > 0.05$) and whitefly nymphs (F

= 1.34, $p > 0.05$). General Linear Model analysis also showed that the interaction between treatments and dates were not significant ($F = 0.80$, $p > 0.05$) for mirids. However, yield for 60 g sprayed at squaring was significantly higher ($F = 2.95$, $p < 0.05$) compared with the unsprayed control and from 60 g sprayed at 4 weeks after flowering, 40 g sprayed at flowering and 60 g sprayed at flowering (Figure 33). Though previous results (1.01.61 CRC155) from the glasshouse and small scale field trials showed kaolin was effective against mirids and stinkbugs, the results from these trials suggest that kaolin may not be an effective management option for these insects.

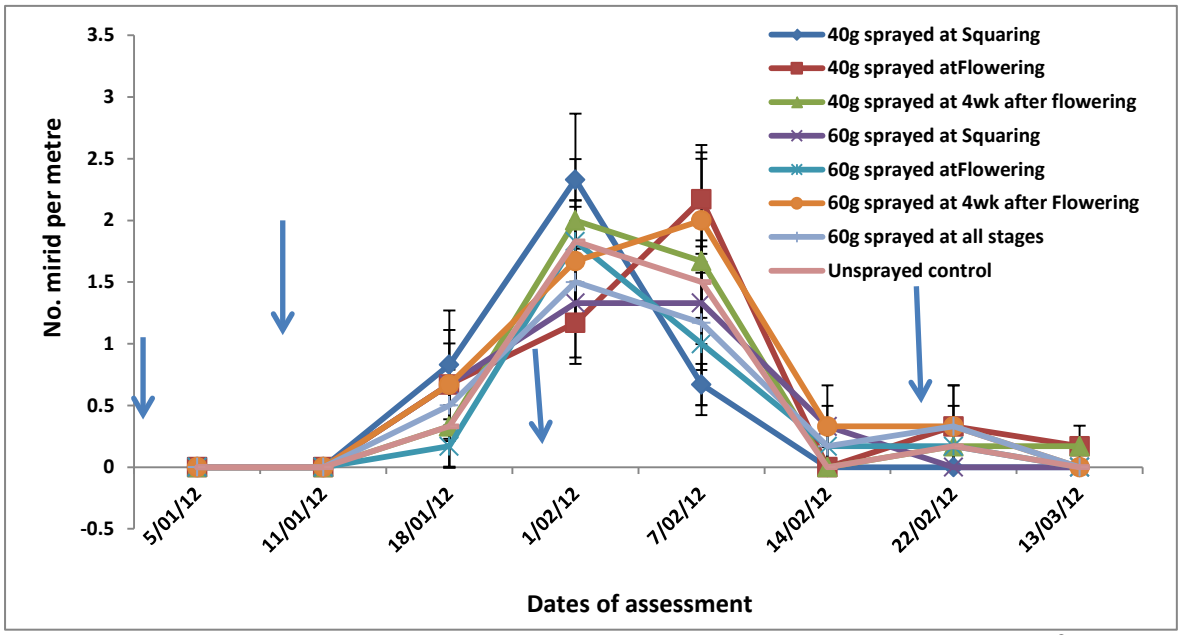


Figure 32. Effect of kaolin aginst mirids sprayed at different crop stages in Bollgard® II. Arrows indicate time of spray. Error bars indicate standard error of means.

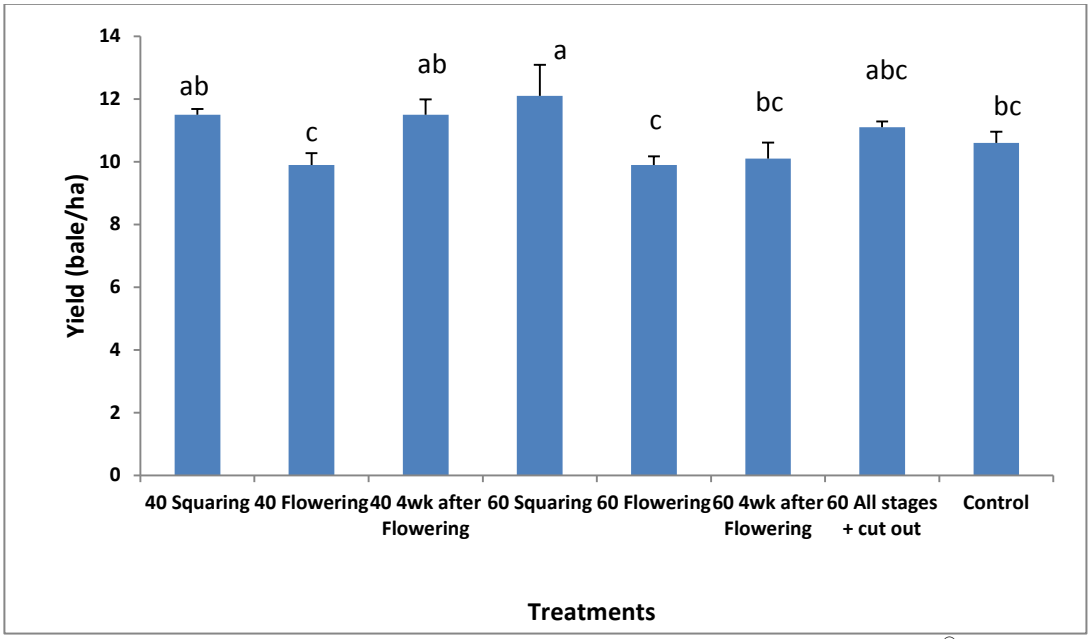


Figure 33. Yield (bale/ha) for kaolin sprayed at different crop stages in Bollgard® II. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

Expt. 4. Evaluation of insecticides against mirids

Methodology

Two experiments were conducted in Bollgard® II cotton at Kingaroy Research Station (KRS) and in Byee using both unregistered and newly registered insecticides. Treatment details are given in Table 14. The treatments were replicated 3 times and each replication measured 8 rows x 20m (row spacing 1 m) at KRS and 10 rows x 20m (row spacing 1 m) in Byee. The insecticides were applied with a Kubota B7100 Highboy sprayer at the rate of 150 L/ha fitted with xl-01 air induction nozzle with 4 bar pressure. Pre-treatment counts were made one day before spray application. Post treatment counts were made at 3 and 8 days after treatment (DAT) at KRS and at 3, 6 and 14 DAT in Byee. Mirids and beneficials were sampled using a beat sheet on 3 x 1m row sections per plot.

Table 14. Treatments used against mirids at KRS during 2012-13 and in Byee during 2013-14 seasons

Treatment	Formulation (g/L)	Rate (g or mL/ha)
KRS		
IKI220	Flonicamid	140 g
IKI3106	Cyclaniliprole	400 mL
IKI3106	Cyclaniliprole	800 mL
Regent® + salt	Fipronil 200 g/L + NaCl	50 mL + 10 g/L
Untreated control	Control	_
Byee		
IKI220	Flonicamid	140 g
IKI3106	Cyclaniliprole	800 mL
Transform®	Sulfloxaflor 240 g/L	300 mL
Transform®	Sulfloxaflor 240 g/L	100 mL
Transform® + salt	Sulfloxaflor 240 g/L + NaCl	300 mL + 10 g/L
Transform® + salt	Sulfloxaflor 240 g/L + NaCl	100 mL + 10 g/L
Regent® + salt	Fipronil 200 g/L + NaCl	50 mL + 10 g/L
Untreated control	Control	_

Results

Pre-spray mirid numbers were very high in Byee (8.5 to 12.1/m) and low at KRS (1.4 to 2.2 per metre). Population structure was predominantly nymphs, at KRS 80% and in Byee 90% were nymphs. Since there was no difference in efficacy between adults and nymphs the data is presented as adults and nymphs combined (Figures 34 and 35). The analysis showed that at KRS 2012-13 there was no significant difference between treatments at 3 DAT ($F = 1.02$, $p > 0.05$) but difference was significant at 8 DAT ($F = 5.59$, $p < 0.05$). However, Fisher's LSD test revealed that the difference between IKI220, IKI3106 and Regent® + salt was not significant ($p > 0.05$) (Figure 34).

