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FINAL REPORT 2007

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Part 1 - Summary Details

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NSW DEPARTMENT OF
PRIMARY INDUSTRIES

AUSTRALIAN COTTON RESEARCH INSTITUTE, NARRABRI

APPROVAL FOR SUBMISSION OF FINAL REPORT
(DAN 179C) - Dr Robert Mensah

Dr Robert Mensah has been granted approval to submit to Cotton Research and Development Corporation (CRDC) the attached final report "IPM in Bollgard cotton-New tools and strategies: A Farming: Systems Approach" (DAN 179C)

The report has been refereed by his peers and considered to be adequate.

Mr Graham Denney **Date:**
(Manager External Funding Unit)

DECLARATION

This report to the best of my knowledge contains no copy or paraphrase of materials previously published by any other person. The studies contained in this report were conducted by myself under the project Code DAN 179C funded by the Australian Cotton Research and Development Corporation (CRDC) and NSW Department of Primary Industries.

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Final Report Executive Summary

This report presents the experimental works and outcomes of my study on the project "**IPM in Bollgard**II® **cotton-New tools and strategies: A Farming: Systems Approach**" under the agreement between NSW Department of Primary Industries and Cotton Research and Development Corporation (Project DAN 179C). The project has refined current tools and developed new tools and strategies to complement beneficial insect's activities in support of IPM program on BollgardII® cotton crops as part of a continuous improvement to the industry's IPM system.

The aims of this project were:

1. Determine the efficacy of Petroleum spray oils (PSOs) as a stand alone product for activity against green mirids and aphids;
2. Determine the efficacy of PSOs as adjuvants for full and reduced label rates of synthetic insecticide for activity against *Helicoverpa* spp. and green mirids;
3. Assess the effect of PSO residues and sprays on the survival and rate of consumption of cotton pests by predatory insects in Bollgard cotton crops;
4. Determine and compare abundance of green mirids on Bollgard crops interplanted with and without lucerne strips or placed adjacent to lucerne blocks or refuge crops interplanted with lucerne strips;
5. Evaluate the efficacy of a new myco-insecticide for activity against green mirids on Bollgard cotton crops;
6. Determine strategic use of Magnet (moth) attractant on Bollgard II® cotton crops on the level of Helicoverpa spp. production and management on conventional cotton crops
7. Determine strategic use of Magnet (moth) attractant on Bollgard II® cotton crops and PSOs on conventional cotton crops on the level of *Helicoverpa* spp. production and management of Helicoverpa spp. on the conventional crops
8. Assist in the review of the cotton industry IPM guidelines.

To achieve these aims a series of laboratory, mesh house and large scale field experiments were conducted in irrigated commercial cotton crops in the lower, Gwydir and Macintyre valleys in New South Wales and Queensland between July 2004 and June 2007. Plot sizes ranged from 3 to 400 hectares.

In studies to assess the efficacy of Petroleum spray oils as a stand alone and in mixtures with reduced rates of synthetic insecticides, the results showed that application of 2% v/v PSOs by themselves can cause direct mortality to *Helicoverpa* spp. larvae particularly 1st -3rd instar stages and green mirid adults and nymphs. The addition of 2% v/v of either Biopest or Canopy® oil to ½ rate insecticides achieved similar control of *Helicoverpa* spp and green mirids as 1% PSO + full label rate insecticide and also the full label rate insecticides alone. In terms of conservation of predatory insects in the cotton cropping system, the application of PSO mixed with ½ label rate of insecticides minimised the impact of the insecticides on beneficial insects compared to the full label rate or 1% plus the full label rate of the insecticides. The prey consumption rate of the predators that survived PSO sprays alone or in combination with reduced rate of insecticides was not affected.

In the study to assess the use of trap crops to manage green mirids on BollgardII® cotton crops, the results showed that green mirids prefer sunflower and lucerne to cotton crops. However, sunflower dries off in January when cotton plants are in their peak squaring, flowering and boll setting stage. The drying of the sunflower crops will force green mirids to move to the cotton crops to cause damage and yield loss. In contrast, lucerne crop has a permanent growth and can maintain the mirids throughout the cotton growing season provided the lucerne is kept fresh by watering. The study showed that lucerne crop can trap and manage green mirid numbers in adjacent BollgardII® cotton crop to a distance of 250-300 metres away from the lucerne crop.

One of the most significant and major breakthroughs in this project is the development of two fungal insecticides (BC 639-*Metarhizium* spp and BC667 – *Beauveria* spp), for the control of green mirids, Helicoverpa and aphids. The result of the study showed that application of 0.5-1.0L/ha of either fungus can cause over 70% mortality of green mired adults and nymphs. In most field trials, application of BC639 and BC667 at rates of 0.5-1.0 L/ha reduced densities of green mirid adults and nymphs from 3.5 per metre to zero within 7 days after treatment. The efficacy of the fungal insecticides applied at these rates against green mirid adults and nymphs was the same as Fipronil applied at the label rate.

The fungus killed green mirids within 3-4 days after application. The fungus is self-perpetuating and can cause secondary infection to green mirids whereby the death of infected mirid or insect can produce spores which will then infect and kill live insects. The fungus is effective against soft bodied insects (mostly pests) so that hard bodied insects (mostly key predatory insects) are often saved. In field trials, I found no significant differences between the number of predatory beetles, bugs, lacewings and spiders per metre in plots treated with fungal insecticides and unsprayed (control) plots. In contrast, the number of predators recorded on plots treated with the fungal insecticides was significantly higher than the commercial insecticide (Fipronil). This indicates that the fungal insecticide is "softer" than Fipronil.

A Commercial partner has been identified and a commercial agreement between the Cotton Research and Development Corporation, NSW Department of Primary Industries and the Commercial partner will be signed on 29 February 2008.

In studies to determine the strategic use of Magnet® (moth attractant) mixed with insecticides to attract and kill Helicoverpa moths on BollgardII® cotton crops, the study showed that the Magnet® formulations killed *Helicoverpa* spp. (moths) resulting in reduction of Helicoverpa moth populations on adjacent conventional cotton crops located 0.5 to 1 km away from the "treated" BollgardII® cotton crops. This strategy resulted in a pest control saving of $11.40 per ha in the "treated" over the "untreated" conventional cotton crops.

The study also showed that application of Magnet® formulation onto the Bollgard cotton crops attracted moths from the environment to the treated area and the residual moths that could not reach the Magnet® treated zone before the Magnet® odour dissipated stayed in the conventional cotton crop that was closest to the "treated" BollgardII® crops and laid eggs. By applying Petroleum spray oil (PSO) on the conventional cotton crops, the number of Helicoverpa moths, eggs and larvae per metre on the conventional cotton crops treated with PSO was significantly lower than the conventional cotton crops not treated with the PSO. The explanation given was that application of PSO on the cotton plants suppressed the quantity of airborne volatiles released by the cotton plants resulting in lower egg and larval numbers on the PSO-treated plants.

The project also provided information and assisted in the review of the Cotton Industry IPM guidelines in 2005. The IPM guidelines was launched by the Hon. John Anderson MP, and Deputy Prime Minister of Australia on 18th February 2005. New information on the use of PSO in managing pests on cotton, trap cropping and predator to beneficial insect ratio has been incorporated into the guidelines.

Overall, the project has developed two new IPM tools (BC639- *Metarhizium* spp. and BC 667 – *Beauveria* spp) for the control of green mirids, Helicoverpa spp. and aphids. The project has also developed other alternative pest control tools (lucerne strips, Petroleum spray oils - as stand alone and adjuvavants with reduced label rates of insecticides) as additional tools for the industry's IPM program. The project has also developed management guidelines for use of lucerne strips, PSOs and Magnet (moth) attractants for cotton growers to manage both their Bollgard and conventional cotton crops. Thus, the project has addressed the issue of sustainable use of pesticides in the cotton industry.

The project has linked with Industry Development Officers (IDOs), reseachers and consultants to provide information and refine the cotton IPM guidelines.

In conclusion, the project has been very successful in developing two new biopesticides (fungi) that can be used by cotton growers to control green mirids, *Helicoverpa* spp. and aphids. Other major project outcomes include enhancement of IPM programs on both transgenic and conventional cotton, reduction of insecticide sprays, delay insect resistance to synthetic insecticide and transgenic cotton and improve profitability, competitiveness and sustainability of the cotton industry.

Full Report

Background

1. Background to the project.

Cotton crops are attacked by a wide range of pests the major one of which has been *Helicoverpa* spp. With the commercial release of BollgardII® cotton crops in the Australian cotton production system, sucking pests such as *Creontiades dilutus* (green mirid) and *Aphis gossypii* (cotton aphid) have emerged as major pests of cotton because they are unaffected by the toxin in the BollgardII® cotton. In fact, the reduction in the use of synthetic insecticide sprays against *Helicoverpa* spp. early in the cotton season as a result of the introduction of BollgardII® cotton crops may unwittingly result in a significant increase in green mirids and aphid populations to economically important levels.

A preliminary survey conducted by consultants on insecticide usage on BollgardII® cotton crops during the 2003-04 cotton season showed that growers applied an average of about 1 Fipronil spray against green mirids. If the reliance on this insecticide continues at this rate, green mirids may develop resistance. In addition, some insecticides will disrupt beneficial insect's activity and flare up other pests such as two-spotted mites and aphids. Furthermore, when the cost of new synthetic insecticides effective against green mirids and/or aphids is added to the price of the BollgardII® licence, grower gross margins may be reduced. Cost could encourage the use of cheaper broadspectrum alternatives, thus reversing the recent progress made by the industry which has had transgenic cotton adoption improved IPM practices.

As a result, cotton growers will need new research in order to maintain integrated pest management (IPM) systems based on alternative pest control tools that complement the activities of beneficial insects on BollgardII® cotton crops. BollgardII® cotton, by itself, is a technology that will provide cotton growers with the necessary platform to conserve and utilise beneficial insects and complement IPM program. Nevertheless, the success of IPM programs in BollgardII® cotton cropping systems will depend on the availability of IPM compatible tools and strategies. Subsequently, it is crucial for new IPM compatible tools to be developed for the cotton industry. The project has developed new tools and refined current tools and strategies to complement beneficial insect's activities in support of IPM program on BollgardII® cotton crops as part of a continuous improvement to the industry's IPM system. Specifically, the project has assessed the following objectives:

1.1 *Objectives*

1. Determine the efficacy of Petroleum spray oils (PSOs) as a stand alone product for activity against green mirids and aphids;
2. Determine the efficacy of PSOs as adjuvants for full and reduced label rates of synthetic insecticide for activity against *Helicoverpa* spp. and green mirids;
3. Assess the effect of PSO residues and sprays on the survival and rate of consumption of cotton pests by predatory insects in BollgardII® cotton crops;
4. Determine and compare abundance of green mirids on BollgardII® crops interplanted with and without lucerne strips or placed adjacent to lucerne blocks

5. Evaluate the efficacy of new myco-insecticide for activity against green mirids on BollgardII® cotton crops;
6. Determine strategic use of Magnet® (moth) attractant on Bollgard II® cotton crops and PSO on conventional cotton crops on the level of *Helicoverpa* spp. production on conventional cotton crops;
7. Develop a management option for PSOs and Magnet against Helicoverpa and green mirids on conventional cotton
8. Assist in the review of the cotton industry IPM guidelines.

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

2.1. Norwood trial, 2004-05

2.1a *Determine the efficacy of Petroleum spray oils (PSOs) as a stand alone product for activity against Helicoverpa spp. and green mirids*

2.1b *Determine efficacy of PSOs as adjuvants for full and reduced label rates of synthetic insecticide for activity against Helicoverpa* spp. *and green mirids.*

The objectives of this study were (1) to determine the level of *Helicoverpa* spp. and green mirid control achieved by different concentrations of PSO in combination with insecticides and (2) to determine the impact of different concentrations of PSO in combinations with insecticides on densities of beneficial insects in cotton (3) to compare the yield and gross margins of cotton crops managed with and without PSOs in combination with synthetic insecticides.

2.1.1 Methodology

The experiment was conducted on a 71.1 ha irrigated commercial cotton field at Norwood near Moree. The cotton variety used for the experiment was Sicot 71. The field was sown on the 20 September 2004 at a rate of 12 plants per metre with row spacing of 1 m. The PSOs used for the studies were Canopy® (an emulsified *n*C27 PSO) supplied by Caltex Australia Pty Ltd. and Biopest oil (an emulsified *n*C24 PSO) supplied by Spray Adjuvants Company of Australia (SACOA) Pty Ltd. The commercial insecticides used for the study were Endosulfan 350EC (Endosulfan 350g/L) and Folimat 800 (Omethoate 800g/L)

The PSOs were evaluated at 1 and 2.0% v/v (Mensah *et al* 2001; Mensah *et al* 2004) in mixtures with half (½) or full label rate synthetic insecticides applied to cotton crops against *Helicoverpa* spp. and green mirids. The treatments evaluated were (1) 2% v/v Canopy® + ½ rate insecticides (2) 2% v/v Biopest + ½ rate insecticides (3) 1% v/v Canopy® + full rate insecticides (4) 1% v/v Biopest + full rate insecticides (5) 2% v/v Canopy® oil alone (6) 2% v/v Biopest oil alone (7) Full rate insecticide alone (treated control) and (8) Unsprayed (untreated control). Plots were arranged in a randomised complete block design with 6 replicates. Each treatment replicate measured 1.48 ha.

Foliar application of each treatment was made on 14 December 2004 and 19 December 2004 (Table 1). The decision to apply these treatments was based on a predator-to-*Helicoverpa* spp. (pest) ratio of 0.5 (Mensah 2002a; Mensah 2002b). The decision to spray against green mirids was made based on the IPM Guidelines and CottonLogic recommended economic threshold of 0.5 green mirids per metre (CottonLogic 1999).

Table 1 *Insecticides applied as indicated to cotton crops within the study site at Norwood near Moree in New South Wales, 2004-05.*

Insecticide treatment	Insecticides were applied with:	Date applied
Half label rate: 1. 05L/ha Endosulfan 0.14L/ha Omethoate	2% Canopy® oil or 2% Biopest or no PSO	 14/12/2004 19/12/2004
Full label rate: 2. 1L/ha Endosulfan 0.28L/ha Omethoate	1% Canopy® oil or 1% Biopest or no PSO	 14/12/2003 19/12/2003

Visual counts of *Helicoverpa* spp. on cotton plants in each treatment were made at approximately weekly intervals in two randomly selected 1 metre lengths of row of each treatment replicate, *i.e.* a total of 8 metres were examined per treatment. Counts were separated into *Helicoverpa* spp. eggs, very small and small (VS+S) larvae and medium and large (M+L) larvae. Data were expressed as numbers per metre and numbers per metre per sample date for each treatment.

Green mirids and beneficial insects were sampled weekly using a D-vac suction tube (Mensah 1997, Mensah and Khan, 1997; 1999). On each sampling occasion, a 20-metre long sample was taken from a single pass of the vacuum tube along the tops of the plants. After each sampling, the contents of the sampler were transferred to separate plastic bags, taken to the laboratory and counted. Data was expressed separately for green mirids, predatory beetles, predatory bugs, predatory lacewings and spiders. Each insect group was expressed as numbers per metre and numbers per metre per sampling date.

Sampling was discontinued on the 12 January 2005 because the crop was hailed off. Hence, the whole crop was slashed by the farmer and no yield data was taken.

2.1.2 *Analysis of the data*

All experimental data were transformed by (X + 0.5) and analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple Comparisons tests were used to separate means. Arithmetic, rather than transformed means are given in the results.

2.1.3 *Results*

2.1.3.1 *Helicoverpa* spp.

Helicoverpa spp. egg and larvae data for this trial are given in Figure 1. The untreated (control) are the only plots that had significantly higher *Helicoverpa* spp. eggs and larvae in them over the season than the other treated plots (Figure 1). All other plots treated with an oil/insecticide mixture had a statistically similar number of *Helicoverpa* spp. eggs and larvae.

Plots treated with 2% v/v PSO alone recorded similar numbers of *Helicoverpa* eggs and larvae per metre per sample date as the oil and insecticide combinations (Figure 1).