The analysis with Byee 2013-14 data showed that there was significant difference between treatments at 3 DAT ($F = 35.35$, $p < 0.001$), at 6 DAT ($F = 44.32$, $p < 0.001$) and at 14 DAT ($F = 4.70$, $p < 0.01$) (Figure 35). When treatment means were separated using Fisher's LSD test, at 3 DAT all rates of Transform® (including with added salt) and Regent® plus salt were significantly different from IKI220, IKI3106 and unsprayed control ($p < 0.05$); at 6 and 14 DAT all rates of Transform® (including with added salt), IKI220 and Regent® plus salt were significantly different from IKI3106 and unsprayed control ($p < 0.05$) (Figure 35).

The results also showed that a reduction of up to 95% for IKI220 and up to 99% for Transform® was obtained which was on par with the standard control. The results also showed that while Transform® acted very quickly (up to 96% reduction at 3 DAT), IKI220 acted slowly (up to 69% reduction at 3 DAT). When a low rate of Transform® (100 mL/ha) was mixed with salt, effectiveness increased by 16% and reached 94% at 3DAT. This indicates that salt can be mixed with an even lower rate of Transform®.

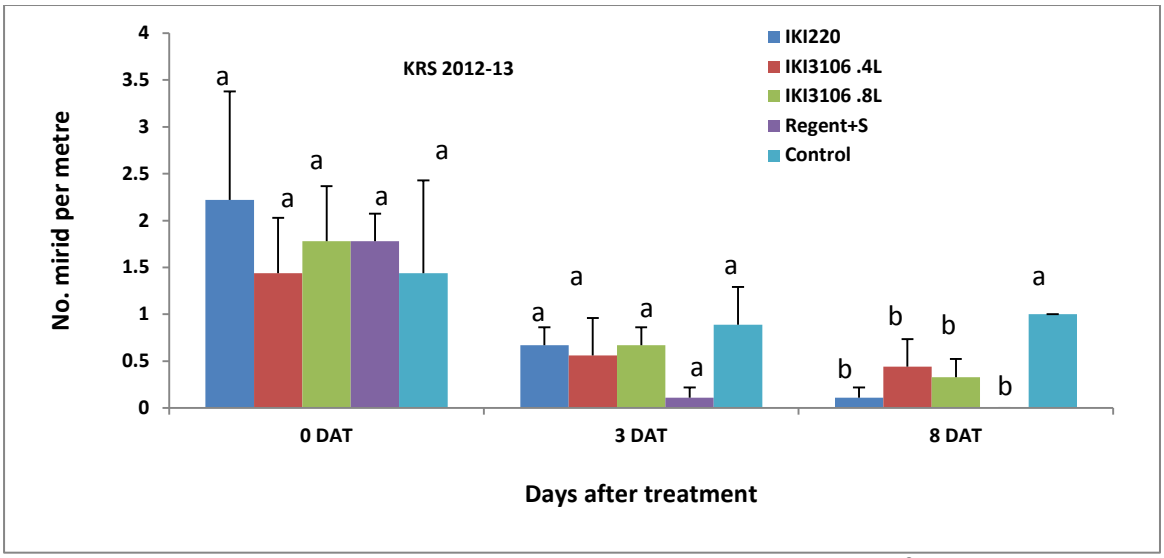


Figure 34. Effect of two unregistered products against mirids in Bollgard® II cotton. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

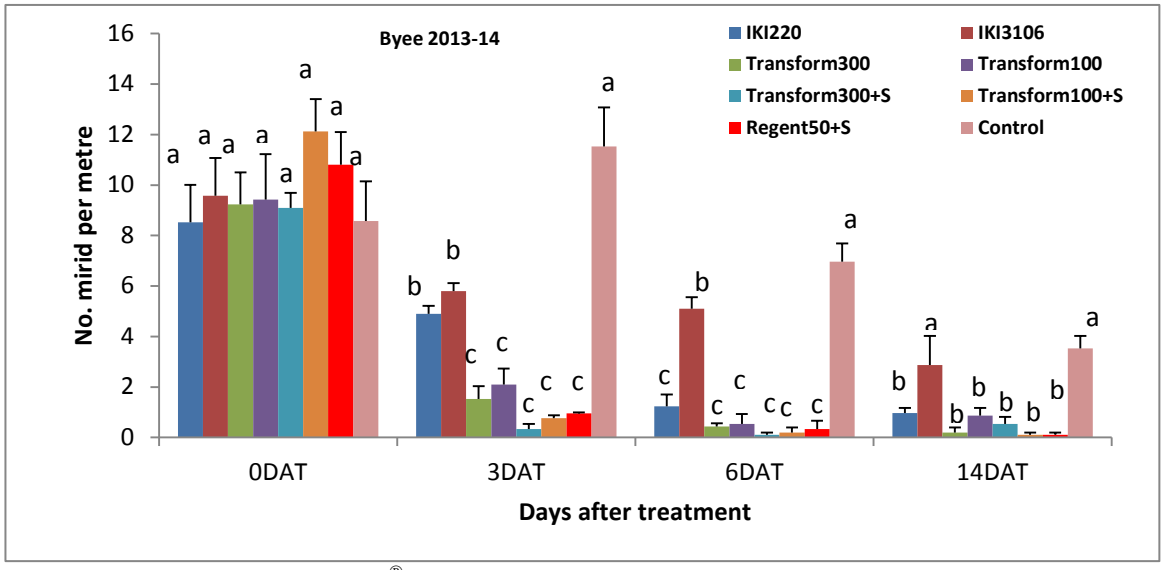
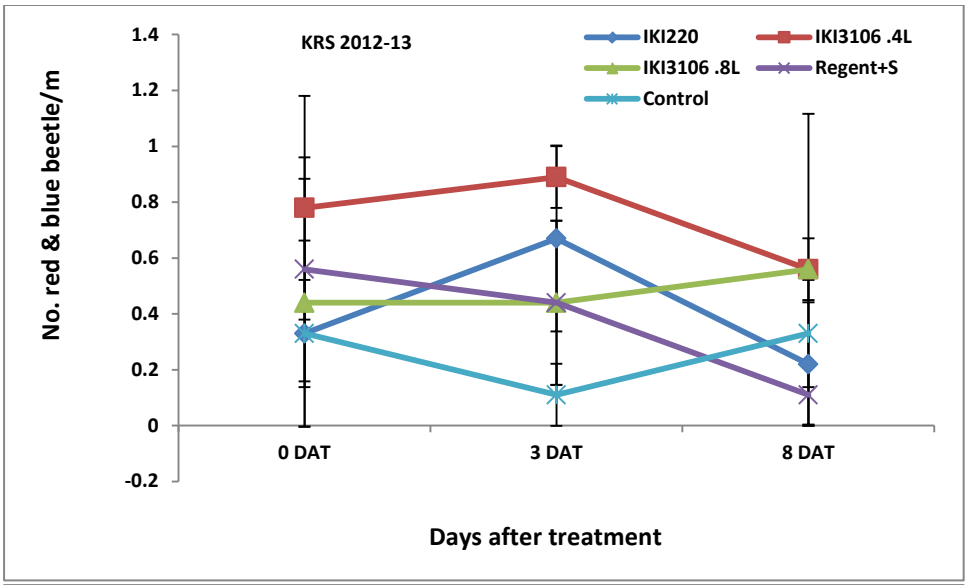
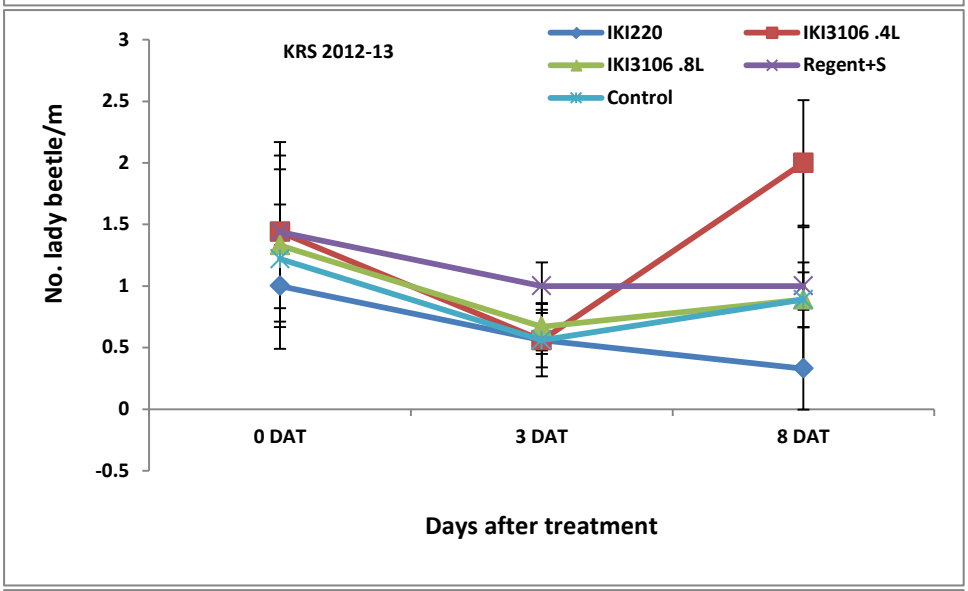


Figure 35. Effect of Transform® (with and without salt) and two unregistered products against mirids in Bollgard® II cotton. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

The most abundant beneficials in both experiments were red and blue beetles (RBB), more than one species of lady beetle (mostly transverse and hippodamia) and a mixture of spiders (mostly lynx spiders). The results are presented in Figures 36 for KRS 2012-13 and in Figure 37 for Byee 2013-14. The analysis (ANOVA) showed that at KRS 2012-13 the difference between treatments was significant at 8 DAT and only for spiders ($F = 5.85$, $p < 0.01$). Fisher's LSD test revealed that IKI3106 (0.8 L) had significantly highest impact on spiders ($p < 0.05$) followed by IKI220, IKI3106 (0.4 L) and Regent® plus salt. The analysis also showed that in Byee 2013-14 the difference between treatments was significant only at 6 DAT and only for spiders ($F = 2.96$, $p < 0.05$). When treatment means were separated using Fisher's LSD test the impact of all test chemicals on spiders were significantly higher than unsprayed control ($p < 0.05$) but the difference between themselves was not significant ($p > 0.05$). When disruption level was expressed as per Cotton Pest Management Guide impact of IKI220, IKI3106 and Transform® on RBB was very low to moderate, the impact IKI220, IKI3106 on LB and spiders was low to very high and the impact of Transform® on LB and spiders was low to high. When salt mixed with Transform® impact on RBB and LB was reduced. However, at 14 DAT beneficial numbers in Byee increased across the treatments indicating there was no residual effect of the test chemicals on these insects.





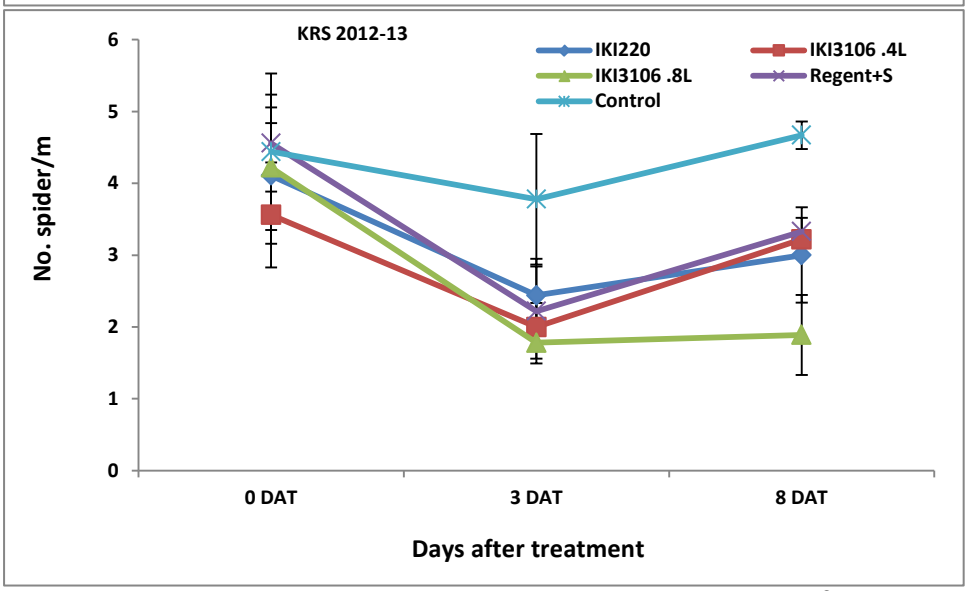


Figure 36. Impact of two unregistered products on beneficials in Bollgard® II cotton at KRS 2012-13. Error bars indicate standard error of means.

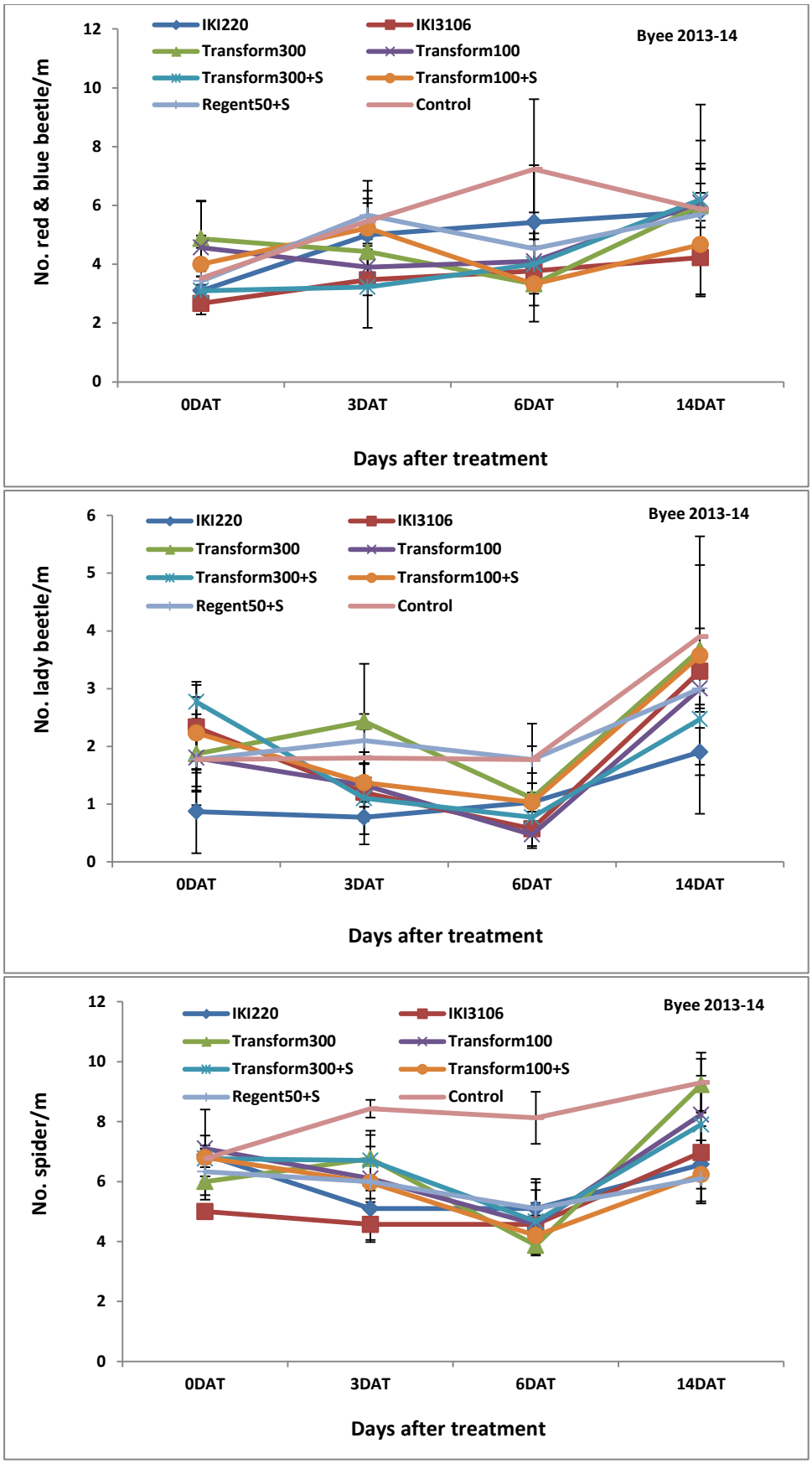


Figure 37. Impact of Transform® (including with added salt) on beneficials in Bollgard® II cotton in Byee 2013-14. Error bars indicate standard error of means.