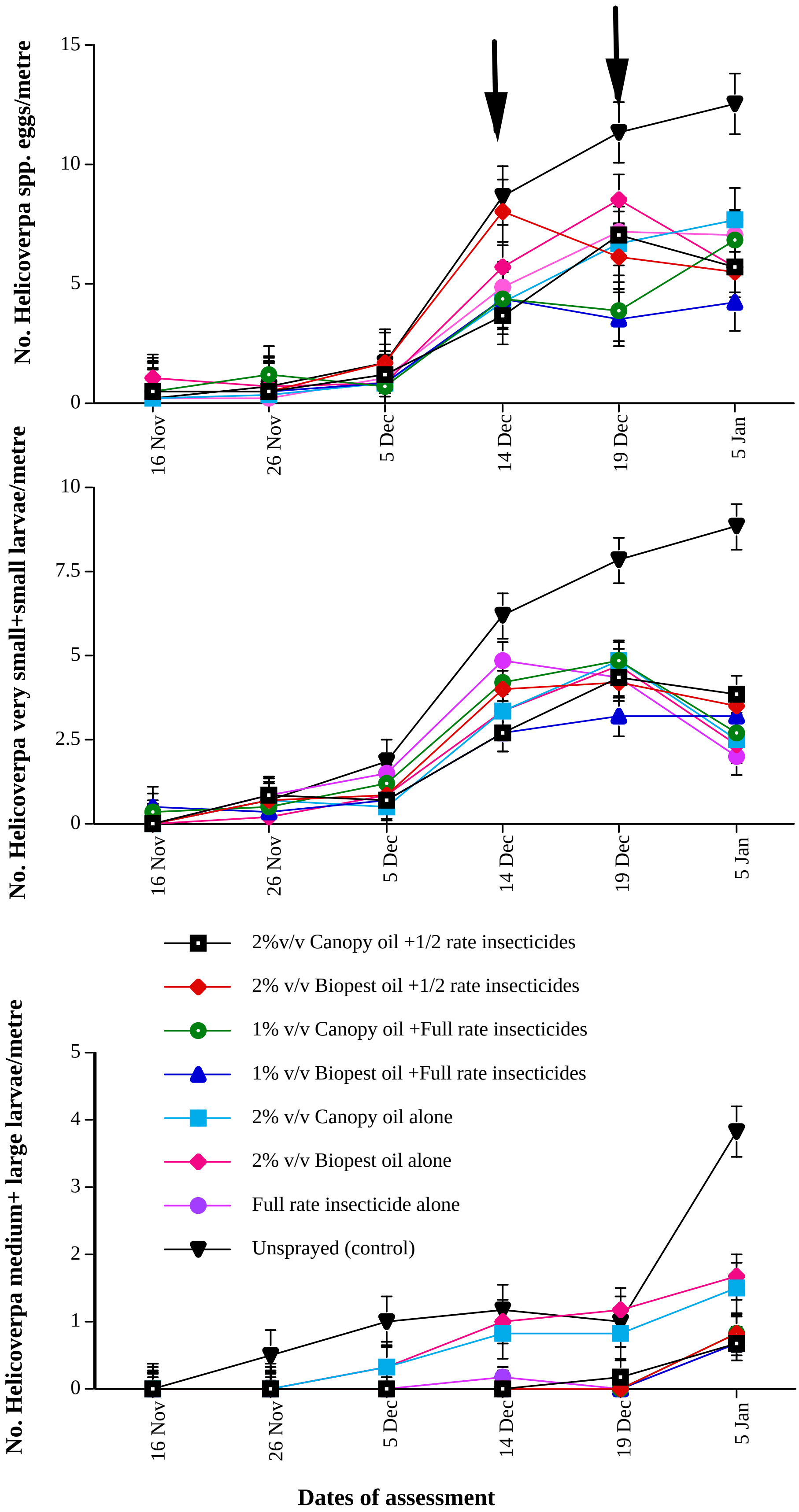


Figure 1. *Number of Helicoverpa spp. eggs, very small and small larvae and medium and large larvae per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy® PSOs, at Norwood near Moree, 2004-05.*

2.1.3.2 *Green mirid adults and nymphs*

Approximately equal numbers of green mirids (adults + nymphs) per metre per sample date were found in plots treated with 2% Biopest + ½ rate insecticides (0.14), 2% Canopy® + ½ rate insecticides (0.19), 1% Biopest + full rate insecticides (0.31), 1% Canopy® + full rate insecticides (0.14), 2% v/v Biopest alone (0.29), 2% Canopy® alone (0.29) and full rate insecticides alone (0.21) (Figure 2). However, the number of green mirids per metre per sample date recorded in the untreated plots (0.60) was significantly higher (P<0.001) than the other treatments (Figure 2).

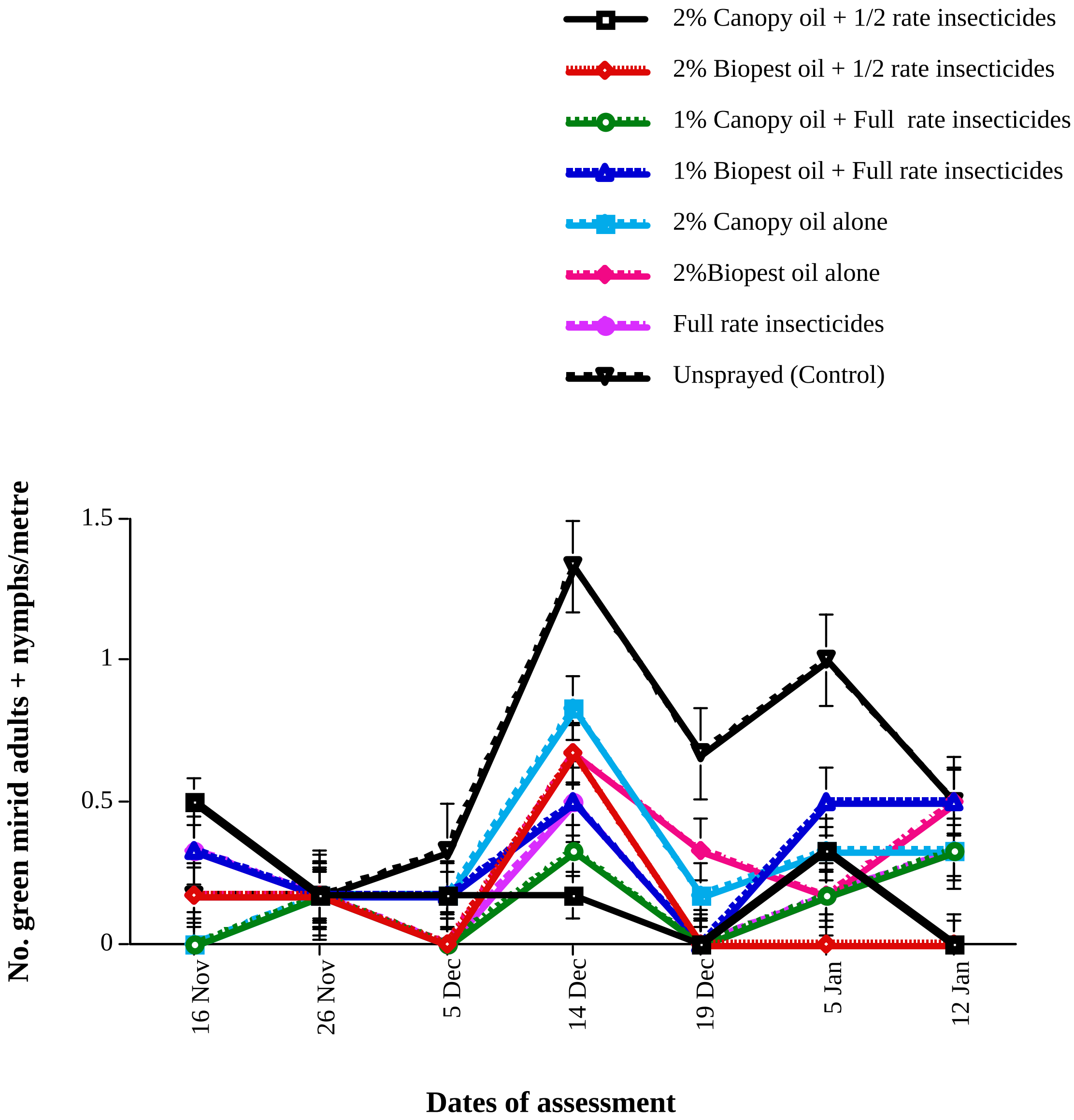


Figure 2. *Number of green mirids adults and nymphs per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2004-05.*

2.1.3.3 *Beneficial insects*

The number of predatory beetles recorded in plots treated with full label rate insecticides were significantly lower (P<0.001) than plots treated with ½ label rate insecticides mixed with 2% PSOs and the untreated control plots (Table 2). No significant difference (P>0.05) was detected on plots treated with full label rate insecticides alone and full label rate insecticides mixed with 1% PSOs (Table 2). The number of predatory beetles recorded in the untreated control plot was not significantly different from plots treated with PSOs alone, ½ label rate insecticides mixed with 2% PSOs (Table 2). Overall, approximately 2.69 and 2.56 times more predatory beetles were recorded in plots treated with ½ label rate insecticides mixed with 2% PSOs than full label rate insecticides alone (Table 2). Furthermore, plots treated with 2% PSOs had 3.00 times more predatory beetles than plots treated with full label rate insecticides (Table 2).

The number of predatory bugs recorded in plots treated with full label rate insecticides were significantly lower (P<0.001) than plots treated with ½ label rate insecticides mixed with 2% PSOs, full label rate insecticides mixed with 1% PSOs and untreated control plots (Table 2). No significant difference was detected between plots treated with PSOs alone and full and ½ label rates insecticides mixed with 1 and 2% PSOs (Table 2). In the case of predatory lacewings, significant differences were found between the treatments and unsprayed (control) plots (Table 2). In addition, the number of predatory lacewings recorded on plots treated with 1% and 2% PSOs alone and full and ½ label rate of insecticides mixed with PSOs were significantly higher than plots treated with full rates of insecticides (Table 2). In contrast, the number of spiders per metre per sample date was not significantly different (P>0.05) among treatments (Table 2).

Table 2. *Number of predators per metre per sample date in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2004-05.*

Treatments	Predatory beetles/m/ sample date	Predatory bugs/m/ sample date	Predatory lacewings/m/sample date	Spiders
2% Biopest oil + ½ rate insecticides	2.48 ± 0.27 a	0.67 ± 0.13 a	0.38 ± 0.06 a	5.89 ± 0.58 a
2% Canopy oil + ½ rate insecticides	2.24 ±0.34 a	0.67 ± 0.09 a	0.39 ±0.08a	7.31 ± 0.89 a
1% Biopest oil + Full rate insecticides	1.50 ±0.29 ab	0.81 ± 0.16 a	0.35 ±0.06ab	7.88 ±2.08 a
1% Canopy oil + Full rate insecticide	1.52 ±0.22 ab	0.60 ± 0.10 a	0.34 ±0.07ab	5.91 ±0.57 a
2% Biopest oil alone	2.71 ±0.29 a	0.60 ± 0.09 a	0.38 ±0.08a	6.12 ±0.56 a
2% Canopy oil alone	2.79 ±0.29 a	0.67 ± 0.12 a	0.45 ±0.08 a	7.05 ±0.76 a
Full rate insecticides	0.93 ±0.16 b	0.26 ± 0.07 b	0.11 ±0.07 b	5.33 ±0.53 a
Unsprayed (control)	2.87 ± 0.29 a	0.88 ± 0.13 a	0.59 ±0.09 a	7.86 ±0.51 a

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

2.1.3.4 Conclusion:

- No significant differences were detected in the number of *Helicoverpa* spp. and green mirids per metre recorded on plots treated with 2% v/v PSOs mixed with ½ rate synthetic insecticides, 1% v/v PSO mixed with Full rate insecticides and full rate insecticides alone.
- The number of predatory beetles, bugs and lacewings recorded on cotton crops treated with 2%v/v PSO + ½ rates of insecticides were not significantly different from the unsprayed plot but was significantly higher than plots treated with full rate insecticides alone.
- The number of predatory beetles, bugs and lacewings recorded on plots treated with 1%v/v PSO+ Full rate insecticides was not significantly different from the full rate insecticides alone.
- No significant difference was detected among treatments on the number of spiders per metre.

2..2 *Norwood trial, 2006-07*

2.2 ***Determine efficacy of different concentrations PSOs as adjuvants for full and reduced label rates of synthetic insecticide for activity against Helicoverpa* spp. *and green mirids.***

2.2.1 Methodology

This trial was undertaken in 2006-07 season because the trial conducted in 2004-05 trial was destroyed by an hailstorm and in the 2005-06 season, not enough conventional cotton was planted by the farmer for the trial. The experiment was set up in an irrigated commercial conventional cotton crops (Field 3: Size 53.5ha) at Norwood near Moree. The objectives of the study was to (1) determine the level of *Helicoverpa* spp. control achieved by different concentrations of PSO in combination with insecticides and (2) determine the level of green mirids control achieved by different concentrations of PSO in combination with insecticides (3) the impact of different concentrations of PSO in combinations with insecticides on beneficial insects in cotton and (4) compare the yield and gross margins of cotton crops managed with and without PSOs in combination with synthetic insecticides.

The PSO products used for the studies were Canopy® and Biopest oil. The commercial insecticides used for the study were Endosulfan 350EC (Endosulfan 350g/L), Affirm (Emamectin benzoate 17g/L EC), Steward (Indoxacarb 200g/L),Ovasyn (Armitraz 200g/L EC), Intruder (Acetamiprid 225g/L SL), Scud (Cypermethrin 200g/L EC) and Wizard (Abamectin 18g/L EC) (Table 3).

The PSOs were evaluated at 1 and 2.% v/v in mixtures with half (½) or full label rate synthetic insecticides applied to cotton crops against *Helicoverpa* spp. and green mirids. The treatments evaluated were (1) 2% v/v Canopy + ½ rate insecticides (2) 2% v/v Biopest + ½ rate insecticides (3) 1% v/v Canopy + full rate insecticides (4) 1% v/v Biopest + full rate insecticides (5) 2% v/v Canopy oil alone (6) 2% v/v Biopest oil alone and (7) Full rate insecticide alone (treated control). Plots were arranged in a randomised complete block design with 6 replicates. Each treatment replicate measured 1.48 ha.

Foliar application of each treatment was made on 14 December 2004 and 19 December 2004 (Table 3). The decision to apply these treatments was based on a predator-to-*Helicoverpa* spp. (pest) ratio of 0.5. The decision to spray against green mirids was made based on the IPM Guidelines and CottonLogic recommended economic threshold of 0.5 green mirids per metre.

Table 3. *Insecticides applied as indicated to cotton crops within the study site at Norwood near Moree in New South Wales, 2006-07.*

Insecticide treatment	Insecticides were applied with:	Date applied
Half label rate:		
1.05L/ha Endosulfan	2% Canopy or 2% Biopest or no PSO	8/12/2006
0.55L/ha Affirm		
0.43L/ha Steward		7/1/07
0.43L/ha Steward		23/1/07
0.3 L/ha Agrimec		9/2/07
		22/2/07
Full label rate:		
2.1L/ha Endosulfan	1% Canopy or 1% Biopest or no PSO	8/12/2006
0.85L/ha Steward		7/1/07
0.70 L/ha Affirm		23/1/07
0.85L/ha Steward + 1L/ha Ovasyn + 0.1L/ha Intruder + 0.060L/ha Pulse		9/2/07
0.500L/ha Alpha Scud Elite + 0.300L/ha Wizard + 0.030L/ha Wetter		22/2/07

Visual counts of *Helicoverpa* spp. on cotton plants in each treatment were made at approximately weekly intervals in two randomly selected 1 metre lengths of row of each treatment replicate, *i.e.* a total of 8 metres were examined per treatment. Counts were separated into *Helicoverpa* spp. eggs, very small and small (VS+S) larvae and medium and large (M+L) larvae. Data were expressed as numbers per metre and numbers per metre per sample date for each treatment.

Green mirids and beneficial insects were sampled weekly using a D-vac suction tube. After each sampling, the contents of the sampler were transferred to separate plastic bags, taken to the laboratory and counted. Data was expressed as number of green mirids per metre and numbers per metre per sampling date.

Cotton in each treated plot was harvested separately using a four-row picker at the end of the season and the average lint yields (bales/ha) were compared between treatments. The gross margin for each treatment was calculated from the lint yield taking into consideration total pesticide (including PSOs) spray costs (namely number of insecticide sprayed + cost of insecticides + insecticide application costs), since all the other farm management or agronomic inputs in each treatment were the same and applied by the farmer.

2.2.2 *Analysis of the data*

All experimental data were transformed by (X + 0.5) and analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means. Arithmetic, rather than transformed means are given in the results.

2.2.3 *Results*

2.2.3.1 *Helicoverpa* spp.

Helicoverpa spp. egg, very small, small, medium and large larvae are given in Figure 3. The only treated plots that had significantly fewer (P>0.005) *Helicoverpa* spp. eggs per metre per sample date over the season than the full rate of insecticides applied alone (1.54 eggs/m/sample date) were the 2% v/v Canopy + ½ label rate (1.06) and 2% Biopest + ½ label rate insecticide (1.29) treated plots. All other plots treated with an oil/insecticide mixture had a statistically similar number of *Helicoverpa* spp. eggs per metre per sample date: the full label rate of insecticides alone (1.54), 1% v/v Canopy + Full rate insecticides (1.19) and 1% v/v Biopest + full label rate insecticides (1.54) (Figure 3).

Approximately equal numbers of very small and small (VS+S) larvae were found in all plots treated with insecticides mixed with a PSO (Figure 3). The number of VS+S larvae per metre per sample date recorded in plots treated with full label rate insecticides (0.68) were not significantly different (P>0.05) from plots treated with ½ label rate insecticides mixed with PSOs. Similarly, full label rate insecticides mixed with either 1% v/v Biopest or Canopy were not significantly different (P>0.05) from full label rate insecticides alone.

The number of medium and large (M+L) larvae recorded in plots treated with full label rate insecticides were the same as plots treated with full label rate insecticides mixed with 1% PSOs and ½ label rate insecticides mixed with 2% PSOs (Figure 3). The number of M+L larvae per metre per sample date recorded in plots treated with ½ label rate insecticide mixed with 2% PSOs and Full rate insecticides mixed with 1% PSOs were the same in all the treatments tested.

2.2.3.2 Green mirid adults and nymphs

Significantly fewer (P<0.007) numbers of green mirids (adults + nymphs) were found in plots treated with 2% Canopy + ½ rate insecticides (0.04) and 1% Canopy + full rate insecticides (0.05) than the full rate insecticides alone (0.18) (Figure 4). Approximately equal numbers of green mirids (adults + nymphs) were found in plots treated with 2% Biopest + ½ rate insecticides (0.12), 2% Canopy + ½ rate insecticides (0.04), 1% Biopest + full rate insecticides (0.17) and 1% Canopy + full rate insecticides (0.05) (Figure 4).

2.2.3.3 Beneficial insects

Approximately equal number of spiders per metre per sample date was found in all plots treated with insecticides mixed with PSOs and full rates insecticides alone (Figure 5).