Conclusions

Sample size at different precision level

Our experiment showed that sample size varied with precision levels. The sample size at higher precision level may give less reliable estimate of the population. Therefore during sampling mirids in the field this needs to be considered.

Temperature effect on mirid feeding

Here we tested 4th instar nymphs only because along with 5th instar nymphs and adults this nymphal stage causes the most damage to cotton (previous study by the principal researcher). From the fitted curvilinear model (see Figure 31) optimum temperature for mirid feeding was calculated as 30 - 31°C. At this temperature range mirid also breed faster (previous study by the principal researcher). Above or below optimum temperature mirid feeding rate will reduce. This is perhaps one of the reasons in the field sometimes mirid damage and mirid density is not linear. However, the results suggest that temperature may play a role in the different rates of damage observed in the field for the same mirid density.

Transform® plus salt and two unregistered chemicals

Low rate of Transform® (100 mL/ha) plus salt (10 g/L of water) was found to be very effective against mirids. Transform® plus salt reduced mirid populations by up to 94% (compared to pre-spray mirid numbers) and 16% mortality was increased due to mixing salt with low rates of the chemical. The impact of Transform® plus salt on beneficials was also lower compare to full rate of Transform® (300 mL/ha). Therefore low label rate of Transform® (100 mL/ha) plus salt can be used as an IPM option for managing mirids.

Among the two unregistered chemicals, IKI220 (flonicamid) was found to be most effective reduced mirid population by up to 95%. However, compared to Transform® and Regent®, IKI220 was slow in acting.

PALE COTTON STAINER BUG

Expt. 1. Pale cotton stainer damage in Bollgard® II

Methodology

This experiment was conducted at the Kingaroy Research Station in Bollgard® II cotton during the 2011-12 and 2012-13 seasons. For both years at 5 weeks after flowering, five different densities of cotton stainer adult (0, 2, 4, 6 and 10 per cage) from a laboratory culture were confined onto plants for 42 days in 2011-12 and for 60 days in 2012-13. Treatments were replicated 5 times. Before the introduction of insects, plants were checked thoroughly and any existing external damage to bolls were recorded. Post release damage was assessed in two steps. Firstly, after insects were removed the plants were examined for external damage and secondly at harvest for internal damage (lint damage). Cotton was hand harvested to determine yield.

Data was analysed using Analysis of Variance (ANOVA) and means were separated using Fisher's Least Significant Difference Test. To determine an action threshold based on the density and damage relationship (damage factor) the data was subjected to regression analysis. From this an Economic Injury Level (EIL) was calculated. The EIL was calculated using following model:

EIL = C/VD

Where, C is the control cost (chemical and application costs), V is value of the crop (price/bale) and D is the damage factor (from the regression analysis).

Results

During the 2011-12 season large numbers of bolls (~80%) were damaged due to boll rot associated with the bacterium *Pantoea agglomerans*. This contamination occurred irrespective of treatments and was difficult to separate from pale cotton stainer damage. Therefore this data was not included in the analysis.

During 2012-13 season, percent boll damage (bolls with spots and warts) was highest at 4 pale cotton stainers per metre. The damage was higher for low densities (4 and 6/m) than for high densities (10/m). This was likely due to competition factors such as overcrowding and competition between insects for food and shelter at the higher (10 per metre) densities.

For yield the analysis showed that there was no significant difference between treatments ($F = 2.51$, $P = 0.075$). However, Fisher's LSD Test revealed that the yield was significantly less ($p < 0.05$) for 4, 6 and 10 pale cotton stainers than the control yield (Figure 38). Since 10 pale cotton stainers per metre produced a higher yield compared to lower densities, treatment 5 was excluded from the regression analysis for calculating economic injury level (EIL). The EIL was calculated using a damage factor obtained from the regression equation ($y = -0.63x + 10.79$; $r^2 = 0.31$) between pale cotton stainer number and yield (Figure 39). Since the insects were allowed to feed for 60 days the damage factor was converted to damage per day. The other two factors in the model were cost of spray, \$20/ha (aerial spray of synthetic pyrethroid) and value of cotton, \$500/bale. The EIL was calculated as 4 per metre.

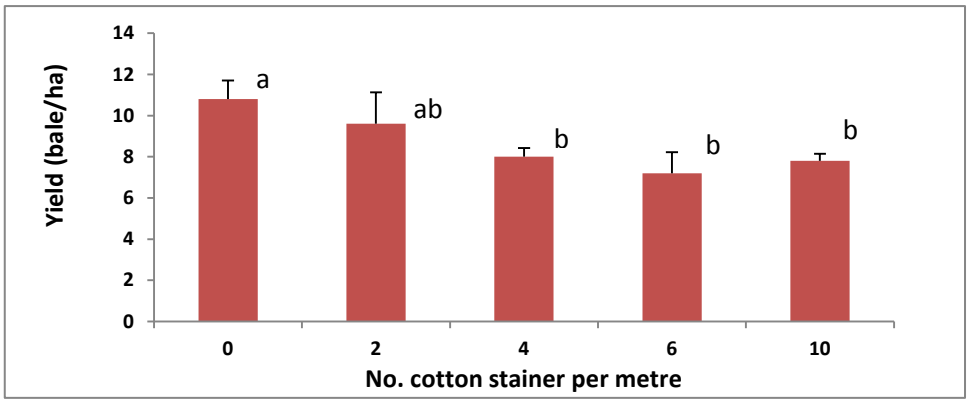


Figure 38. Yield (bale/ha) in a field cage trial with pale cotton stainer during 2012-13 season at Kingaroy Research Station. Error bars indicate standard error of means. The means with same letter in the bars are not significantly different ($p > 0.05$), Fisher's LSD test.

In theory EIL is considered as above action threshold level, which means action threshold should be below EIL level to prevent insect to reach economic injury level. Also in the experiment insects were confined inside cage which might prevent insects to fly around feeding on more bolls therefore causing more damage. Also there was no significant difference ($p > 0.05$) in yield between 2 pale cotton stainers per metre and the control (Figure 38). Considering all these factors an action threshold of 3 pale cotton strainers per metre is proposed which is similar to recommended threshold in the Cotton Pest Management Guide which was developed by the principal researcher from the damage relationship between green vegetable bug and pale cotton stainer.

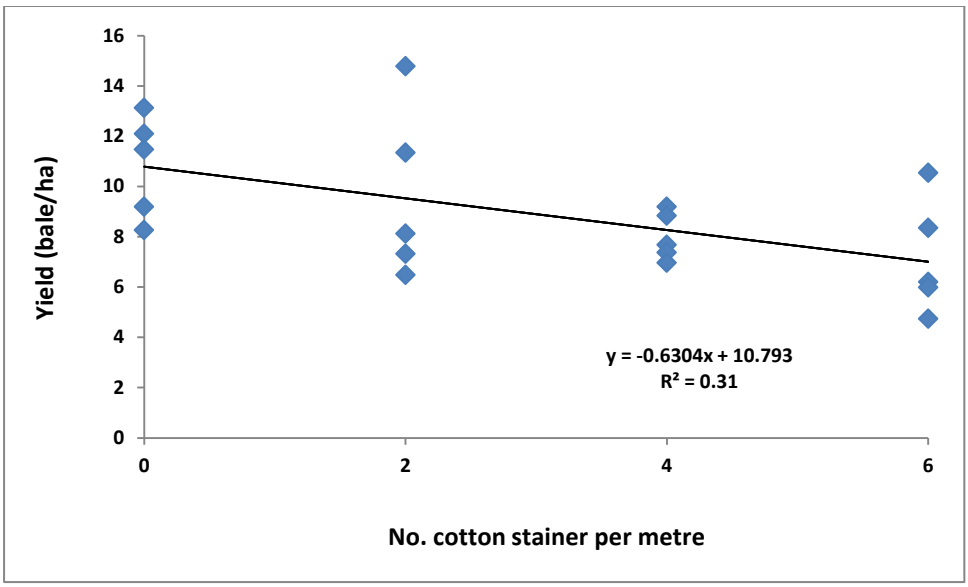


Figure 39. Relationship between pale cotton stainer density and yield. Each dot point represents yield per replication for each treatment.