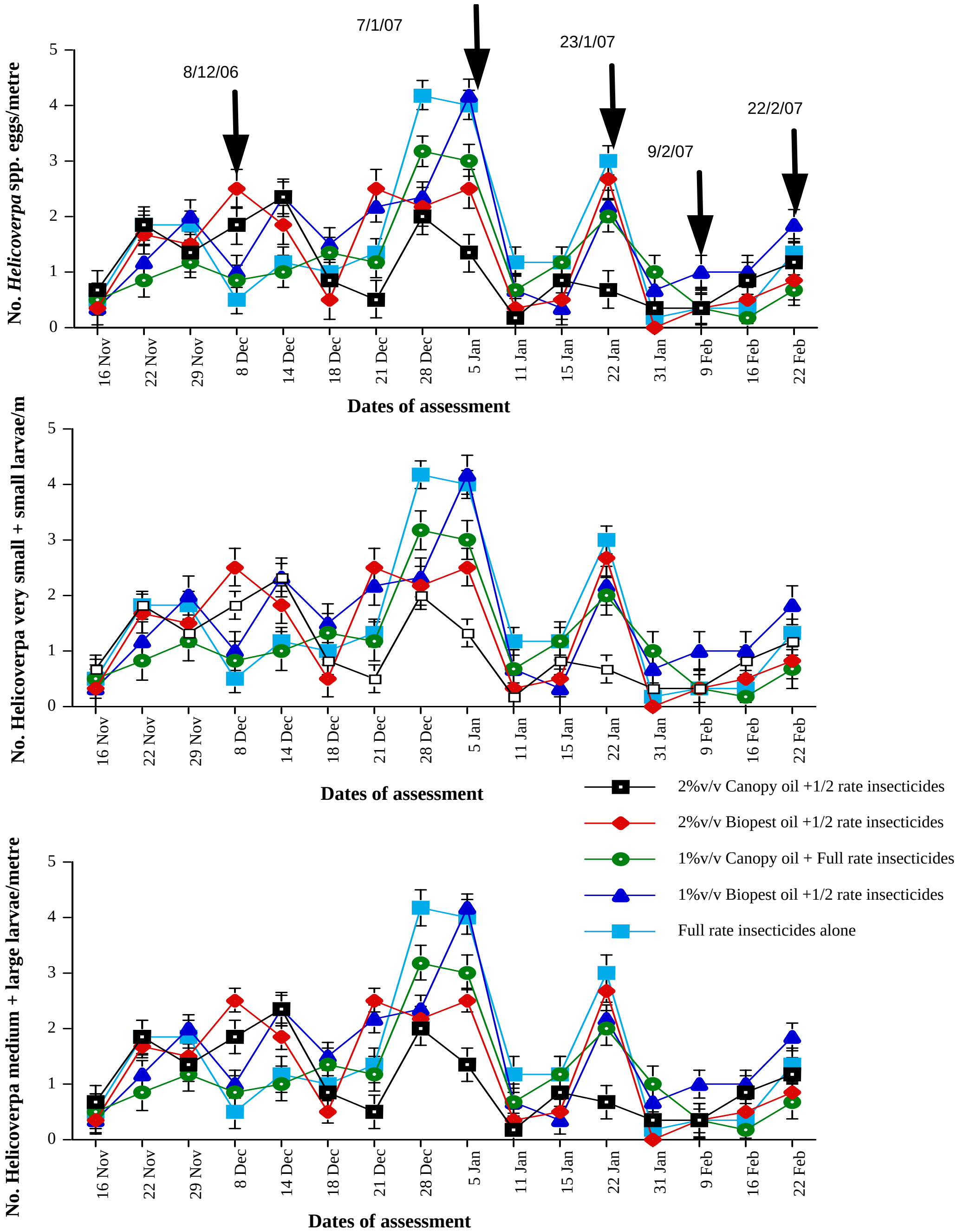


Figure 3. *Number of Helicoverpa spp. eggs, very small and small larvae and medium and large larvae per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2006-07.*

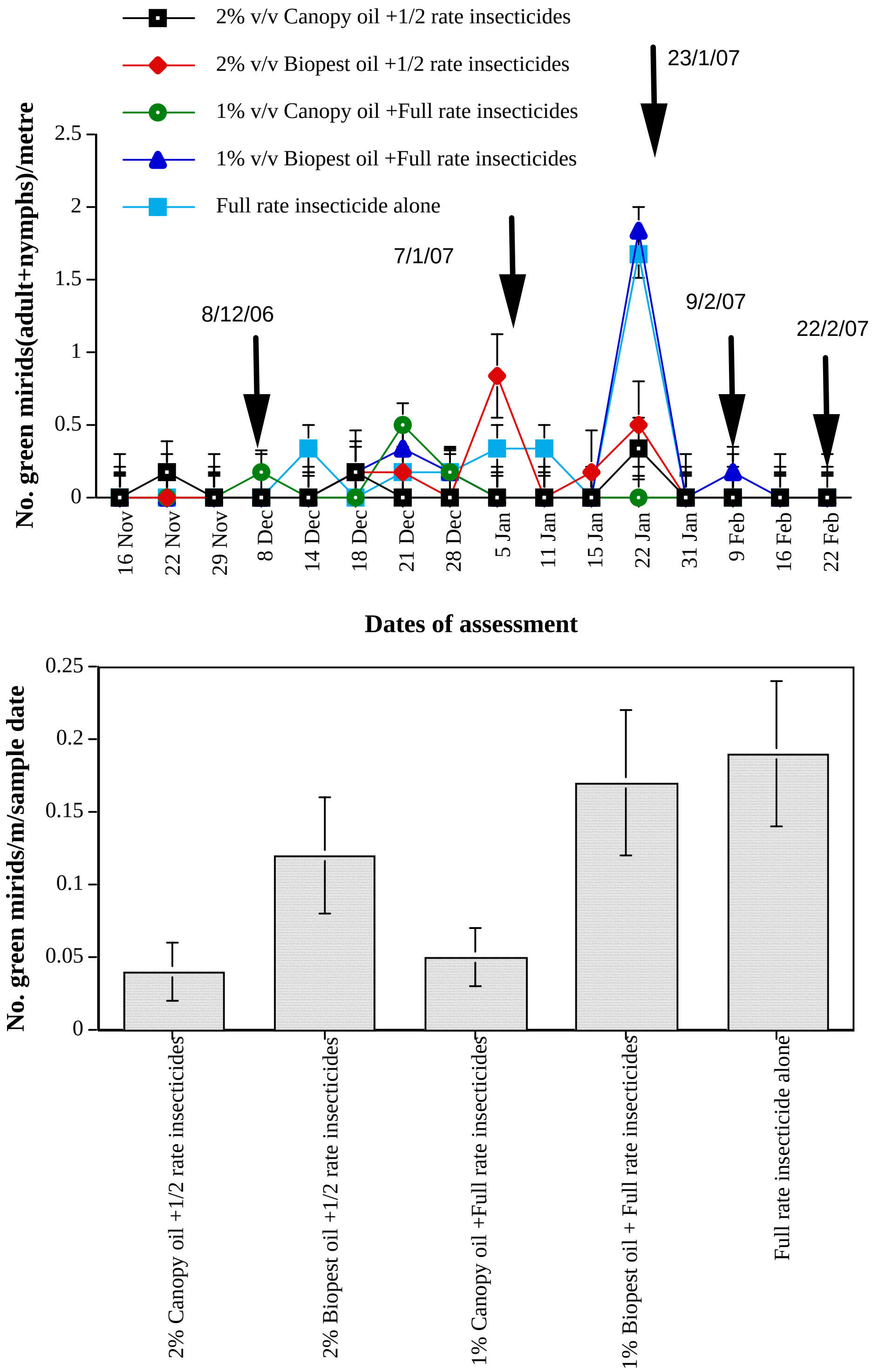


Figure 4. *Number of green mirids adults and nymphs per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2006-07.*

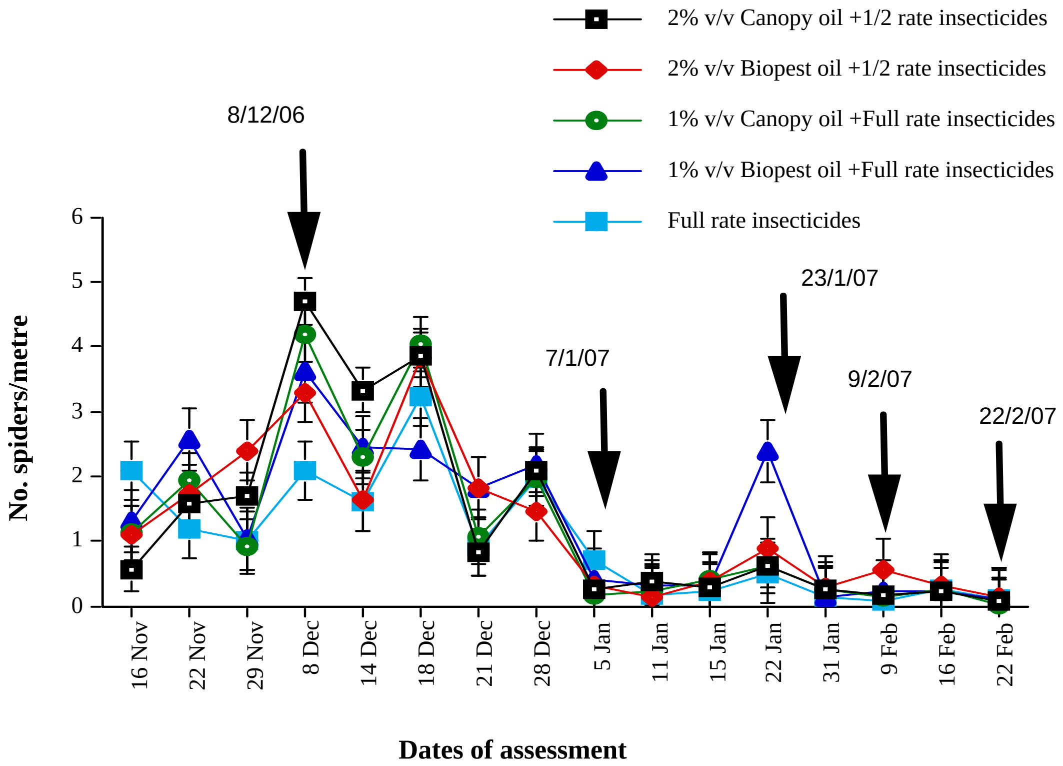


Figure 5. *Number of predatory beetles, bugs and lacewings per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2006-07.*

Predatory beetles, bugs and lacewings data for this trial are given in Figure 6. The only treated plots that had significantly higher numbers (P<0.03) of predatory beetles over the season than the full rate of insecticides applied alone (0.28) were the full label rate of insecticides mixed with 1% v/v Canopy (0.40) and Biopest (0.34). All other plots treated with 2% v/v PSOs + ½ insecticides had a statistically similar number of predatory beetles as the full label rate of insecticides alone (0.28).

Significantly higher (P<0.001) numbers of predatory bugs were found in all plots treated with ½ label rate of insecticides mixed with 2% v/v PSOs (Figure 6). The number of predatory bugs per metre per sample date recorded in plots treated with ½ label rate insecticides mixed with 2% v/v PSOs was significantly different (P<0.001) from plots treated with Full label rate insecticides alone and full label rate of insecticides mixed with 1% v/v PSOs. Overall, approximately 2.09and 2.18 times more predatory bugs were recorded in plots treated with 2% v/v Canopy oil + ½ rate insecticide and 2% v/v Biopest oil + ½ rate insecticides than in plots treated with full rate insecticides alone, respectively.

The number of predatory lacewings recorded in plots treated with full label rate insecticides were the same as plots treated with full label rate insecticides mixed with 1% PSOs but significantly different (P<0.04) from ½ label rate insecticides mixed with 2% PSOs (Figure 6).

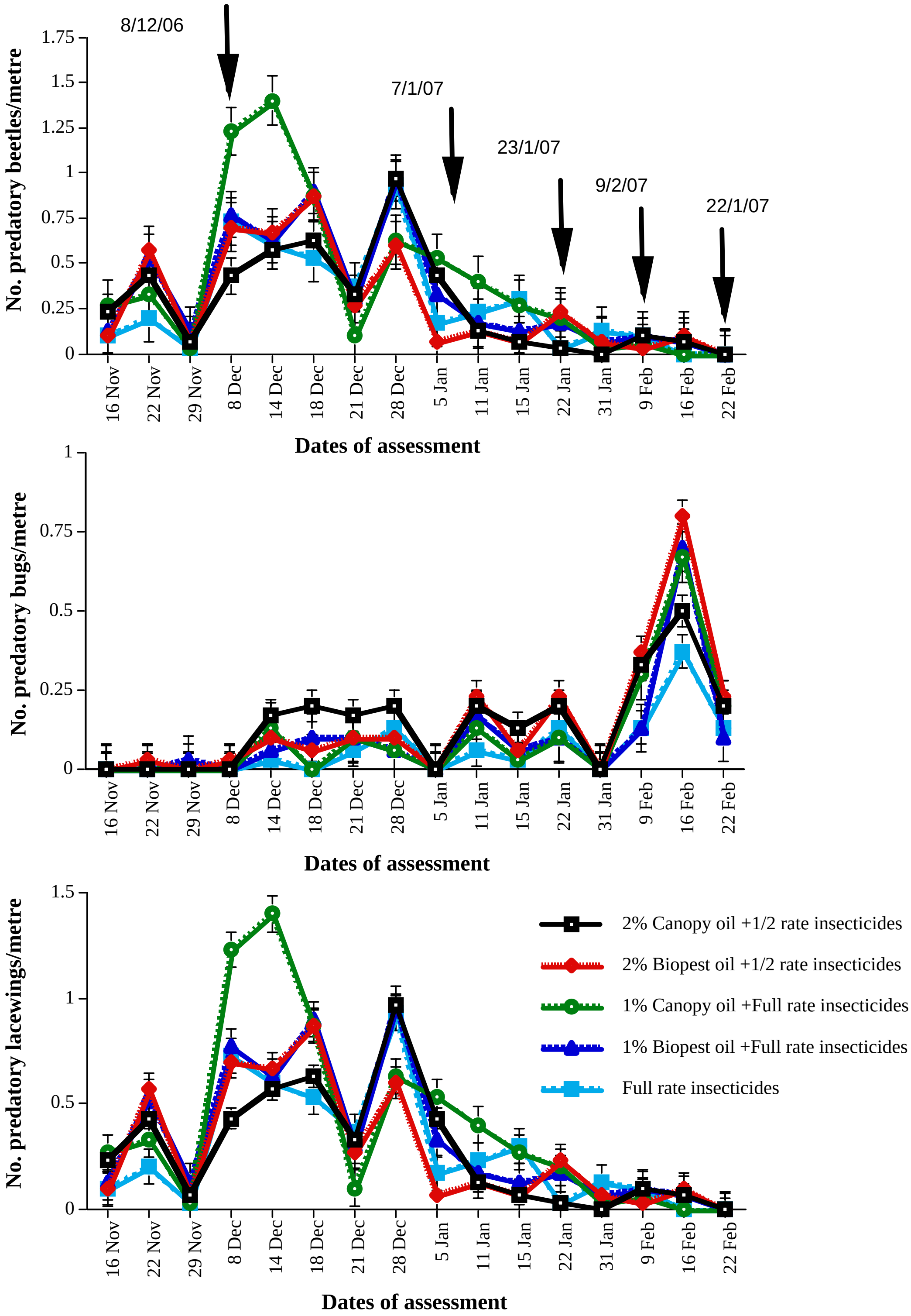


Figure 6. *Number of predatory beetles, bugs and lacewings per metre in commercial cotton crops managed with full and half (½) label rates of synthetic insecticides in combinations with Biopest and Canopy PSOs, at Norwood near Moree, 2006-07.*

Table 4. Cotton yield (bales/acre) harvested from commercial cotton crops managed with PSO and insecticide mixture at the Australian Cotton Research Institute in Narrabri, 2006-07.

Treatments	Yield (bales/acre)
2%v/v Canopy oil + ½ rate insecticides	4.60 ± 0.08 a
2%v/v Biopest oil + ½ rate insecticides	4.49 ± 0.12 a
1%v/v Canopy oil + Full rate insecticides	4.57 ± 0.09 a
1%v/v Biopest oil + Full rate insecticides	4.53 ± 0.18 a
Full rate insecticide alone	4.39 ± 0.16 a

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

2.2.3.4 Conclusion

- The only treated plots that had significantly fewer *Helicoverpa* spp. eggs per metre per sample date over the season than the full rate of insecticides applied alone eggs/m/sample date) were the 2% v/v Canopy + ½ label rate (1.06) and 2% Biopest + ½ label rate insecticide treated plots.
- All other plots treated with an oil/insecticide mixture had a statistically similar number of *Helicoverpa* spp. eggs per metre per sample date: the full label rate of insecticides alone, 1% v/v Canopy + Full rate insecticides (1.19) and 1% v/v Biopest + full label rate insecticides
- Approximately equal number of Helicoverpa spp. vs+S and M+L larvae per metre per sample date were found in all treatments
- Significantly fewer (P<0.007) numbers of green mirids (adults + nymphs) were found in plots treated with 2% Canopy + ½ rate insecticides and 1% Canopy + full rate insecticides than the full rate insecticides alone and all insecticide treatments mixed with Biopest oil

3.0 Australian Cotton Research Institute trials, 2005-06

3.1 *Assess the effect of PSO residues and sprays on the survival and rate of consumption of cotton pests by predatory insects in Bollgard II cotton crops;*

PSOs are known to have minimal impact on beneficial insects particularly predators than most synthetic insecticides. In Bollgard II cotton crop systems which may not require many synthetic insecticide sprays, there is the need to assess the degree or impact of the oil spray on the consumption rate of survivors of individual key predatory insect species such ladybird beetles on preys such as *Helicoverpa* spp. eggs, green mirids or aphids.
Therefore the objective of the study was to assess the impact of PSOs on predatory insect densities and effect on the consumption rate of survivors of PSO sprays.

3.1.1. Methodology

The study was conducted in 2005-06 cotton season. *Coccinella transversalis* adults were used in the study. Two studies were conducted. The first trial was conducted on 17 May 2005 and the second on 23 May 2005. The ladybirds used in the studies were collected from adjacent lucerne crops at the Australian Cotton Research Institute farm in Myall Vale in Narrabri. The lady birds were starved for 24 hours and separated into 2 groups, each of 60 ladybird adults. A 2%v/v PSO (Canopy® oil) was applied to one group of adults and water to the second group (control). Sixteen petridishes each containing 50 *Helicoverpa armigera* eggs were set up on the laboratory bench. Ladybird adult survivors from PSO-treated and untreated (control) representing one hour, 1 day, 2, 3 and 4 days after treatment, were placed separately in each petridish containing 50 eggs (i.e. 1 adult/petridish). The petridishes were then placed in a temperature cabinet maintained at 25^{o}C and relative humidity of 60%. The number of eggs consumed by each ladybird was recorded daily over 7 days. Eggs in each petridish were replaced every two days. The experiment was replicated 5 times for both treated and untreated (control). At the end of the study, the number of eggs consumed over 7 days was compared between treatments.

3.1.2 *Analysis of the data*

All experimental data were analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means.

3.1.3 Results

Helicoverpa spp. eggs consumed in this study are given in Figure 7. The number of *C. transversalis* adults that survived PSO spray one hour prior to exposure to Helicoverpa eggs consumed significantly lower ($P<0.001$) eggs than adults that survived PSO sprays 1 to 4 days after treatment (Figure 7). The number of eggs consumed by ladybird adult survivors one hour after PSO treatment was significantly lower ($P<0.05$) than the 1, 2 and 3 days after treatment survivors (Figure 7). No significant difference was detected among the treatments on day 4 of egg exposure (Figure 7).

In trial 1, the number of eggs per day consumed by 1, 2, 3 and 4 DAT adults were not significantly different ($P>0.05$) from the control (34.75) and one hour after treatment (1 HAT) adults (21.46). In contrast, 1 HAT adults consumed significantly higher number of eggs per day (21.46) than the untreated adults (34.75). In trial 2, significantly fewer (P<0.02) eggs per day was consumed by the 1 HAT adults than the other treatments and control. No significant difference was detected between 1, 2, 3 and 4 DAT adults and the untreated (control) adults. Overall, approximately 1.64 and 1.40 times less eggs per day in trial 1 and 2 respectively were consumed over the untreated (control) adults.

3.1.4 Conclusions

- PSO can reduce the consumption rate of *C. transversalis* adults on Helicoverpa eggs by 1.5 times within one hour after spray application
- However, the effect of the PSO residue 4 days after treatment did not affect the consumption rate of adult ladybird beetles on Helicoverpa eggs

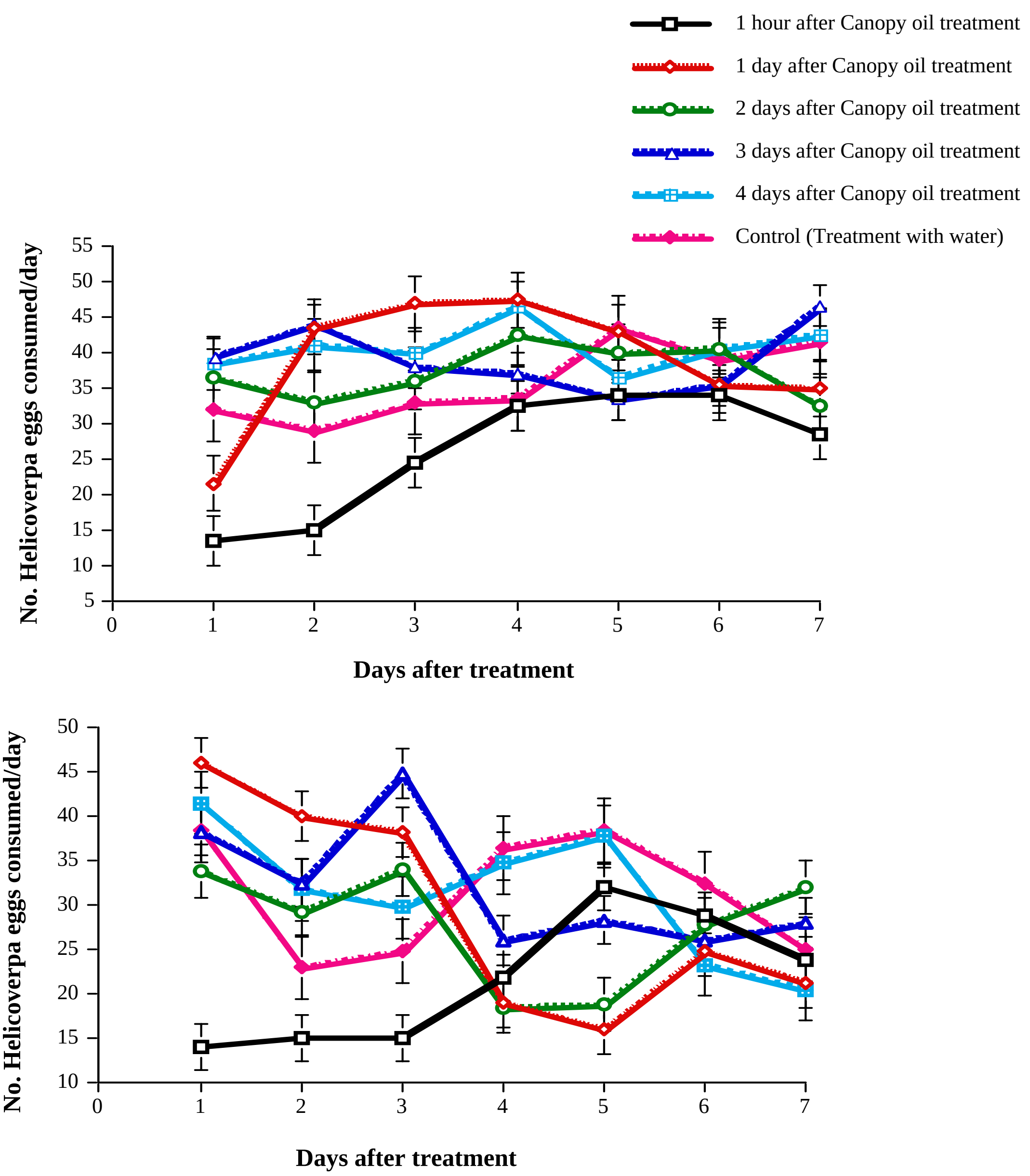


Figure 7. *The number of eggs per day consumed over 7 days by C. transversalis adults treated with Canopy oil in the laboratory at ACRI in Narrabri, 2005-06.*

4.1 Evaluation of the utility of locally grown alternative crops as trap crop for green mirids in cotton

4.1.1 Methodology

The study was conducted in a 2 ha irrigated cotton field at the Australian Cotton Research Institute (ACRI) farm at Narrabri between November 2004 and February 2005. The alternative crops evaluated were lucerne, sorghum, sunflower, soybean, mungbean, pigeon pea, conventional cotton and Bollgard II (Bt) cotton. Most of these crops are grown in monoculture in the cotton growing regions. Each of the crops were planted in strips, 8 m (or rows) wide and 150 m long and adjacent to and separated by cotton strips 20 m (or rows) wide and 150 m long. In total 4 strips of each crop were planted across the field. The cotton crop was planted on 15 October 2004 whereas the rest of the alternative crops were planted on 1 and 2 November 2004. All crops were irrigated at the same time as cotton; irrigation depended on the soil moisture level.

Green mirids adults and nymphs were sampled from each crop once every week from 16 December 2004 to 10 February 2005 by using a small portable suction sampler, D-vac (Homelite Textron Inc., NC, USA). On each sampling date, one sample was taken from each crop strip. The D-vac has a 120 mm diameter cone and a nozzle speed of approximately 10 m per second. A gauze bag (25 cm deep) was inserted into the suction tube to collect insects sucked from the plants. The sample was collected from a single pass of the tube of the vacuum sampler along the tops of the plants in a 20 m of row. After each sampling, the contents of the D-vac were transferred to a plastic bag, chilled, taken to the laboratory, and frozen until the contents were counted. Data was expressed as numbers per metre per sample date.

4.1.2 Analysis of data

All experimental data were analysed using repeated measures ANOVA on transformed data (X=log (X+1). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple Comparisons tests were used to separate the means. Arithmetic rather than transformed means are given in the text.

4.1.3 Results

The number of green mirid adults and nymphs found in the plots are given in Figure 8. Significantly higher ($P<0.001$) numbers of green mirids per metre per sample date were found on lucerne crops than any of the crops tested (Figure 8). The number of mirids recorded on sunflower and soybean were not significantly different ($P>0.05$) but they were significantly different ($P<0.01$) from cotton, sorghum, mung bean and pigeon pea (Figure 8).

4.1.4 Conclusion

- Green mirids prefer lucerne to cotton, sorghum, soybean, sunflower, mung bean, pigeon pea.
- Following, lucerne, Sunflower was the next preferred crop by green mirids.
- Lucerne could be the best crop to use as trap for green mirids in commercial cotton farms.

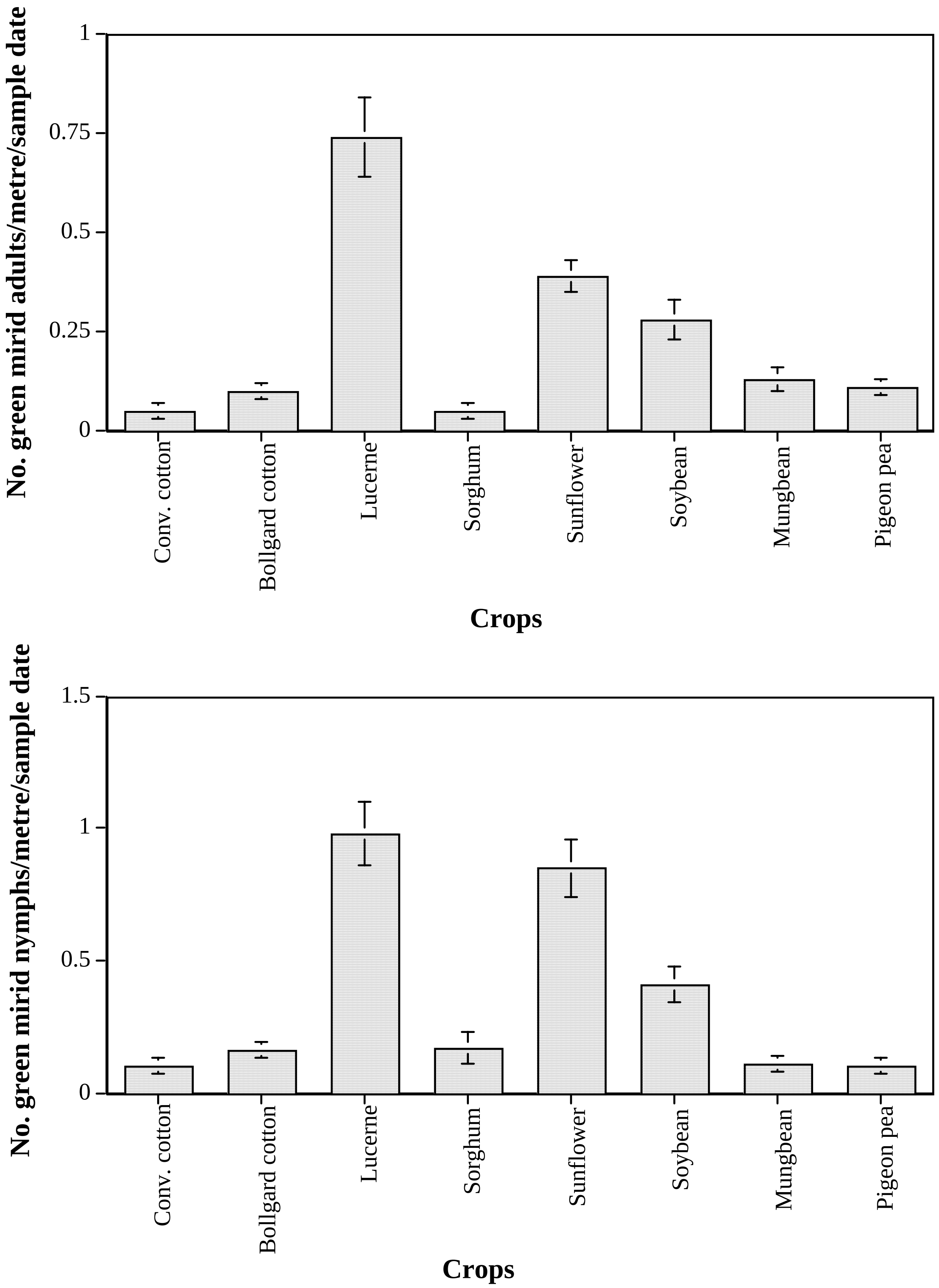


Figure 8. *Responses of green mirids to alternative crops interplanted in commercial cotton in the Australian Cotton Research Institute farm at Narrabri, 2004-05.*

4.2 Determine and compare levels of green mirids control on Bollgard crops interplanted with and without lucerne strips

Studies in experiment 2.4a showed that lucerne crop planted as strips or blocks in conventional cotton crops can be used to conserve beneficial insects and manage green mirids in adjacent cotton farm. Prior to the introduction of transgenic cotton crops in the cotton cropping system, insecticide sprays against *Helicoverpa* spp. in conventional cotton crops drifts onto the lucerne strips and blocks killing the beneficial insects and as a result making the lucerne crops ineffective in conserving these beneficial insects. The insecticide drifts to the lucerne strips usually occur during the mid and late cotton seasons when Helicoverpa populations on the conventional crops are very high and growers are continuously applying insecticides to control the pest. However, with the introduction of Bollgard crops, insecticide use against *Helicoverpa* spp. has reduced and lucerne strips or blocks will have a greater potential in trapping and managing green mirids on Bollgard crops.
The study assessed the feasibility of interplanting lucerne strips in Bollgard crops or strategically placing lucerne blocks to Bollgard fields in the management of green mirids in the Bollgard crops.

4.2.1 Methodology

The experiment was conducted in two irrigated Bollgard cotton fields (Fields 11 and 12) at Norwood near Moree. The size of each field was 800 metres or rows long and they were adjacent to each other separated by a 20 m access road. A lucerne strip or block 24 m wide and 502 metres long were planted as borders or adjacent to each cotton field. The lucerne strip was half slashed alternatively every 4 weeks to stimulate growth and prevent the lucerne from haying off.

Green mirid adults were sampled weekly by taking a 20 m row vacuum sample from the Bollgard cotton crop at 10, 50, 100, 150, 250, 400, 550, 650, 750 and 800 metres away from the edge of the lucerne strip. A sample was also taken in the lucerne strip at the same time. These were repeated 4 times at each distance. Sampling started on 16 November 2005 and finished on 25 February 2006. Data was expressed as numbers of green mirids (adults + nymphs) per metre per sampling date.

4.2.2 Analysis of data

All experimental data were analysed using repeated measures analysis of variance on transformed data ($X = \log(X+1)$ and Tukey-Kramer Multiple Comparisons test was used to separate the means. Arithmetic rather than transformed means are presented.

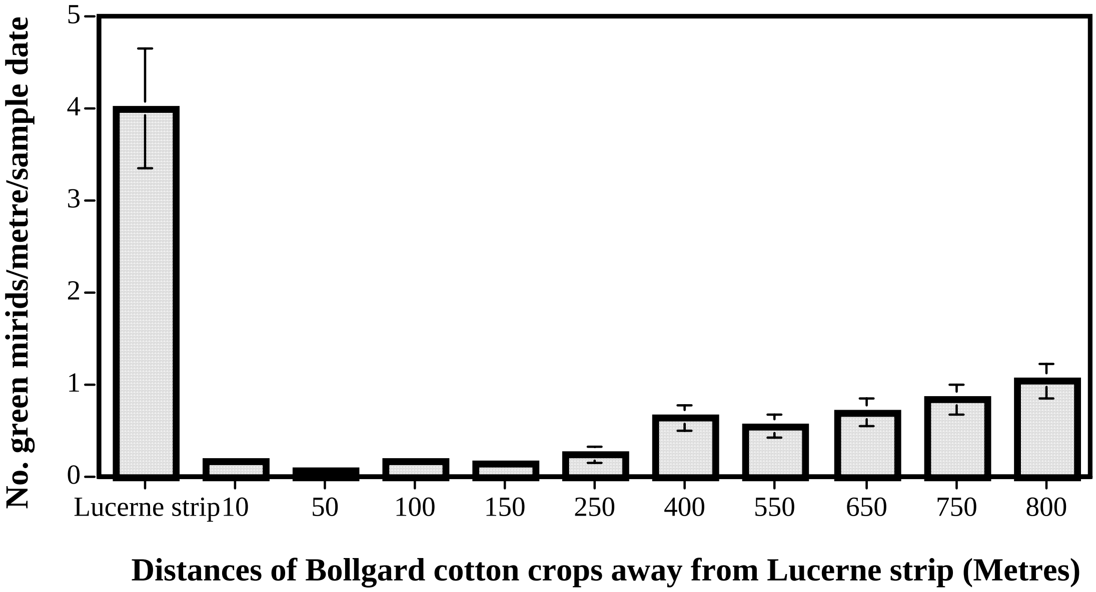
4.2.3 Results

Populations of green mirids in the lucerne strip was significantly higher ($P<0.001$) than on the adjacent Bollgard cotton and declined with increasing distance from the lucerne strip to reach their lowest level at 250 metres away (Figure 9). In general, the predator numbers recorded on the cotton crop 400-800 m away from the lucerne strip were significantly different ($P<0.05$) from each other but were significantly higher ($P<0.01$) than those in cotton

10-250 m away (Figure 9). This indicates that the lucerne strip was acting as a trap for green mirids minimizing green mirid infestation on the cotton located 10-250 m away.