Expt. 2. Evaluation of insecticides plus salt against pale cotton stainer bug

Methodology

The experiment was conducted using different insecticides with and without salt at KRS in the 2011-12 season at cut out stage. Treatment details are given in Table 15. Each treatment was applied in a 5 row x 15m plot with 3 replications in a RCB design. The insecticides were applied using a Kubota B7100 Highboy sprayer with a spray volume of 110 L/ha fitted with xl-01 air induction nozzle with 4 bar pressure. Pre-treatment counts were made one day before spray application and post treatment counts were made at 4, 9 and 14 days after treatment (DAT). Cotton stainer bugs and beneficials were sampled using a beat sheet on 3 x 1m row sections per plot.

Table 15. Treatments used against pale cotton stainer bug at KRS during 2011-12 season

Treatment	Formulation (g/L)	Rate (g or mL/ha)
Pegasus®	Diafenthiuron 500 g/L	400 mL
Pegasus®	Diafenthiuron 500 g/L	800 mL
Pegasus® + salt	Diafenthiuron 500 g/L + NaCl	400 mL + 10 g/L
Shield®	Clothianidin 200 g/L	125 mL
Shield® + Maxx	Clothianidin 200 g/L + Maxx	250 mL + 2% (v/v)
Shield® + salt	Clothianidin 200 g/L + NaCl	125 mL + 10 g/L
Rogor®	Dimethoate 400 g/L	300 mL
Rogor®	Dimethoate 400 g/L	500 mL
Rogor® + salt	Dimethoate 400 g/L + NaCl	300 mL + 10 g /L
Untreated control	Control	_

Data was transformed into log transformation before analysis. Transformed data was subjected to Analysis of Variance (ANOVA) and means were separated by using Fisher's Least Significant Difference Test. Data was also subjected to General Linear Model (GLM) analysis to find out if there was any interaction between insecticides and days after treatment.

Results

The pre-spray pale cotton stainer numbers were 2.2 to 6.7 per metre, of which 95 per cent were adults. The results are summarised in Figure 40. The results showed that Pegasus® @800 mL/ha was most effective against pale cotton stainer followed by Pegasus® @400 mL/ha and Shield® @125 mL/ha plus salt. Pegasus® @800 mL/ha reduced pale cotton stainer population by up to 83% at 9 DAT compared to pre-spray count. The analysis showed that except at 9 DAT the difference between treatments were not significant (F = 1.91, P = 0.110 at 4 DAT; F = 3.21, P = 0.014 at 9 DAT; F = 1.43, P = 0.241 at 14 DAT). The Fisher's LSD Test revealed that at 9 DAT Pegasus® @800 mL/ha, Pegasus® @400 mL/ha, Shield® @125 mL/ha plus salt and Shield® @125 mL/ha reduced pale cotton stainer population more than other treatments.

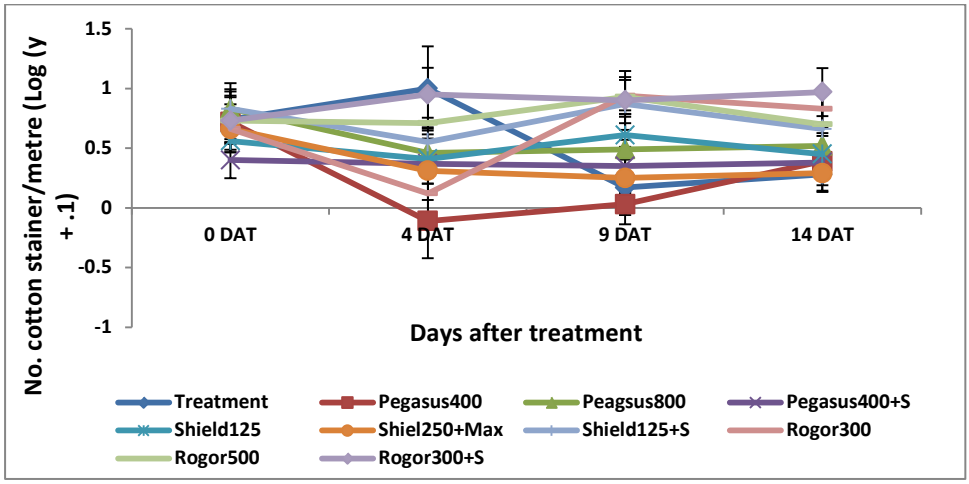


Figure 40. Effect of insecticides against pale cotton stainer bug in Bollgard® cotton at cut out stage at Kingaroy Research Station during 2011-1 season. Error bars indicate standard error of means.

The GLM analysis showed that there was no significant interaction between treatments and dates (F = 0.93, P = 0.575). However, analysis on pooled data for all dates there was a significant difference between treatments (F = 2.63, P = 0.034) and LSD Test revealed that the effect of Pegasus® @800 mL/ha and Pegasus® @400 mL/ha was significantly more than other treatments (Figure 41).

Results also showed that while efficacy increased when salt was mixed with a low rate of Shield® efficacy did not increase when salt is mixed with a low rate of Pegasus® and Rogor®.

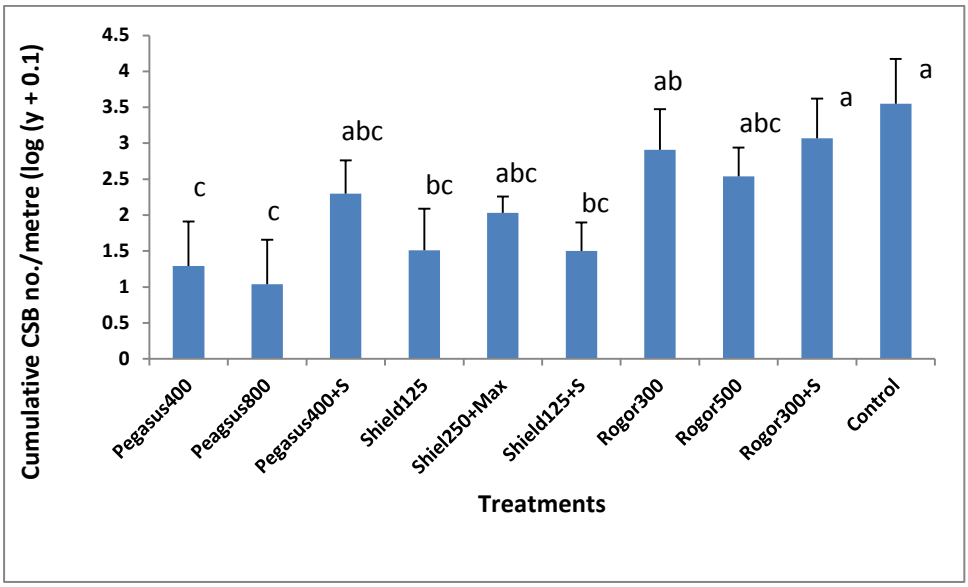


Figure 41. Treatment means for all dates together against pale cotton stainer bug in Bollgard® cotton at cut out stage at Kingaroy Research Station during 2011-1 season. Error bars indicate standard error of means. Means in the bars followed by same letter are not significantly different (p > 0.05), Fisher's LSD Test.

Survey on causal agents of boll rot and potential link with bug transmitted pathogens

Survey on causal agents of boll rot

Methodology

Surveys were conducted during the 2011-12 and 2012-13 seasons at peak flowering stage. During 2011-12, bolls were randomly collected from 3 different sites in Central Queensland and St George, 1 site in Darling Downs and 2 sites in South Burnett (Table 16). From each site 50 young, 50 old and 20 sting bolls (bolls with insect feeding spots) were collected walking across the field. Insects (mirids and stinkbugs) feeding spots are characterised by shiny black spots are different from brown coloured natural blemish. In addition, assessment also made on bolls from 12 sites in NSW and QLD including 2 fields at ACRI was collected by disease survey team (Table 16). But these were not separated as young or old or sting bolls. During 2012-13 surveys were only conducted at Kingaroy Research Station (KRS). Half of the bolls were dissected for damage and the remaining half were sent to Dr Nandita Pathania, Bacteriologist, QDAFF, Mareeba for bacterial identification.