4.2.4 Conclusion

- Lucerne planted as strips or borders to Bollgard cotton fields can manage green mirids in adjacent Bollgard cotton crops from 10-250 metres away.
- Lucerne crop can serve as a trap for green mirids



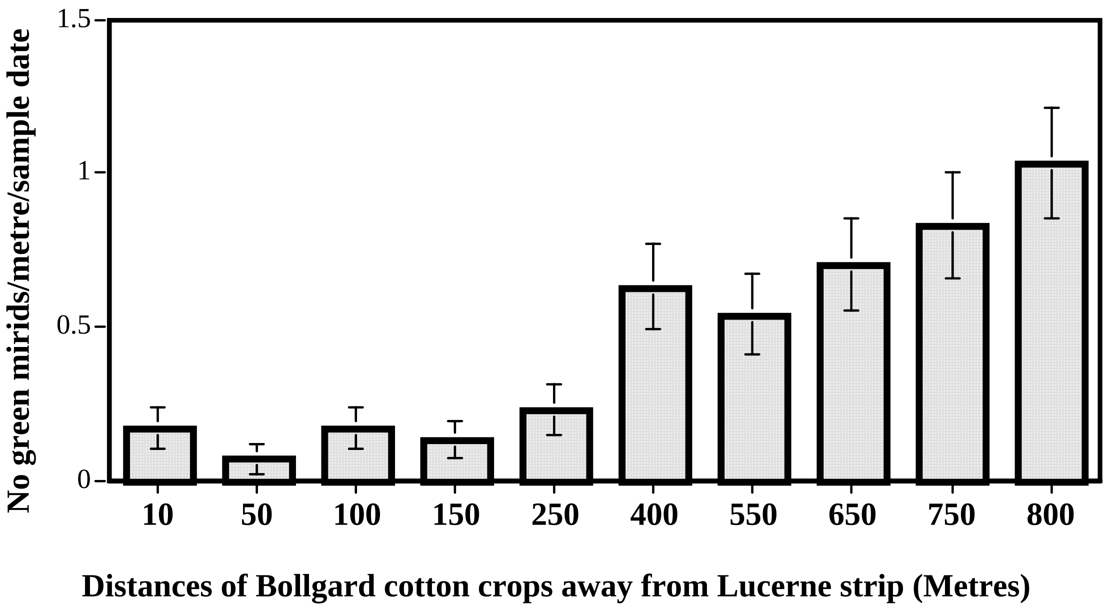


Figure 9. *Effect of Lucerne strips on the number of green mirids per metre per sample date on Bollgard cotton crops located 10 to 800m away from the lucerne strip at Norwood near Moree, 2005-06.*

5.0 Evaluation of the efficacy of a new myco-insecticide for activity against green mirids on Bollgard cotton crops

Studies undertaken by Dr Robert Mensah in collaboration with BioCare Pty Ltd in 2001-2003 identified 3 fungal isolates (BC 667 = *Beauveria* spp.; BC 639 = *Metarhizium* spp. and BC 614 = *Metarhizium* spp) to be efficacious against green mirids and *Helicoverpa* spp. However, only BC 667 was tested in commercial conventional cotton crops at ACRI in 2003-2004 season. The results of the study showed that BC667 mixed with PSO provided 55% control of green mirids 3 to 7 days after application during early and late season. A 55% kill caused by the fungal insecticide was regarded as suitable for use in cotton IPM programmes.
The fungal insecticides (myco-insecticides) provide advantage over the PSOs because they can control mirids both early, mid and late seasons. The fungus can also build up in the cotton system to provide secondary infection particularly after crop closure when humidity within the crops is high after irrigation or natural rainfall. The impact of BC667 on aphids has not been assessed and this will be undertaken in collaboration with Dr Lewis Wilson.

The objective of this project was to formulate the fungal insecticides in a vegetable oil and assess its efficacy against green mirids, aphids and their natural enemies. This study will provide efficacy data to support product registration and commercialization. The following experiments conducted on the mycoinsecticides were:

5.1 *Assessment of the efficacy of BC667, BC 639 and BC 614 fungal insecticides against green miridsand beneficial insects on Bollgard cotton crops*

5.1.1 Methodology

The trial was conducted in an irrigated commercial Bollgard II cotton crops at the Australian Cotton Research Institute farm (River block 3) in Narrabri in 2004-05 season. A 2-12 rows of sunflower crop were planted as strips on both sides of the cotton field (i.e. sunflower strips sandwich the field) to attract green mirids to the study site. The treatments evaluated were (1) Fipronil (applied at 62.5ml/ha (treated control), (2) 1L/ha BC 667 (Beauveria spp.) + 2% v/v Canopy oil, (3) 1L/ha BC639 (Metarhizium spp) + 2% v/v Canopy oil, (4) 1L/ha BC614 (Metarhizium spp.) + 2% v/v Canopy oil, (5) 1L/ha BC667 + 2% v/v Horticultural oil, (6) 1L/ha BC 639 + Horticultural oil formulation, (7) 1 L/ha BC 614 + Horticultural oil and (8) Unsprayed (control). The treatment plots were arranged in a randomized complete block design with 6 replicates per treatment. Each replicated plot measured 8 m wide and 220 metres long.

Green mirids and beneficial insects (mainly predators) were sampled weekly using a D-vac suction tube. On each sampling occasion, a 20-metre long sample was taken from a single pass of the vacuum tube along the tops of the plants. After each sampling, the contents of the sampler were transferred to separate plastic bags, taken to the laboratory and counted. The predatory insects were grouped into predatory beetles, bugs, lacewings and spiders. Data was expressed separately for green mirids, predatory beetles, predatory bugs, predatory lacewings and spiders. Each insect group was expressed as numbers per metre and numbers per metre per sampling date.

Sampling was discontinued on the 1 February 2005. Cotton in each treated plot was harvested separately using a four-row picker at the end of the season and the average lint yields (bales/ha) were compared between treatments.

5.1.2 Analysis of the data

All experimental data were transformed by (X + 0.5) and analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means. Arithmetic, rather than transformed means are given in the results.

5.1.3 Results

The results of the study are given in Figure 10. The fungi BC 667 and BC 639 in Canopy oil and Horticultural oil formulations achieved similar efficacy against green mirids as Fipronil treatments Figure 10. After two applications of BC 667 and BC 639 in Canopy and Horticultural oils, the fungal formulation suppressed green mirid numbers similar to and in some cases better than Fipronil applied at half rate (Figure 10). The fungus BC 614 was not as effective as BC 667 and 639 (Figure 10). Between 21 December and 7 January 2005, the number of green mirids recorded on plots treated with BC 614 and the unsprayed plots were not significantly different ($P>0.05$) (Figure 10). In contrast, the number of green mirids recorded on plots treated with BC 667 and 639 mixed in Canopy oil were consistently lower than the unsprayed plots (Figure 10).

The number of predators recorded in cotton crops treated with the fungal insecticides are given in table 5. Approximately equal number of predatory beetles, bugs, lacewings and spiders per metre per sample date were recorded on plots treated with BC667, 639 and 614 mixed with oils and the unsprayed plot (Table 5). The number of predatory beetles, bugs, lacewings and spiders recorded on plots treated with Fipronil insecticide were significantly lower ($P<0.01$) than plots treated with the fungal insecticides and the unsprayed plot (Table 5). In terms of cotton yields, there was no significant difference ($P>0.05$) in yields (bales/acre) harvested from plots treated with insecticides and fungus (Table 6). However, the cotton yields harvested from the plots treated with the fungal insecticides were significantly higher ($P<0.05$) than the unsprayed plots (Table 6).

5.1.4 Conclusion

- IL/ha BC 667 and BC639 mixed with either Canopy oil or Horticultural oil provided effective control of green mirid adults and nymphs similar to Fipronil insecticide.
- 1L/ha BC 614 fungal insecticides mixed in either Canopy or Horticultural oil was not effective in controlling green mirids on cotton as did BC 667, 639 and Fipronil insecticide.
- The efficacy of Fipronil insecticide was significantly higher on the predatory beetles, bugs, lacewings and spiders than the fungal insecticides.
- No significant difference in yield (bales/acre) was found between plots treated with fungal insecticides and Fipronil.
- Cotton crops treated with the fungal insecticides yielded higher than the unsprayed plots.

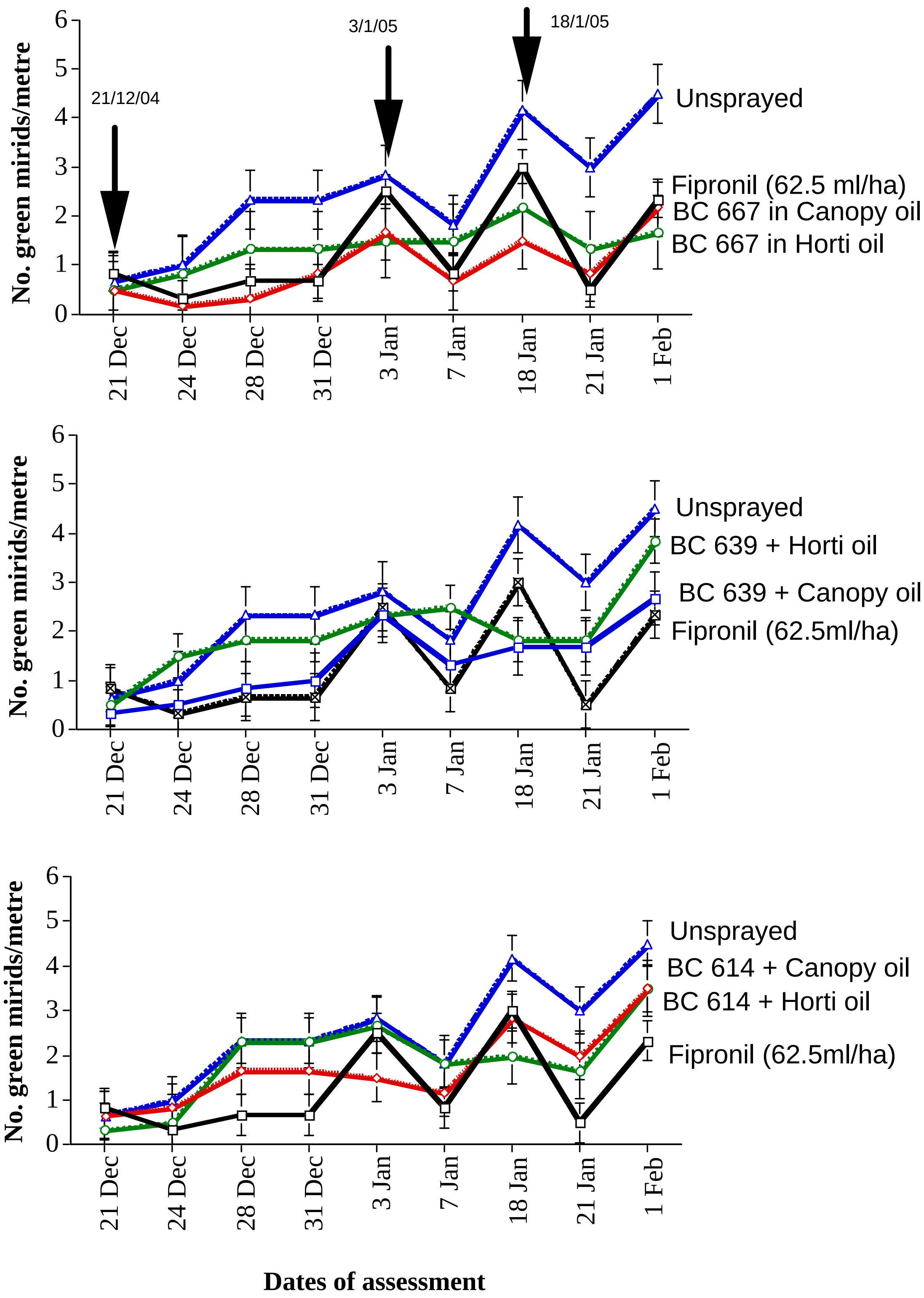


Figure 10. *Efficacy of Fungal insecticide formulations against green mirids on cotton, ACRI, Narrabri, 2004-05*

Table 5. The number of predators per metre per sample date in commercial cotton crops treated with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2004-05.

Treatments	Predatory beetles/m/ sample date	Predatory bugs/m/ sample date	Predatory lacewings/m/sample date	Spiders/m/ sample date
1L/ha BC667 + 2%v/v Canopy oil	0.63 ± 0.09 a	0.71 ± 0.12 a	0.69 ± 0.10 a	6.39 ± 0.47 b
1L/ha BC639 + 2%v/v Horti oil	0.87 ± 0.11a	0.86 ± 0.14 a	0.67 ± 0.09 a	8.67 ± 1.61 b
1L/ha BC667 + 2%v/v Canopy oil	0.72 ± 0.10 a	0.80 ± 0.15 a	0.69 ± 0.08 a	6.76 ± 0.52 b
1L/ha BC639 + 2%v/v Horti oil	0.80 ± 0.09 a	0.87 ± 0.12 a	0.69 ± 0.08 a	6.89 ± 0.58 b
1L/ha BC 614 + 2%v/v Canopy oil	0.63 ± 0.08 a	0.89 ± 0.14 a	0.78 ± 0.10 a	7.80 ± 0.49 b
1L/ha BC614 + 2%v/v Horti oil	0.78 ± 0.10 a	0.75 ± 0.10 a	0.67 ± 0.09 a	6.96 ± 0.54 b
2% v/v Horti oil alone	0.81 ± 0.08 a	0.85 ± 0.15 a	0.76 ± 0.10 a	7.78 ± 0.60 b
62.5ml/ha Fipronil	0.37 ± 0.08 b	0.43 ± 0.09 b	0.67 ± 0.11 a	4.61 ± 0.43 a
Controlunsprayed)	0.92 ± 0.09 a	1.04 ± 0.16 a	0.87 ± 0.21 a	7.44 ± 0.49 b

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

Table 6. Cotton yield (bales/acre) harvested from commercial cotton crops managed with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2004-05.

Treatments	Yield (bales/acre)
1L/ha BC667 + 2%v/v Canopy oil	4.13 ± 0.20 a
1L/ha BC639 + 2%v/v Horti oil	4.12 ± 0.20 a
1L/ha BC667 + 2%v/v Canopy oil	3.97 ± 0.29 a
1L/ha BC639 + 2%v/v Horti oil	3.60 ± 0.55 a
1L/ha BC 614 + 2%v/v Canopy oil	3.57 ± 0.50 a
1L/ha BC614 + 2%v/v Horti oil	3.40 ± 0.40 a
2% v/v Horti oil alone	3.50 ± 0.23 a
62.5ml/ha Fiprinol	4.20 ± 0.19 a
Control (unsprayed)	2.91 ± 0.12 b

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

5.2 *To assess the efficacy BC 667 and BC 639 at two concentrations for the control of green mirids on Bollgard cotton crops*

5.2.1 Methodology

The trial was conducted in an irrigated commercial Bollgard® cotton crop at the Australian Cotton Research Institute farm at Narrabri. A 2-12 rows of sunflower strips were planted on both sides of the field (i.e. sunflower strips sandwich the field) to generate green mirids..

The following treatments were evaluated against green mirids: (1) Fipronil (applied at 62.5ml/ha (treated control), (2) 1L/ha BC 667 (Beaveria spp.) in Horti oil formulation, (3) 1L/ha BC639 (Metarhizium spp) in Horti oil formulation, (4) 1.5L/ha BC667 in Horti oil formulation, (5) 1.5L/ha BC639 in Horti oil formulation, (6) Horticultural oil alone and (7) Unsprayed (untreated) control. The treatment plots were arranged in a randomized complete block design with 3 replicates per treatment. Each replicated plot measured 8 m wide and 220 metres long.

Counts of insects were made 24 h before treatment application and then approximately every 7 days until the end of the study. Foliar application of each treatment was made on 13 December 2005, 9 January 2006 and 23 January 2006 using a tractor (ground rig). On each occasion treatments were applied using 100 litres water per hectare. The untreated (control) plot was left unsprayed and the synthetic insecticide treated standard plot received 3 applications at the same date as the fungal insecticides.

Visual counts of green mirids (adults and nymphs) and beneficial insects (mainly predators) on whole cotton plants were made in two selected 1 – m lengths of row of each treatment replicate, i.e 6 m per treatment. The predatory insects were grouped into predatory beetles, bugs, lacewings and spiders. Data was expressed separately for green mirids, predatory beetles, predatory bugs, predatory lacewings and spiders. Each insect group was expressed as numbers per metre and numbers per metre per sampling date.

Sampling commenced on 29 November 2005 was discontinued on 6 March 2006. Cotton in each treated plot was harvested separately using a four-row picker at the end of the season and the average lint yields (bales/ha) were compared between treatments.

5.2.2 Analysis of the data

All experimental data were analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means.

5.2.3 Results

The efficacy of BC 667 and BC 639 formulations against green mirid adults and nymphs are given in Figure 11. The study showed that BC667 and BC639 applied at either 1.5 or 1.0 L/ha achieved similar efficacy against green mirids as Fipronil applied at half the label rate (Figure 11). The efficacy of the fungal insecticides did not vary ($P>0.05$) with concentrations (Figure 11). The efficacy of Horticultural oil alone against green mirids was low and not significantly different ($P>0.05$) from the unsprayed (control) plots (Figure 11). BC 667 and BC 639 killed green mirids within 3 days of application (Figures 12 and 13).

Approximately equal numbers of predatory beetles, bugs, lacewings and spiders per metre were found in all treated plots (table 7). The number of predators found in the Fipronil treated plots was the same in all the treatments tested (table 7).

Cotton yield harvested from the Horticultural oil and unsprayed (control) plots were significantly lower (P<0.01) than the fungal insecticides and Fipronil treated plots (table 8).

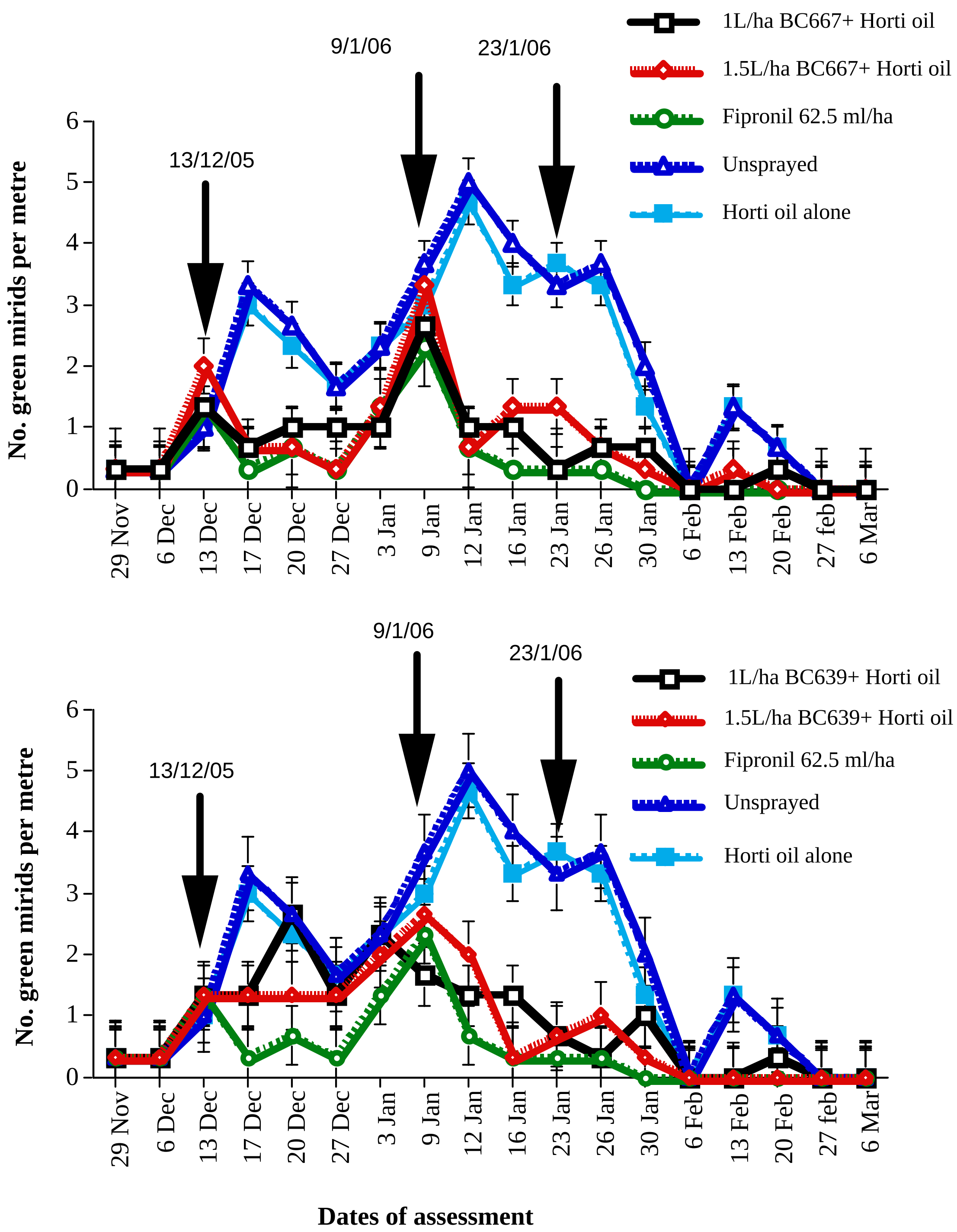


Figure 11. Efficacy of fungal insecticide formulations against green mirids on cotton, ACRI, Narrabri, 2005-06

Table 7. The number of predators per metre per sample date in commercial cotton crops treated with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2005-06.

Treatments	Predatory beetles/m/ sample date	Predatory bugs/m/ sample date	Predatory lacewings/m/sample date	Spiders/m/ sample date
1L/ha BC667 + 2%v/v Horti oil	0.08 ± 0.01 a	0.06 ± 0.01 a	0.11 ± 0.02 a	2.53 ± 0.13 a
1L/ha BC639 + 2%v/v Horti oil	0.07 ± 0.02 a	0.04 ± 0.01 a	0.09 ± 0.02 a	2.57 ± 0.16 a
1.5L/ha BC667 + 2%v/v Horti oil	0.08 ± 0.02 a	0.06 ± 0.01 a	0.08 ± 0.02 a	2.63 ± 0.17 a
1.5L/ha BC639 + 2%v/v Horti oil	0.08 ± 0.02 a	0.07 ± 0.01 a	0.07 ± 0.01 a	2.57 ± 0.15 a
2% v/v Horti oil alone	0.10 ± 0.02 a	0.05 ± 0.01 a	0.08 ± 0.01 a	2.47 ± 0.13 a
62.5ml/ha Fiprinol	0.11 ± 0.02 a	0.05 ± 0.01 a	0.08 ± 0.02 a	2.42 ± 0.17 a
Control (unsprayed)	0.10 ± 0.02 a	0.06 ± 0.01 a	0.09 ± 0.02 a	2.87 ± 0.16 a

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

Table 8. Cotton yield (bales/acre) harvested from commercial cotton crops managed with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2005-06.

Treatments	Yield (bales/acre)
1L/ha BC667 + 2%v/v Horti oil	2.87 ± 0.20 a
1L/ha BC639 + 2%v/v Horti oil	2.76 ± 0.10 a
1.5L/ha BC667 + 2%v/v Horti oil	2.90 ± 0.29 a
1.5L/ha BC639 + 2%v/v Horti oil	2.87 ± 0.25 a
2% v/v Horti oil alone	2.41 ± 0.23 b
62.5ml/ha Fiprinol	2.97 ± 0.19 a
Control (unsprayed)	2.31 ± 0.12 b

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

Figure 12. Green mirid adult collected from Bollgard cotton crops treated with BC 639 fungus (The fungus killed the green mirids 3 days after treatment application)

Figure 13. Green mirid adult collected from Bollgard cotton crops treated with BC 667 fungus (The fungus killed the green mirids 3 days after treatment application)

5.2.4 Conclusion

- Efficacy of BC667 and BC639 applied at 1.5 and 1.0 L/ha against green mirids is the same as Fipronil applied at half the label rate
- BC667 and BC639 applied at 1.5 and 1.0 L/ha did not have any negative effect against predatory beetles, bugs, lacewings and spiders
- Application of 1.5 and 1.0 L/ha BC667 and BC639 on cotton plants managed green mirids and achieved similar cotton yields as cotton crops treated with Fipronil to manage green mirids

5.3 *To assess the efficacy of different concentrations of BC667 and BC 639 for the control of green mirids on Bollgard cotton crops*

5.3.1 ACRI Trials 2006-07

The trial was conducted in an irrigated commercial Bollgard II cotton crops at the Australian Cotton Research Institute farm (River block 3) in Narrabri. A 3-12 rows of sunflower strips were planted on both sides of the field (i.e. sunflower strips sandwich the field) to generate green mirids.

5.3.1.1 Methodology

The following treatments were evaluated against green mirids: (1) 1L/ha BC 639 (Metarhizium spp.), (2) 0.75L BC 639 (Metarhizium spp.), (3) 0.50L BC 667 (Beauveria spp.), (4) 0.50L BC 639 (Metarhizium spp.), (5) 62.5ml Fipronil and (6) Unsprayed (untreated) control. The treatment plots were arranged in a randomized complete block design with 6 replicates per treatment. Each replicated plot measured 8 m wide and 220 metres longs.

Foliar application of each treatment was made from 22 January 2007. The decision to apply the treatment was made based on the IPM Guidelines and CottonLogic recommended economic threshold of 0.5 green mirids per metre.

Visual counts of green mirid adults and nymphs on cotton plants in each treatment were made at approximately weekly intervals in two randomly selected 1 metre lengths of row of each treatment replicate, *i.e.* a total of 8 metres were examined per treatment. Counts were separated into nymphs and adults. Data were expressed as numbers per metre and numbers per metre per sample date for each treatment.

Cotton in each treated plot was harvested separately using a four-row picker at the end of the season and the average lint yields (bales/ha) were compared between treatments.

5.3.1.2 Analysis of data

All experimental data were transformed by (X + 0.5) and analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means. Arithmetic, rather than transformed means are given in the results.

5.3.1.3 Results

The number of green mirid adults and nymphs recorded in plots treated with different rates of BC 667 and BC 639 were the same as plots treated with Fipronil (Figure 14). The number of green mirid adults and nymphs per metre recorded in the unsprayed plot was significantly higher ($P<0.008$) than the plots treated with fungal insecticides and Fipronil insecticide (Figure 14). The number of green mirids per metre in plots treated with Fipronil was not significantly different ($P>0.05$) from plots treated with the different rates of BC667 and BC 639 (Figure 14). Overall, approximately 2.5 and 2.2 times more green mirid adults and nymphs were recorded in the untreated plots than in plots treated with fungal insecticides.

The number of predatory beetles, bugs, lacewings and spiders per metre per sample date is given in Table 9. No significant difference (P>0.05) in the number of predatory insects and spiders were found among treatment and control plots (Table 9).

Significantly higher (P<0.09) yields were harvested from the synthetic (Fipronil) and fungal (BC 667 and BC 639) insecticide treated plots than the unsprayed plots (Table 10). The fibre quality characteristics of cotton harvested in the treated and control plots is given in Table 11. No significant difference (P>0.05) was detected in fibre strength, length, uniformity and micronaire (Table 11).

5.3.1.4 Conclusion

- The number of green mirids per metre in plots treated with Fipronil was not significantly different (P>0.05) from plots treated with the different rates of BC667 and BC 639
- Approximately 2.5 and 2.2 times more green mirid adults and nymphs were recorded in the untreated plots than in plots treated with fungal insecticides.
- The fungal insecticides did not have any significant effect on the number of predatory beetles, bugs, lacewings and spiders per metre on the cotton crops.
- Cotton crops treated managed with the fungal insecticides had the same yield and quality as crops managed the synthetic (Fipronil) insecticide

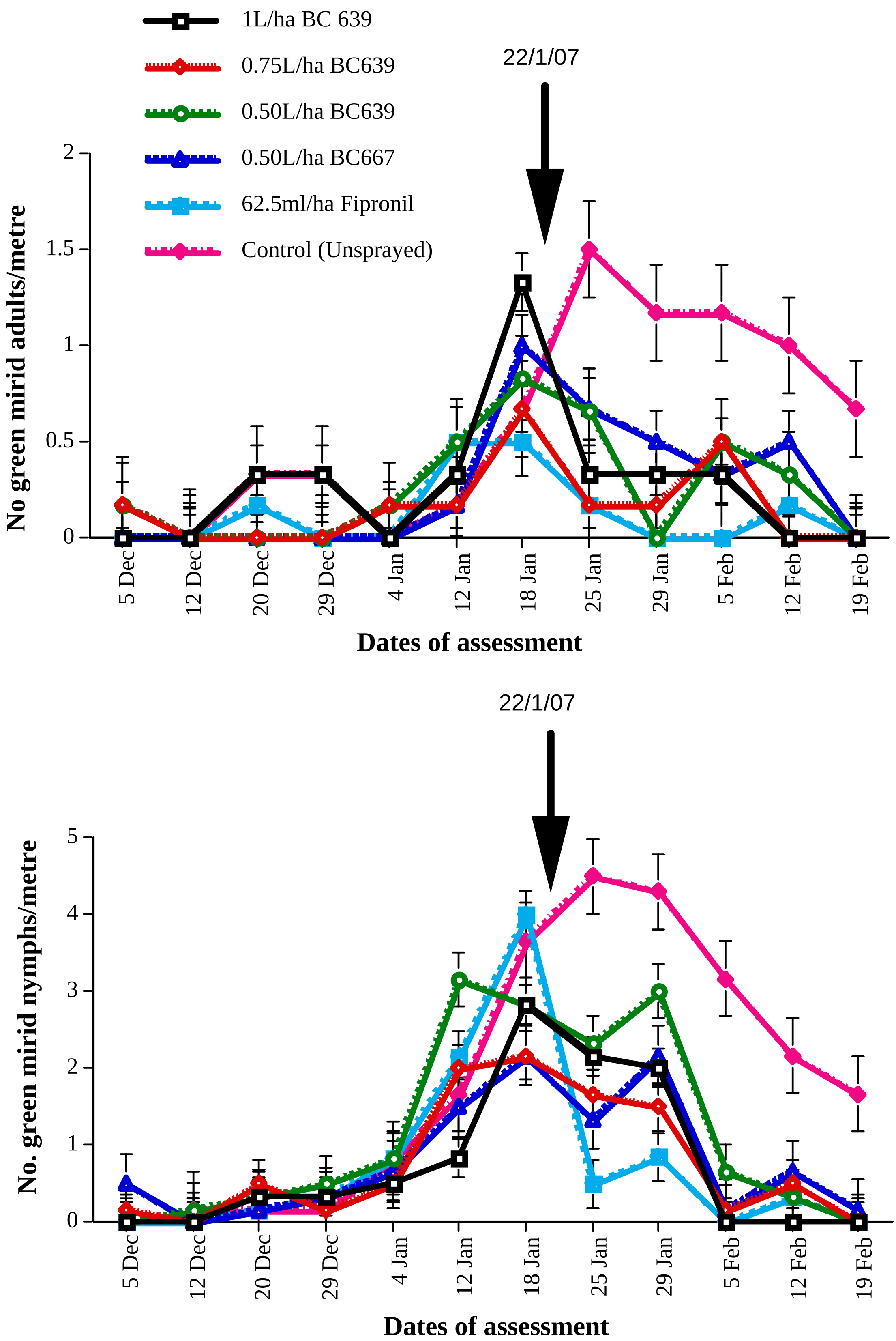


Figure 14. The efficacy of different rates of BC639 and BC 667 fungal insecticides against green mirid adults and nymphs in Bollgard cotton crops at ACRI in Narrabri, 2006-07.

Table 9. The number of predators per metre per sample date in commercial cotton crops treated with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2006-07.

Treatments	Predatory beetles/m/ sample date	Predatory bugs/m/ sample date	Predatory lacewings/m/sample date	Spiders/m/ sample date
1L/ha BC639	0.39 ± 0.06 a	0.17 ± 0.03 ab	0.025 ± 0.006 a	1.26 ± 0.08 a
0.75 L/ha BC639	0.45 ± 0.07 a	0.19 ± 0.04 ab	0.015 ± 0.005 a	1.48 ± 0.08 b
0.5L/ha BC639	0.41 ± 0.02 a	0.18 ± 0.03 ab	0.025 ± 0.005 a	1.20 ± 0.08 a
0.5L/ha BC667	0.33 ± 0.05 a	0.19 ± 0.04 ab	0.022 ± 0.005 a	1.33 ± 0.08 a
62.5ml/ha Fipronil	0.44 ± 0.02 a	0.15 ± 0.02 b	0.025 ± 0.005 a	1.12 ± 0.08 a
Control (unsprayed)	0.42 ± 0.02 a	0.24 ± 0.04 a	0.022 ± 0.005 a	1.19 ± 0.09 a

Means within columns followed by the same letters are not significantly different (P>0.05), Tukey-Kramer Multiple comparison test.

Table 10. Cotton yield (bales/acre) harvested from commercial cotton crops managed with fungal insecticides at Norwood near Moree, 2006 - 07.

Treatments	Yield (bales/acre)
1L/ha BC 639 + Horti oil	3.28 ± 0.11 a
0.75 L/ha BC 639 + Horti oil	3.35 ± 0.09 a
0.50 L/ha BC 639 + Horti oil	3.37 ± 0.19 a
0.50L/ha BC 667 + oil	3.15 ± 0.11 a
62.5 ml/ha Fipronil	3.42 ± 0.13 a
Unsprayed (Control)	2.84 ± 0.05 b

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Krammer Multiple Comparison Tests.

Table 11. Fibre quality characteristics from commercial cotton crops managed with fungal insecticides at the Australian Cotton Research Institute in Narrabri, 2006-07.

Treatments	Length (mm)	Strength (g/tex)	Micronaire	Uniformity (%)
1L/ha BC639	1.19 ± 0.003 a	32.5 ± 0.49 a	4.83 ± 0.09 a	83.1 ± 0.35 a
0.75 L/ha BC639	1.19 ± 0.017 a	32.9 ± 0.63 a	4.70 ± 0.12 a	82.2 ± 0.81 a
0.5L/ha BC639	1.17 ± 0.013 a	32.5 ± 0.45 a	4.77 ± 0.03 a	81.5 ± 0.55 a
0.5L/ha BC667	1.18 ± 0.015 a	33.0 ± 0.35 a	4.77 ± 0.03 a	83.1 ± 0.44 a
62.5ml/ha Fipronil	1.16 ± 0.008 a	33.6 ± 0.47 a	4.73 ± 0.03 a	82.6 ± 0.52 a
Control (unsprayed)	1.19 ± 0.010 a	33.1 ± 0.23 a	4.67 ± 0.09 a	83.3 ± 0.43 a

Means within columns followed by the same letters are not significantly different (P>0.05), Tukey-Kramer Multiple comparison test.

5.3.2 Trials at Norwood near Moree

The trial was conducted in a commercial Bollgard II cotton farms at Norwood near Moree (Field 1). Trials at each site commenced on 16 November 2006 until 28 February 2007. Sucking pests and beneficial insects from each treated plots were sampled visually and by suction sampling. Cotton in each treated plot was harvested and yield expressed in bales per acre.

5.3.2.1 Methodology

The following treatments were evaluated against green mirids: (1) 1L/ha BC 639 (Metarhizium spp.), (2) 0.75L BC 639 (Metarhizium spp.), (3) 0.50L BC 667 (Beauveria spp.), (4) 0.50L BC 639 (Metarhizium spp.), (5) 62.5ml Fipronil and (6) Unsprayed (untreated) control. The treatment plots were arranged in a randomized complete block design with 6 replicates per treatment. Each replicated plot measured 40 m (rows) wide and 90 metres long (average).

Foliar application of each treatment was made from 16 and 23 January 2007. The decision to apply the treatment was made based on the IPM recommended economic threshold of 0.5 green mirids per metre.