Results

Survey data from the 2011-12 season including the names of causal agents for all sites are presented in Table 16. Survey results from all sites found the bacteria *Pantoeae agglomerans* to be the predominant organism in rotten bolls. *P. agglomerans* has also been identified in the USA as one of the causal agents for boll rot. During the 2012-13 season, boll rot occurred in 10 – 20% of bolls with young bolls incurring more damage than older bolls. The assessment on bacterial identification revealed that 50% of the damaged bolls carried the *P. agglomerans*. The lower percentage of boll rot during the 2012-13 season compared to 2011-12 season was perhaps due to drier weather conditions (low rain fall) in the latter season. Per cent boll rot was highest at Kingaroy. Here, in addition to *P. agglomerans*, flies (described below) were also associated with boll rot particularly with insect damaged bolls. This may have exacerbated the boll rot problem.

Table 16. Survey of boll rot at different sites in NSW and QLD during 2011-12. *dominant species

Location	*% damaged boll*			% boll rot			Organism found*
	Young boll	*Old boll*	*Sting boll*	Young boll	Old boll	Sting boll	
Kingaroy Research Station	*86*	*98*	*100*	50	80	100	*P. agglomerans** & *Flavobacterium spp*
Byee	*14*	*22*	-	10	25	-	*P. agglomerans** & *Flavobacterium spp*
Gebur, Jandowae	*66*	*16*	*60*	50	25	60	*P. agglomerans** & *E. cowanii*
Anderson, Emerald	*4*	*12*	*40*	4	12	40	*P. agglomerans*
Karraman, CQ	*20*	*12*	*40*	20	12	40	*P. agglomerans*
Farm26, StGeorge	*4*	*10*	*10*	10	25	50	*P. agglomerans*
Bundoran, StGeogre	*30*	*40*	*80*	20	35	43	*P. agglomerans*
Opposite Farm 158, StGeorge	*4*	*34*	*15*	30	50	50	*P. agglomerans** & *Falvobacterium spp*
Cambooya	*78*			50			*P. agglomerans** & *Flavimonas spp*
Field 2, ACRI	*86*			23			*P. agglomerans** & *Flavimonas spp*
Field 4, ACRI	*78*			35			*P. agglomerans*
Red bank	*29*			5			*P. agglomerans*
Top Box 46	*50*			11			*P. agglomerans*
Royston	*40*			5			*P. agglomerans*
Wyadrigah	*83*			5			*P. agglomerans*
Warentdi	*80*			5			*P. agglomerans*
Currawiogen	*70*			5			*P. agglomerans*
Drayton	*100*			5			*P. agglomerans*
Wanlea	*0*			0			
5-Mile	*63*			30			*P. agglomerans*

Boll rot and potential link with bug transmitted pathogens

Methodology

An experiment was conducted in a glasshouse to determine if pale cotton stainer bugs are capable of transmitting the boll rot producing bacteria *Pantoea agglomerans* to cotton bolls during feeding. The bacterium was isolated by Dr Pathania from the field samples described above. Field collected pale cotton stainers were cultured in the laboratory for several generations to remove any naturally occurring *Pantoea* from their systems. The trials were conducted in three steps. Firstly, cotton seeds were dipped into a solution of the bacterium for five minutes. Cotton stainer bugs from the laboratory culture were then allowed to feed on those seeds. Finally, these insects, along with fresh cotton stainers from the same culture, were each confined to 20 day old fresh bolls (one insect per boll) for 7 days. At every step the bacterium was isolated and cultured in media plates for confirmation of the bacterium species.

Damage was assessed by assessing lock and lint damage. Percent of lock damage was calculated for each boll. For lint damage, an assessment of each lock was made for different category of brown coloured lint and an score was assigned to each lock for each damage category: no damage = 0, specks of yellow/brown lint = 1, 1/4th of a lock with brown lint = 2, 1/3rd of a lock with brown lint = 3,

½ of a lock with brown lint = 4, 2/3rd of a lock with brown lint = 5 and a whole lock with brown lint = 6.

Data was analysed using Analysis of Variance (ANOVA) and means were separated using Fisher's Least Significant Difference Test.

Results

The results are summarised in Figure 45. There were a greater number of lock (Figure 42A) and lint (Figure 42B) damage in bolls exposed to cotton stainer bug with bacteria. Analysis also revealed that there was significant difference between treatments for per cent lock (F = 12.88, P = 0.0001) and lint (F = 9.45, P = 0.001) damage. However, caution should be exercised when applying this to a field situation.

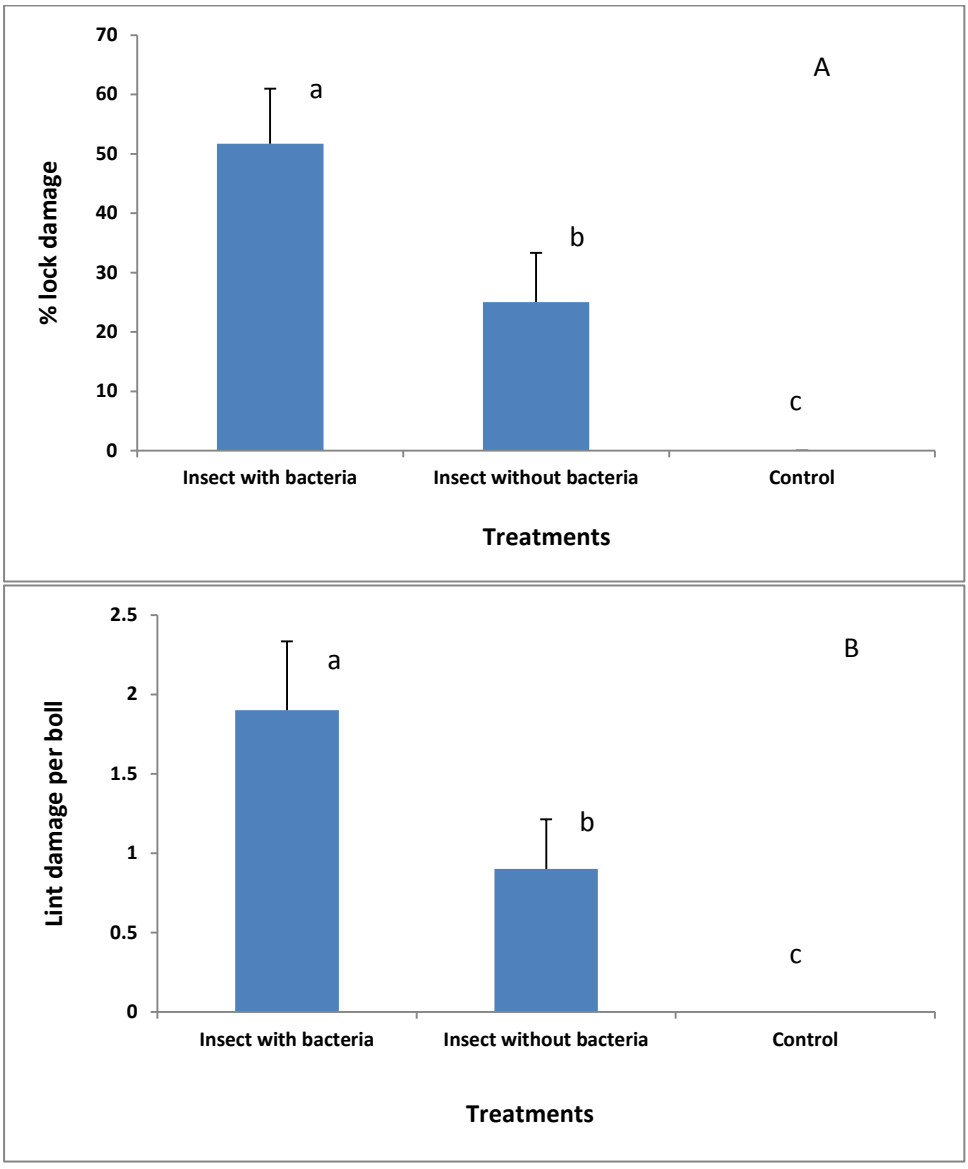


Figure 42. Percent lock (A) and lint (B) damage caused by pale cotton stainer bug with and without bacteria in the glasshouse. Error bars indicate standard error of means. Means in the bars followed by same letter are not significantly different (p > 0.05), Fisher's LSD Test

Boll rot and potential link with Queensland fruit fly

Methodology

In mid March 2012 at the KRS trial site fly maggots were collected from inside bolls that appeared to be undamaged on the surface. These maggots were reared to adult flies and were sent to QDAFF taxonomy unit at EcoSciences Precinct, Brisbane for identification. Following the first detection in

March, fly activity was monitored until mid-May. Weekly assessments were made on 50 bolls each from the top 7 to 10 nodes and from the bottom of the plant (below 10 nodes).

Results

Maggots were observed at Kingaroy inside normal looking bolls containing complete rot. These maggots were reared to adult flies and identified as *Bactrocera tryoni*, *Atherigona orientalis* and *Chloropsina sp. (*near *turneri)* (Plate 8)

B. tryoni

A. orientalis

Chloprpsina sp

Plate 8. Flies associated with boll rot in Bollgard® II cotton in Kingaroy

B. tryoni is an established pest of fruits and some vegetables in Australia and *A. orientalis* has been reported as a pest of peppers. Little is known about the pest status of *Chloropsina*.

Infected bolls appear undamaged on the surface, however they become softer and paler than undamaged bolls as infection advances. These bolls feel soft when squeezed. Inside the damaged bolls maggots are easily visible and locks turn brown or pinkish-brown and squishy, usually watery in younger bolls (15 to 20 days old). More mature bolls (above 20 days old) are drier with maggot feeding holes clearly visible in affected locks (Plate 9). Once the affected bolls open, the locks are tight and un-harvestable (Plate 10).

Plate 9. Young boll- brown locks, squishy, watery Mature boll- drier with maggot feeding hole

Plate 10. Tight, rotten locks in an open boll

The results on weekly assessment showed that bottom (older) bolls were infested more often than top (younger bolls) (Figure 43) suggesting flies prefer older bolls. It is worth to mention that this was an extremely wet season which along with flies might influence this level of boll rot.

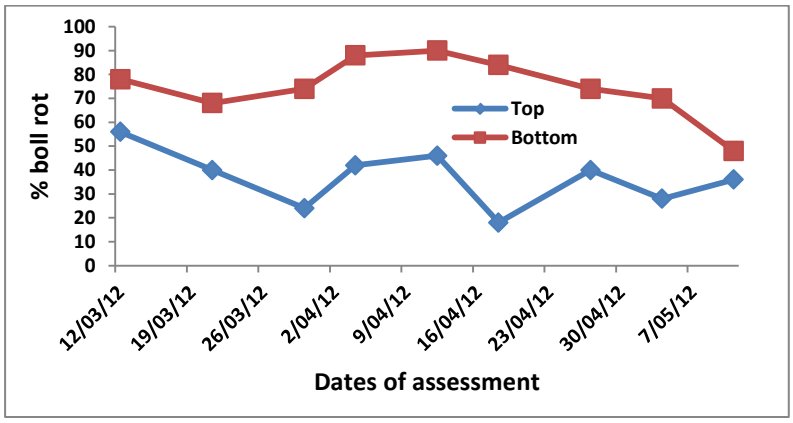


Figure 43. Maggot infestation over the time at top and bottom level bolls in Bollgard® cotton at Kingaroy Research Station during 2011-12 season

Conclusions

Pale cotton stainer damage and action threshold

We propose an action threshold for pale cotton stainer is 3 adults per metre. For nymphs the same calculation as green vegetable bug nymphs should be applied (see Cotton Pest Management Guide); that is 4th and 5th instars causing similar damage to adults, 3rd instars causing half the damage caused by adults and so on.

Boll rot and potential link with bug transmitted pathogens

An organism, *Pantoea agglomerans* was identified causing boll rot. It is important to mention that there may be other organism or other means involving boll rot which we did not explore. It was found from our investigation that pale cotton strainers are able to transmit *P. agglomerans*. During wet years the association with this organism causing boll rot increase substantially. Three flies were also identified as being associated with boll rot. These flies are *Bactrocera tryoni*, *Atherigona orientalis* and *Chloropsina sp. (*near *turneri)*.

Reference cited

Arif, M. I., Rafiq, M. and Ghaffar, A. (2009). Host plants of cotton mealybug (*Phenacoccus solenopsis*): A new menace to cotton agroecosystem of Punjab, Pakistan. International Journal of agriculture & Biology, 11: 163-167

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Nagrare, V. S., Kranthi, S., Biradar, V. K., Zade, N. N., Sangode, V., Kakde, G., Shukla, R. M., Shivare, D., Khadi, B. M. and Kranthi, K. R. (2009). Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* (Tinseley) (Hemiptera: Pseudococcidae) on cotton in India. Bulletin of Entomological Research, 99: 537 – 541

Whitehouse, M. (2006). Benchmarking what triggers mirid sprays in cotton- the results from the pilot study. The 13th Australian Cotton Conference, Gold Cost

Outcomes

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

Almost all of 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.*
A basic management strategy for solenopsis mealybug	Understanding of basic ecology that underpins an effective monitoring and population management program	Growers have sufficient information and knowledge to minimise the impact of Solenopsis mealybug on their crops
Determine optimum sample sizes at different precision levels at key crop stages and identify if temperature affects mirids feeding behaviour	Researchers gain knowledge on sampling, distribution pattern and feeding behaviour of mirids	Judicious and timely application of control method
Identify environmentally friendly management tools for the pests	New IPM tools identified	Reduce reliance on broad spectrum insecticides
Effective control options evaluated and results communicated to industry. Data made available for registration or permits, where appropriate	Knowledge of effective chemistry and timing of application	Growers have knowledge of effective option for mealybug control
Determine action threshold for pale cotton stainer	Researchers gain knowledge and adoption of their research	Improved knowledge of pale cotton stainer impact and management

The major outputs that were generated through this project for three important pests in Bollgard® cotton include:

Mealybug: Some aspects of basic mealybug ecology such as overwintering strategy were determined. Impact of mealybug establishment at different life and crop stages and impact of volunteer cotton for overwintering hosts were also determined. Mealybug distribution patterns in the field and within plant were also established. Until detail sampling protocols are developed a preliminary assessment methods for searching a crops canopy are proposed. The mealybug parasitoid, *Aenasius Bambawalei*, which found very effective in overseas was identified at all mealybug infested areas. An insecticide Clap® (buprofezin) was found to be effective against mealybug.

Mirids: Required sample sizes at different precision levels for different mirid densities at squaring and flowering stages were determined. Distribution patterns of mirids in the field were confirmed. Mirid feeding behaviour was found to be influenced by temperature and an optimum temperature was determined. Identified an insecticide (Transform®) that can be mixed with salt to increase the efficacy of the chemical against mirids and reduce its impact on beneficials.

Pale cotton stainer: Action threshold for pale cotton stainer was proposed. An organism, *Pantoea agglomerans* was identified causing boll rot which was found to be transmitted by pale cotton stainer. It is worth to mention that this organism might transmit by other means as well which is not known yet.

These outputs will substantially contribute to the planned outcomes. For example, knowledge of different aspects of mealybug behaviour such as effects of overwintering hosts and volunteer cotton on establishment, distribution pattern in the field and within plant will improve industry knowledge to minimise mealybug impacts. Mirid sample sizes for different mirid densities, temperature related mirid feeding behaviour and an action threshold for pale cotton stainer bug will further improve grower and consultant decision making. This will facilitate more timely chemical applications. Use of salt mixtures will enable a further reduction in chemical use for mirid control. There will be follow on effects from this management approach. A reduced rate of chemical plus salt is comparatively softer than a broad-spectrum chemical, boosting survival of beneficials and reducing the likelihood of secondary pest outbreaks such as whitefly, aphids, and mealybugs.