Visual counts of green mirid adults and nymphs on cotton plants in each treatment were made at approximately weekly intervals in two randomly selected 1 metre lengths of row of each treatment replicate, *i.e.* a total of 8 metres were examined per treatment. Counts were separated into nymphs and adults. Data were expressed as numbers per metre and numbers per metre per sample date for each treatment.

Cotton in each treated plot was harvested separately at the end of the season and the average lint yields (bales/ha) were compared between treatments.

5.3.2.2 Analysis of data

All experimental data were transformed by (X + 0.5) and analysed using repeated measures ANOVA (Graphpad Instat and Prism Software, Inc. v. 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer Multiple comparisons tests were used to separate means. Arithmetic, rather than transformed means are given in the results.

5.3.2.3 Results

The number of green mirid adults and nymphs per metre recorded on plots treated with synthetic (Fipronil) insecticide, different rates of fungal insecticides and unsprayed control is given in Figure 15. The first spray application of the treatments occurred when green mirid numbers per metre in the treated plots ranged from 0.25 - 1.0 (adults) and 1.5-3.5 (nymphs) per metre (Figure 15). The mirid numbers on all treated plots except the unsprayed plot declined to 0.125 (adults) and 0.25 per metre (nymphs) 3 days after treatment (3 DAT) (Figure 15). After 7 DAT, the number of mirid adults recorded on plots treated with 0.75 and 0.50 L/ha BC 667 and 639 increased to 0.25 and 0.50 per metre respectively, whereas the plots treated with 1L/ha BC639 and Fipronil were zero. In contrast, the green mirid numbers in the unsprayed plot rose from 0.25 to 1.5 per metre after 7 DAT whereas the number of green mirid nymphs per metre declined to 0.125 per metre on plots treated with 1L/ha BC 639 and Fipronil (Figure 15). The green mirid adults and nymphs per metre continued to decline after the second treatment application (23 January 2007) on all treatments and control till the end of study. Nevertheless, the number of adults and nymphs per metre recorded on the unsprayed plots was significantly higher ($P<0.01$) than the fungal and synthetic insecticide treated plots (Figure 15).

The results showed that the fungal insecticides are selective against hard bodied predatory insects particularly predatory beetles and bugs (mainly adults) (Figure 16). The fungal insecticides were found to be less selective against lacewings (Figure 17). Overall, the fungal insecticides were more selective to predatory insects than Fipronil insecticide (Figures 16 and 17).

Significantly higher ($P<0.05$) cotton yields were harvested from the Fipronil (4.27 bales/ha), 0.75L/ha BC 639 (4.04 bales/ha), 0.50 L/ha BC 639 (3.67 bales/ha), 0.50L/ha BC 667 (3.94 bales/ha) treated plots than the unsprayed plots (2.80 bales/ha). The cotton yield harvested from the Fipronil treated plots was not significantly different ($P>0.05$) from the fungal insecticide treated plots.

5.3.2.4 Conclusions

- Application of 0.5-1.0 L/ha of either BC667 or BC 639 fungal insecticide can reduce densities of green mirids adults and nymphs from over 3.5 per metre to zero within 14 days after application.
- The efficacy of fungal insecticides applied at 0.50 – 1 L/ha against green mirids adults and nymphs was the same as Fipronil applied at ½ label rate.
- Cotton crops with green mirids managed with 0.5 – 1.0 L/ha BC 639 and BC 667 had the same yields as crops where mirids were managed with Fipronil applied at ½ label rate.
- Application of the fungus can kill green mirid adults and nymphs within 3-4 days.
- The fungus can cause secondary infection to green mirids whereby the death of infected mirids can produce more spores which will continue to infect and kill insects.
- The fungus can continue to control insects over 28 days after application.
- The fungus is effective only against soft (not hard) bodied insects.

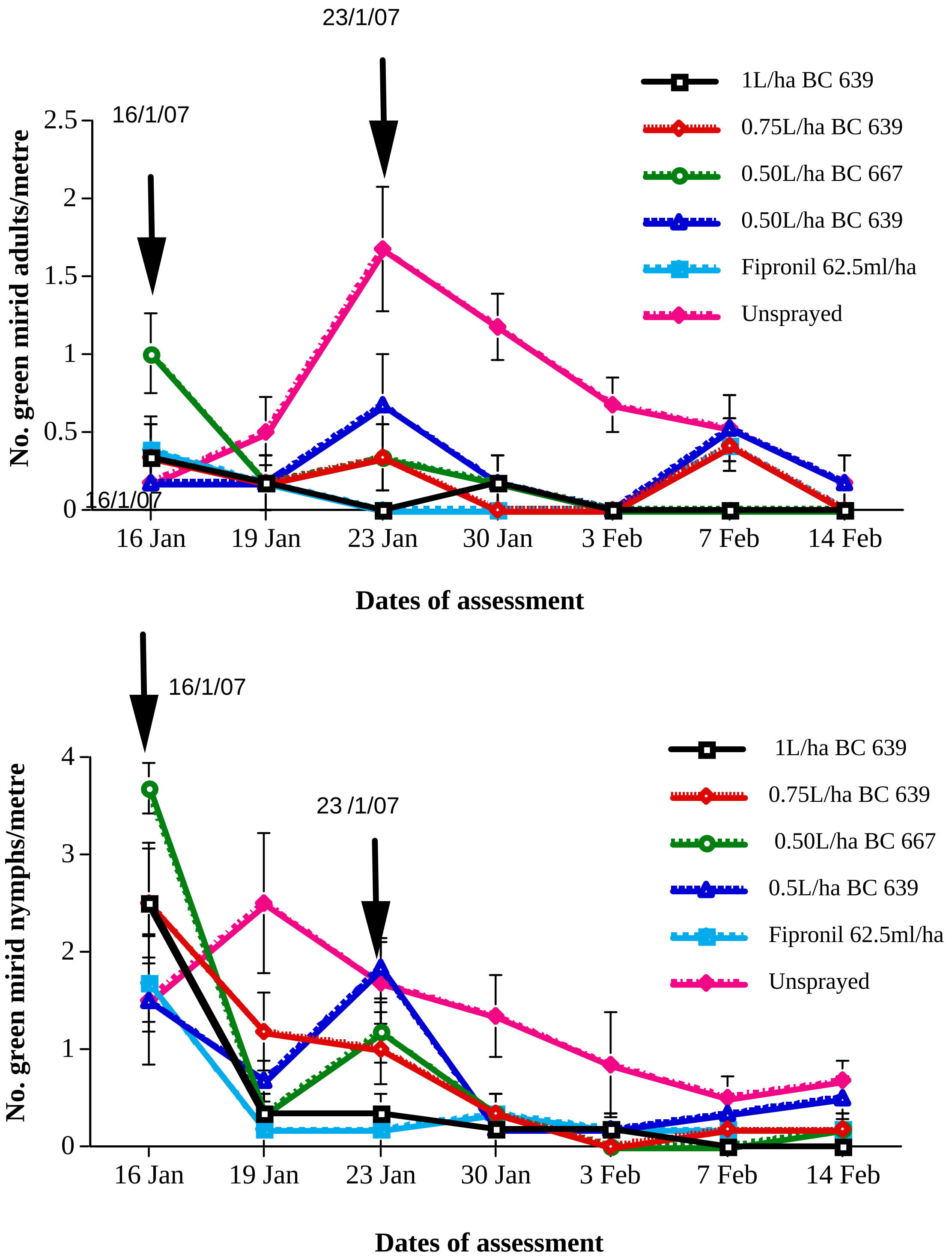


Figure 15. Efficacy of different rates of fungal insecticides against green mirid adults and nymphs on Bollgard cotton crops at Norwood near Moree, 2006-07.

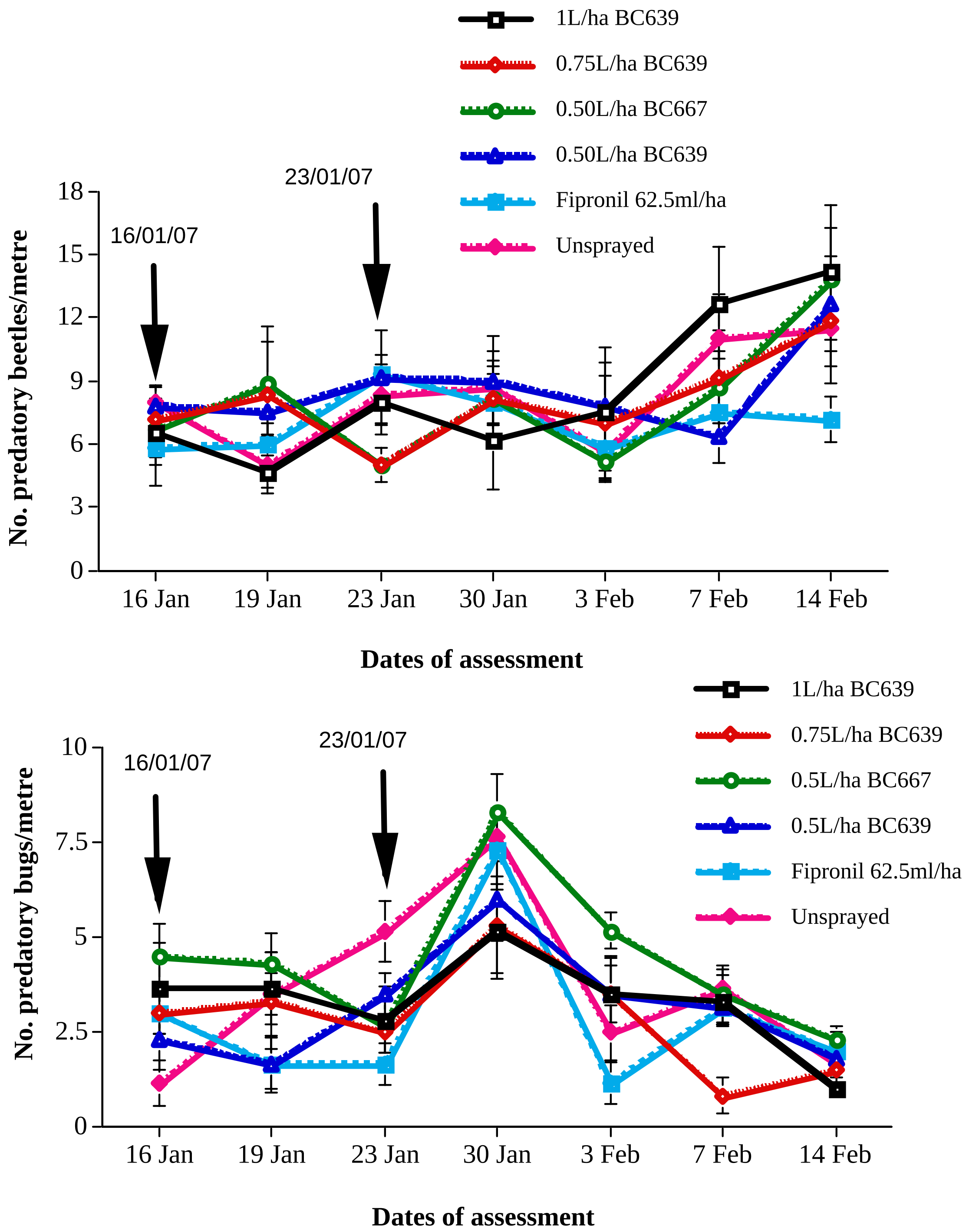
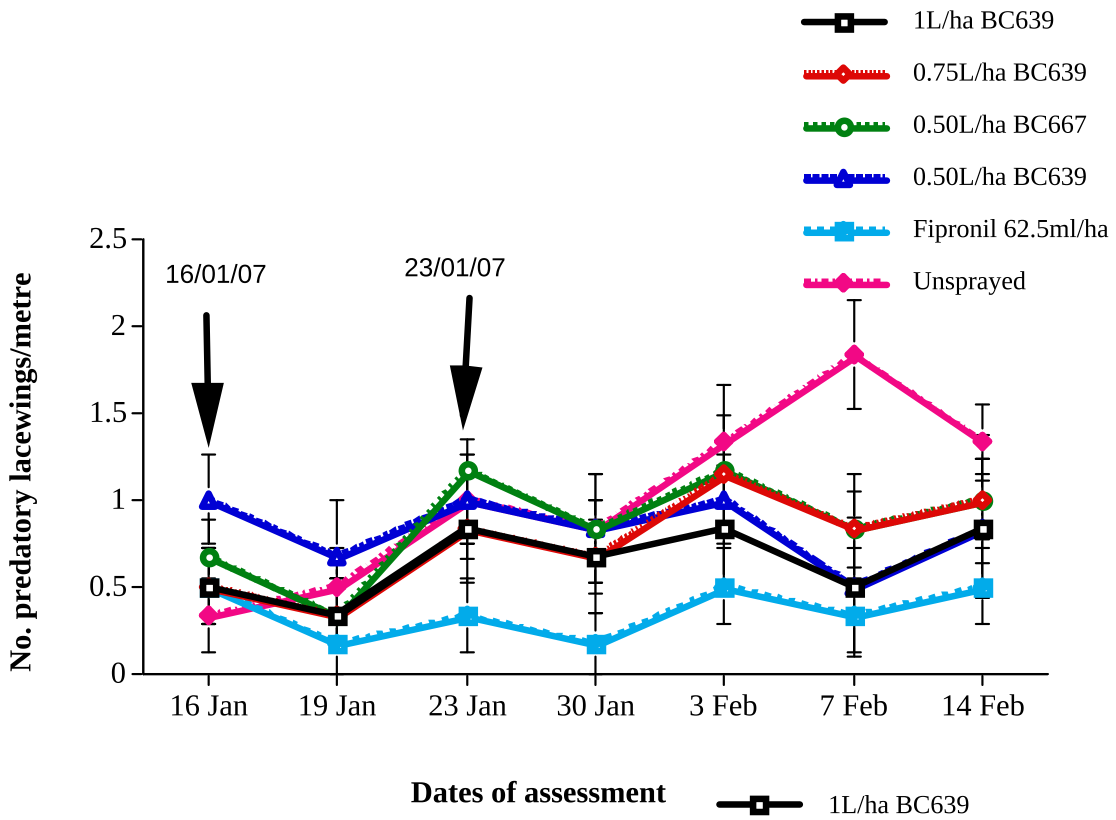


Figure 16. The efficacy of fungal insecticides against predatory beetles and bugs in Bollgard cotton crops at Norwood near Moree, 2006-07.



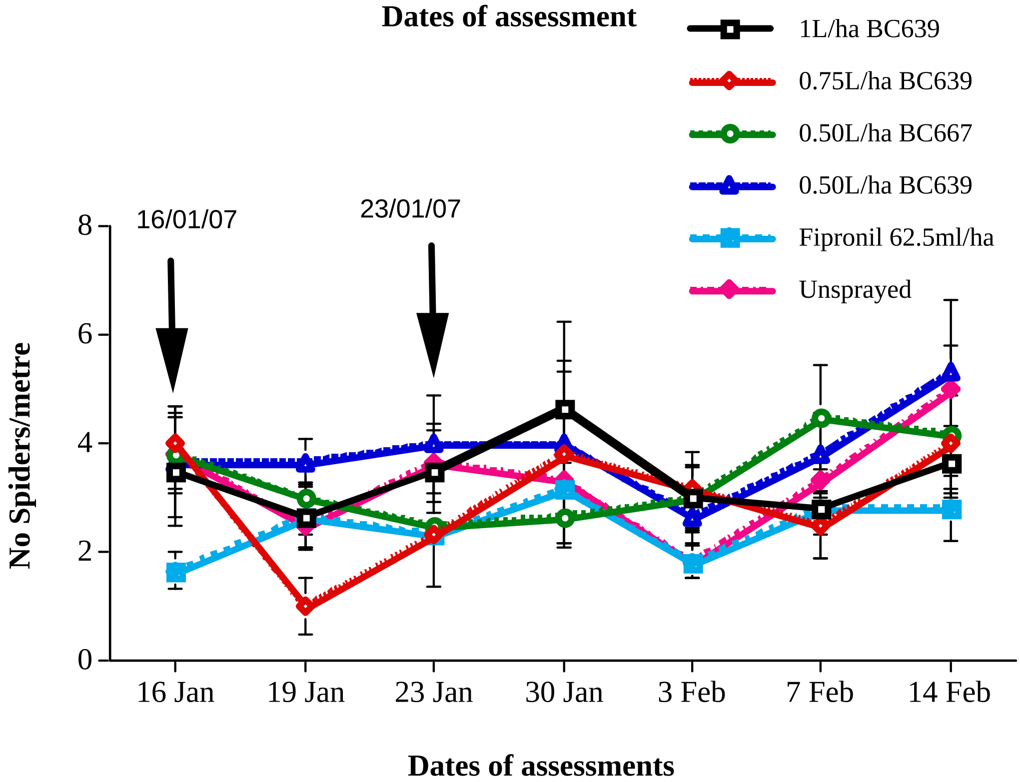


Figure 17. The efficacy of fungal insecticides against predatory lacewings and spiders in Bollgard cotton crops at Norwood near Moree, 2006-07.

6.0 Determine strategic use of Magnet (moth) attractant on Bollgard II cotton crops on the level of *Helicoverpa* spp. production and management on conventional cotton crops

In Australia about 85% of cotton farms are planted with Bollgard crops. This is because the Bollgard cotton crops can control *Helicoverpa* spp. larvae without excessive use of synthetic insecticides. Recently, a moth attractant (Magnet®) formulation consisting of a volatile blend and feeding stimulants that mimic the type of signals that lepidopteran adults look for when seeking nectar from flowers has been developed to manage *Helicoverpa* spp. and other lepidopteran pests in a wide range of crops. Magnet® formulation was successfully used to concentrate *Helicoverpa* spp. moths on conventional cotton crops adjacent to transgenic (BollgardII®) cotton crops as a resistance management strategy for *Helicoverpa* spp. on Bollgard® crops. The strategy of applying Magnet® formulation onto strategically placed BollgardII® cotton crops surrounded by conventional cotton crops may lure *Helicoverpa* moths from the environment onto the BollgardII® cotton crops where they are killed by the attracticide. By doing this, *Helicoverpa* moth populations on the surrounding conventional cotton crops may be reduced and could offer cost-effective control on the conventional crops.