5. 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

6. 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 on mealybug ecology such as overwintering strategy, impact of volunteer cotton mealybug ecology and understanding of their damage potential will improve grower and consultant knowledge of this new pest, helping to minimise their impacts on cotton crops through practicing farm hygiene.

The results of this project, such as a mirid sample sizes at different precision levels and an action threshold for pale cotton stainer bug, will help growers and consultants in taking management decision timely manner therefore will further improve the grower and consultant decision making process.

The take home messages are outlined below:

- All stages of mealybug can cause damage. Mealybug establishment on the cotton plants as adults causing more damage.
- The earlier in the life of a crop that mealybug establish on cotton the more damage they cause. Therefore any steps that a grower takes to minimise overwintering populations will impede re-infestation and reduce the potential for damage.
- Mealybug can infest any time of cotton's growth stage but usually start soon after sowing from alternate hosts.
- Mealybugs overwinter both as adults and nymphs on a wide variety of weed hosts in and around cotton fields. During winter months they move into the soil and live on the root zone of the hosts.
- Keeping fields clean after harvest through until next planting will reduce mealybug establishment in cotton.
- Volunteer cotton must be removed as they can initiate infestation of mealybug on cotton.
- Assessing the underside of leaves and inside bracts of squares and bolls of the top 10 nodes may give a reliable estimation of the mealybug presence in the field.
- The mealybug parasitoid *Aenasius bambawalei* was identified at all mealybug infested areas. In overseas this parasiotid is found to be very effective.
- An insecticide, Clap® (buprofezin) was found to be effective against mealybug.

- Sample size varied with precision level. The sample size at higher precision level will give less reliable estimate of the population. Therefore during sampling mirids in the field this need to be considered.
- The optimum temperatures for mirid feeding are 30 - 31°C. Within this temperature range mirids feed more than in any other temperature range suggesting temperature may play a role in the different rates of damage observed in the field for the same mirid density.
- The action threshold for pale cotton stainer is proposed as 3 adults per metre.
- An organism, *Pantoea agglomerans* was identified causing boll rot. During wet years the association with this organism causing boll rot increase substantially. Three flies were also identified as being associated with boll rot. These flies are *Bactrocera tryoni*, *Atherigona orientalis* and *Chloropsina sp. (*near *turneri)*.
- A low rate of Transform® (100 mL/ha) plus salt increased mortality by 16% 3 days after spraying compared to a low rate Transform® alone.

Extension Opportunities

7. 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.

A new research project has been funded which will further investigate into mealybug ecology and management. In this project investigation into mealybug incidence and population characterisation including distribution, dynamics, survival and inter-seasonal relationship has been planned. It is also planned to researching on treating hot spots.

There is a plan to publish the information generated from this project in the Australian Cotton Grower magazine. The project outcomes will further disseminate through grower meetings, field days, CCA meetings, farm walks and conferences. The principal researcher will also write scientific journal articles on research outcomes.

As much is still unknown about mealybug, future research needs to remain focussed on this pest. Specific factors affecting mealybug population dynamics need to be identified. How natural enemies and plant health (stress vs normal plant) affect mealybug populations requires further investigation. The potential for augmentation of key predators such as lacewings and Cryptolaemus lady beetles also need to be investigated. To understand how mealybugs spread in the field (through plants or by air) needs further investigation. Investigation is also necessary to understand from where they move to cotton. Chemicals evaluated against mealybug in this project were tested at high population levels and at late growth stages of the cotton crop. These chemicals need to be tested at low population levels and at earlier crop stages to realise their potential effectiveness. This information will assist with the development of management strategies for Solenopsis mealybug.

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., Byers, K. and Spargo, G. 2014. Solenopsis mealybug damage at different developmental stages of Bollgard® II cotton. *Proceedings of the 17th Australian Cotton Conference*, Gold Coast.
2. Khan, M., Byers, K. and Spargo, G. 2014. Evaluation of insecticides against solenopsis mealybug. *Proceedings of the 17th Australian Cotton Conference,* Gold Coast.
3. Khan, M., Byers, K. and Spargo, G. 2013. Understanding solenopsis mealybug damage on Bollgard II. *The Australian cottongrower*, 34 (5): 14 – 20.
4. Khan, M., Byers, K. and Spargo, G. 2013. Understanding solenopsis mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) damage and within

plant distribution on Bollgard cotton. *Proceedings of the inaugural Australian Cotton Research Conference*, Narrabri.

5. Spargo, G., Khan M. and Byers K. 2013. A parasitoid of solenopsis mealybug found at Emerald. *The Australian cottongrower*, 34 (2): 22- 23.
6. Khan M., Byers K., Bartlett J. and Pathania N. 2012. Queensland fruit fly and boll rot. *The Australian cottongrower*, 33 (3): 14-15.
7. Khan M. and Byers K. 2012. Evaluation of insecticides for controlling pale cotton stainer bug. *The Australian cottongrower*, 33 (4): 55-56.
8. Khan M., Miles M., Maas S., Byers K. and Spargo G. 2012. Overwintering strategy of solenopsis mealybug. *The Australian cottongrower*, 33 (6): 22-24.
9. Khan M., Quade A., Byers K. and Hall Z. 2012. Record of *Aenasius bambawalei* Hayat, a parasitoid of solenopsis mealybug, in Australia. *Proceedings of the 16th Australian Cotton Conference*, Gold Coast.
10. Khan M., Byers K., Maas S. and Spargo G. 2012. Cotton seed and boll rot-Queensland fruit fly may carry causal organism. *Proceedings of the 16th Australian Cotton Conference*, Gold Coast.

In addition, following paper was published into a refereed journal

Wilson L., Downes S., Khan M., Whitehouse M., Baker J., Grundy P. and Maas S. 2013. IPM in the transgenic era: A review of the challenges from emerging pests in Australian cotton systems. *Crop and Pasture Science*, 64 (8): 737 - 749.

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

Yes, two fact sheets on melaybug.

1. Solenopsis mealybug in Australia- an overview
   (http://thebeatsheet.com.au/wp-content/uploads/2012/06/SplenosisOverview.pdf)
2. Management strategies for solenopsis mealybug in the Australian cotton farming system
   (http://thebeatsheet.com.au/wp-content/uploads/2012/06/Solenopsis-in-Cotton.pdf)

Part 4 – Final Report Executive Summary

The research undertaken in this project has addressed three sucking pests of cotton: Solenopsis mealybug, mirids and pale cotton stainers. The main aim of this project was to provide research outcomes that underpin the successful implementation of IPM in cotton. As Solenopsis mealybug is a new pest objectives were from understanding their ecology and damage potential to exploring possible management options. For mirids investigations were made to determine sample sizes for mirids at different precision levels to further improve management dicision making and to understand if mirid feeding is affected by temperature. An action threshold for pale cotton stainers was developed. A summary of key findings is given below:

1. All stages of mealybug caused damage; however, mealybug establishment on the cotton plants as adults cause more damage than infestations spawned by juveniles. The level of damage caused by mealybug is directly linked to the timing of infestation

with earlier infestations giving rise to greater crop damage. Mealybug outbreaks late in a crops development is unlikely to cause major losses in yield.

2. Mealybugs overwinter both as adults and nymphs on a wide variety of weed hosts in and around cotton fields. During winter months they move into the soil and live on the root zone. Keeping fields clean after harvest until next planting will reduce mealybug establishment on cotton.
3. The mealybug parsitoid, *Aenasius bambawalei* was identified from all mealybug infested areas. In overseas this parasitoid is found to be very effective. Conserving this parasitoid along with key predators such as lacewings, Cryptolaemus and three banded lady beetles may key to successful management of mealybug.
4. An insecticide, Clap® (buprofezin) was found to be effective against mealybug.
5. Sample size varies with precision level. Higher the precision level the less reliable is population estimate. This need to take into consideration during mirid assessment in the field.
6. Temperature may play a role in the different rates of damage observed in the field for the same mirid density. The optimum temperature for mirid feeding was determined as 30 - 31°C. Within this range mirids fed more than in other temperature ranges.
7. The action threshold for pale cotton stainers was proposed as 3 adults per metre. For nymphs, the same calculations described in the Cotton Pest Management Guide for green vegetable bug nymphs may be applied.

Futher information:

Moazzem Khan
DAF, Toowoomba
07 46881310 or 0428600705