This study reports on field trials using Magnet® formulation with insecticide (attracticide) to determine the effect of applying the attracticide to a centrally located BollgardII® (Bt) cotton crop on levels and management of *Helicoverpa* spp. adults, eggs and larval populations on surrounding conventional cotton crops. The study also determined the cost effectiveness of the "attract and kill" strategy with Magnet.

6.1 Materials and methods

6.1.1 *Attracticide formulation*

Magnet® was an attracticide product used in this study. The product consists of a blend of plant synthetic plant volatiles (3.1%), feeding stimulant (20% sucrose), toxicant (0.5% Thiodicarb (Larvin® 375) insecticide) and 0.1% blue food dye to mark moths that had fed on the material. Magnet® mixture was applied at 500ml per 100 metre row of cotton plants in 50 cm bands with 72 metre spacing between each band. The plant volatiles in Magnet® mimic the type of odour that *Helicoverpa* moths respond to when seeking nectar from flowers. Thus, the product works by attracting *Helicoverpa* moths to the Magnet® treated cotton plants where they feed on the product and then ingest the added insecticide causing death. Any moth in the treated field or that is attracted to the treated area and feeds on the product are affected. In Australia, Magnet® is licensed for commercial release by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

6.1.2 Layout of experiment

The experiment was conducted on irrigated conventional and BollgardII® cotton fields at Carbucky near Goondiwindi (28° 30'S, 150° 21'E) in Queensland in Australia during the 2004-05 and 2005-06 cotton growing seasons. The layout of the study site is given in Figure 18. In the 2004-2005 cotton growing season, the cotton crops were planted on 7 October 2004 and in 2005-06 they were planted on 10 October 2005. In each year of the study, we selected a 120 ha transgenic (Bollgard II®) cotton field on a commercial cotton farm for treatment with Magnet®. Six conventional cotton fields (each measuring approximately 120 ha) and located at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 km away from, and perpendicular to the “treated” BollgardII® cotton field were selected to assess *Helicoverpa* spp. population. The “treated” BollgardII® and the conventional cotton fields were each divided into four subplots and each subplot (27 ha cotton) was considered as a replicate. Each subplot or replicate was separated by a buffer of 4 ha cotton.

At a second location, an untreated BollgardII® cotton field and six conventional cotton fields of similar sizes, layout but located at least 6 km away from the “treated” BollgardII® cotton field, were selected as the control (untreated).

In this paper, the BollgardII® cotton field treated with Magnet® formulation and the conventional cotton fields located 0.5 to 3 km away are referred to as “treated” BollgardII® and conventional cotton fields. The BollgardII® cotton field that was not treated with the Magnet® formulation and the conventional cotton fields located 0.5 to 3 km away from the untreated BollgardII® field are referred to as “untreated (control)” BollgardII® and conventional cotton fields.

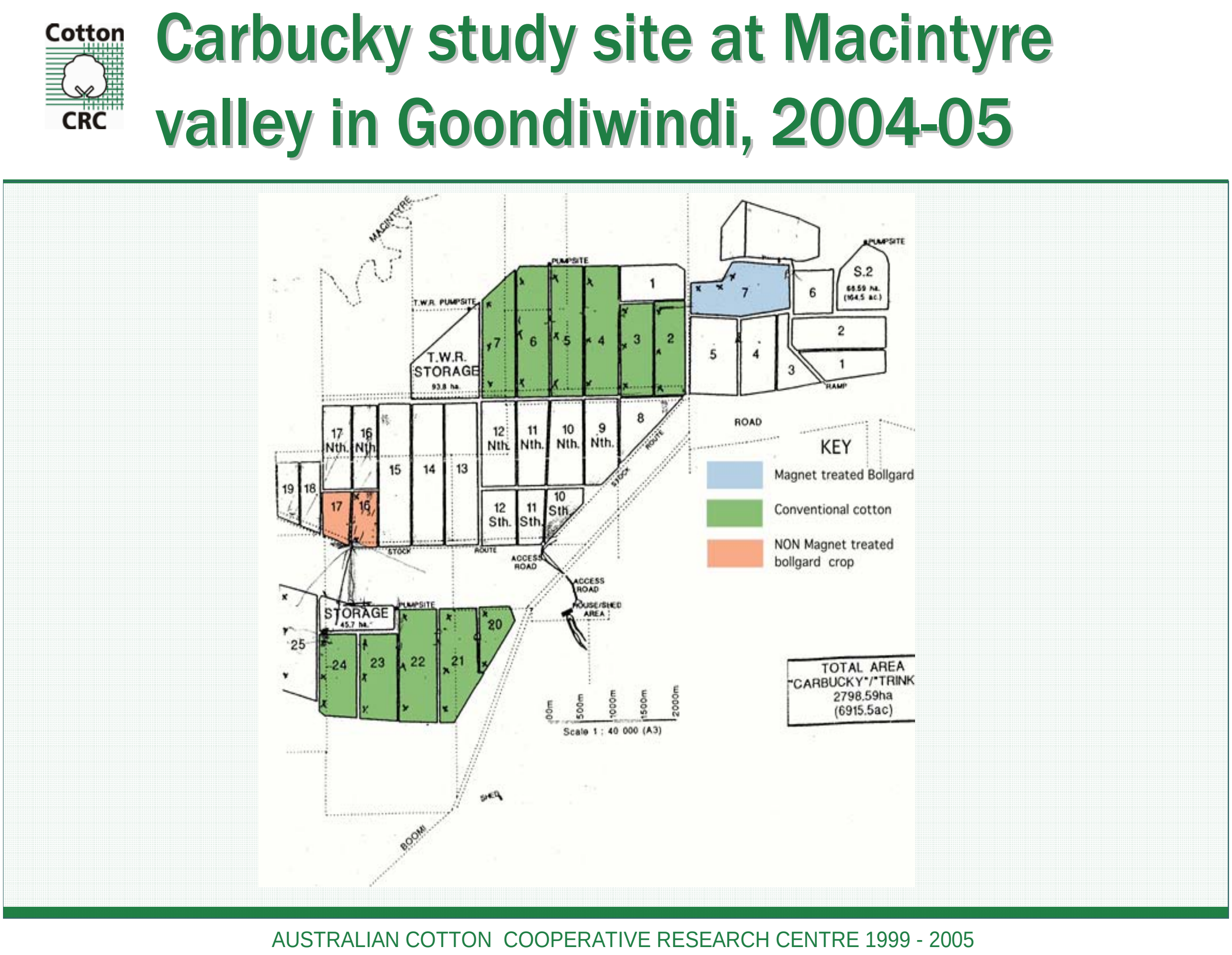


Figure 18. Moth attractant study site at Carbucky near Goondiwindi, 2004-05.

6.1.3 Application of treatments

A 20 L Magnet®) formulation was applied at 500ml per 100 m row of cotton plants in a narrow (50 cm wide) band on the foliage of single rows of the “treated” BollgardII® cotton crop at 72 m spacings using a rig fitted to a motor bike. The motor bike was fitted with a third wheel to allow operations in a row crop. The motorbike speed was between 15-20 km/hr. In 2004-05 season, three applications were made on 26 November, 15 December 2004 and 7 January 2005 using the motor bike rig.

In 2005-06, application of the Magnet® formulation was made on 8 December 2005 and 12 January 2006 using the motor bike rig. In both 2004-05 and 2005-06 seasons, the application dates coincided with the pre and peak squaring periods of the cotton plants when *Helicoverpa* moths were abundant in the study area. The decision to apply the Magnet® was based on consultant and grower observations of moths flying around in the farm.

6.1.4 Sampling

6.1.4.1 Flush counts of Helicoverpa adult moths, eggs and larvae

In assessing *Helicoverpa* moth numbers in the "treated" and "untreated" BollgardII® and conventional cotton fields, 4 m (rows) wide by 10 m long plots were marked with pegs in each subplot or replicate (27 ha) of the "treated" and "untreated" BollgardII® and conventional cotton fields. Flushing moths from each of the 4 by 10 m subplots (replicates) assessed the *Helicoverpa* adult population in the "treated" and "untreated (control)" BollgardII® and conventional cotton crops. The number of moths per ha was estimated from these counts.

In 2004-05, *Helicoverpa* moth counts were taken on 25 November, 14 December, 6 and 13 January (i.e. 24 hours prior to application of the Magnet® formulation). In 2005-06, the counts were taken on 7 December 2005, 11 and 17 January 2006. These were done by walking 50 m into the field and throwing one handful of dry gravely soil across each plot and counting the number of *Helicoverpa* moths that were disturbed and emerged from the canopy.[21]

Visual counts of *Helicoverpa* spp. eggs and larvae on whole cotton plants in each of the "treated" and "untreated (control)" fields were made twice a week commencing at 24 hours of after each treatment in four randomly selected 1m lengths of row of each treatment replicate, i.e. a total of 4m per row of cotton in each treatment. Counts were separated into *Helicoverpa* spp. eggs and larvae. Data were expressed as numbers per metre for each treatment.

6.1.4.2 Dead moth counts

Dead moths were assessed 3 days after each Magnet® spray in the "treated" and "untreated" BollgardII® and conventional cotton crops by walking 50 m into the crop in the furrows beside the rows where the Magnet® formulation was applied (in the case of "treated" BollgardII® crop) and at 72 m spacing in the "treated" conventional and the "untreated" BollgardII® and conventional cotton crops. A metre stick was placed in the furrow and all dead moths in the one metre length of furrow were counted. This was repeated in each of the 4 plots in the treated and control plots.

6.1.5 Control of Helicoverpa spp. on "treated" and "untreated" conventional cotton crops and cost effectiveness of the attract and kill strategy

The aim of the study was to determine whether *Helicoverpa* spp. infestation and the cost of controlling *Helicoverpa* spp. on the conventional cotton crops located near the "treated"

BollgardII® cotton field were lower than the conventional cotton crops located near the "untreated" BollgardII® crops. Therefore, the conventional cotton crops in both "treated" and "untreated (control)" cotton fields were managed through the season using IPM strategies previously described in the IPM Guidelines.

Foliar application of pesticides on the "treated" conventional cotton crops commenced on 7 November 2004 and "untreated (control)" on 1 December 2004 (Table 12). The last spray application on the "treated" conventional cotton crops occurred on 2 March 2005 and the "untreated" on 2 April 2005 (Table 12). In 2005-06, application of pesticides to control *Helicoverpa* spp. on the "treated" and "untreated" conventional cotton crops commenced on 9 November 2005 but was completed on 19 January 2006 because the grower decided to terminate the trial after the attracticide spray on 12 January 2006. This was due to consistent high moth pressure affecting the whole cotton growing region as a result of early season rainfall, availability of weeds and host plants supporting the build up of *Helicoverpa* moth population. The decision to apply pesticides to control *Helicoverpa* spp. on each treatment was based on a predator-to- *Helicoverpa* spp. eggs and larvae (pest) ratio of 0.5. The grower did all pesticide applications. In addition, all farm management or agronomic inputs in each treatment were the same and applied by the farmer.

At the end of the season, the benefit (in terms of pest control) to the grower of the "treated" and "untreated (control)" conventional cotton crops was calculated on the quantity of insecticides sprayed, cost of insecticides and insecticide application costs (Table 12).

6.1.5.1 Analysis of data

All data was analysed using repeated measures ANOVA (Graphpad Instat Software, Inc., Version 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer multiple comparisons test was used to separate the means.
In the analysis of dead moths, all data collected 3 days after treatment was transformed by (X + 0.5) before analysis. Arithmetic, rather than transformed means are given in the results.

6.2 Results

6.2.1 Flush counts of adult moths

The number of *Helicoverpa* moths per ha recorded on the "treated" and "untreated" BollgardII® and conventional cotton crops in 2004-05 season is given in Figure 19. After the first application of Magnet® formulation (i.e. 26 November 2004), the number of *Helicoverpa* moths recorded on the "treated" BollgardII® cotton crop increased from 500 to 2,485 per ha and that on the "untreated" BollgardII® crop from 438 to 610 per ha (Figure 19). In the conventional cotton crop located 500 m away from the "treated" BollgardII®, the number of *Helicoverpa* moths increased from 510 to 950 per ha compared to 408 to 625 per ha in "untreated" conventional cotton crop (Figure 19).

After the second treatment application the number of *Helicoverpa* moths on the "treated" BollgardII® and "treated" conventional cotton crops (located 0.5 km away) declined significantly ($P<0.01$) from 2,485 to 1,860 and 950 to 408 per ha respectively (Figure 1). In contrast, the number of moths on the "untreated" BollgardII® and "untreated" conventional cotton crops (located 0.5 km away) increased from 610 to 750 and 625 to 855 per ha respectively (Figure 19).

The third treatment application resulted in the decline of moths in all treatments (Figure 19). The number of moths on the “treated” BollgardII® and “treated” conventional cotton crops again declined from 1860 to 980 and 408 to 85 respectively and that of the “untreated” BollgardII® and conventional cotton crops reduced from 750 to 405 and 855 to 510 respectively (Figure 19). The higher decline in moth numbers occurred on the “treated” BollgardII® and conventional cotton crops indicating moths attracted to the “treated” BollgardII® field were killed by the Magnet® formulation thereby significantly reducing moth numbers (P<0.01) on the adjacent conventional cotton crops and to a lesser extent on the “untreated” fields. This was supported by the higher number of dead moths recorded in the “treated” BollgardII® and conventional cotton crops (2.75 ± 0.48 and 0.25 ± 0.15 per metre respectively) compared with 0.01± 0.01 per metre recorded on the “untreated” BollgardII® and conventional cotton crops (Table 13).

Overall, the highest number of moths per ha per sample date was recorded on the “treated” BollgardII® crop (1456.3 ± 443.71), followed by the “untreated” conventional crops (599.50 ± 96.01) and “untreated” BollgardII® crop (550.75 ± 80.19) (Figure 19). The “treated” conventional cotton crop located 0.5 km away recorded the lowest number of moths per ha per sample date (488.25 ± 178.59) (Figure 19). This was not significantly different (P>0.05) from the conventional crop located 1.0 km away but was significantly different (P<0.01) from the “treated” conventional crops located 1.5 to 3.0 km away. No significant difference (P>0.05) was detected between the number of moths recorded on the “untreated” BollgardII® and conventional cotton crops (Figure 19).

The estimated number of moths per ha recorded on the “treated” BollgardII® and conventional cotton crops during 2005-06 season is given in Figure 2. No significant difference (P>0.05) was detected between the number of moths per ha recorded on the “treated” Bollgard® and conventional cotton crops (Figure 20) despite the high number of dead moths (5.12 ± 0.88 per metre) recorded in the treated BollgardII® crop (Table 13).

Overall, the number of moths recorded in the study area ranged from 3,000 to 5,500 per ha in 2005-06 season compared to 500 to 2,500 in the 2004-05 season (Figures 19 and 20) indicating a higher moth pressure during the 2005-06 season (Figure 19 and 20). Due to the high moth pressure in 2005-06 season the study was discontinued on 19 January 2006 upon the advice of the grower. Prior to the termination of the 2005-06 study, all treatments had very high infestation of moths and no significant difference (P>0.05) were detected in moth numbers among the treated (Figure 20) and “untreated” BollgardII® and conventional cotton crops.

Table 12. *Cost effectiveness of Attract and kill formulation of Magnet® and chemicals applied to conventional cotton crops located 500m from "treated" and "untreated" Bollgard® cotton fields at Carbucky near Goondiwindi from October 2004 to March 2005.*

Treatments	Product/Chemical	Date applied	Cost of pest control (Pesticide + Spray costs) ($/ha)
Treated conventional cotton crops	0.60L//ha Canopy oil + 2L/ha Dipel SC (Bt) by Groundrig	7/11/04	29.00
	0.60L//ha Canopy oil + 2L/ha Dipel SC (Bt) by Aircraft	23/11/04	28.90
	2L/ha Canopy oil	28/11/04	18.00
	3 applications of 0.50L//ha Magnet® *to* BollgardII® *crops* by Groundrig	26/11/04;15/12/04 & 7/1/05	30.00
	0.60L/ha Canopy oil + 0.20L/ha Spinosad by Aircraft	8/12/04	84.30
	0.60L/ha Canopy oil + 0.85L/ha Indoxacarb by Aircraft	22/12/04	81.65
	0.60L/ha Canopy oil + 0.20L/ha Spinosad by Aircraft	5/1/05	84.30
	0.70L/ha Emamectin benzoate by Aircraft	17/1/05	89.70
	0.60L/ha Canopy oil + 0.85L/ha Indoxacarb by Aircraft	2/2/05	81.65
	0.60L/ha Diafenthiuron by Aircraft	2/3/05	61.70
	0.50L/ha Canopy oil by air	23/3/05	17.50
Total			606.70
Untreated conventional cotton crops	3L//ha Canopy oil + 2L/ha Dipel SC (Bt) by groundrig	1/12/04	33.00
	0.60L/ha Canopy oil + 0.20L/ha Spinosad by Air	8/12/04	84.30
	0.60L/ha Canopy oil + 0.85L/ha Indoxacarb by Air	22/12/04	81.65
	0.60L/ha Canopy oil + 0.20L/ha Spinosad by Air	1/1/05	84.30
	0.70L/ha Emamectin benzoate	11/1/05	89.70
	0.60L/ha Canopy oil + 0.85L/ha Indoxacarb by Air	21/1/05	81.65
	0.60L/ha Canopy oil + 0.20L/ha Spinosad by Air	12/2/05	84.30
	0.60L/ha Diafenthiuron by Air	2/3/05	61.70
	0.50L/ha Canopy oil by Air	2/4/05	17.50
Total			618.10/ha

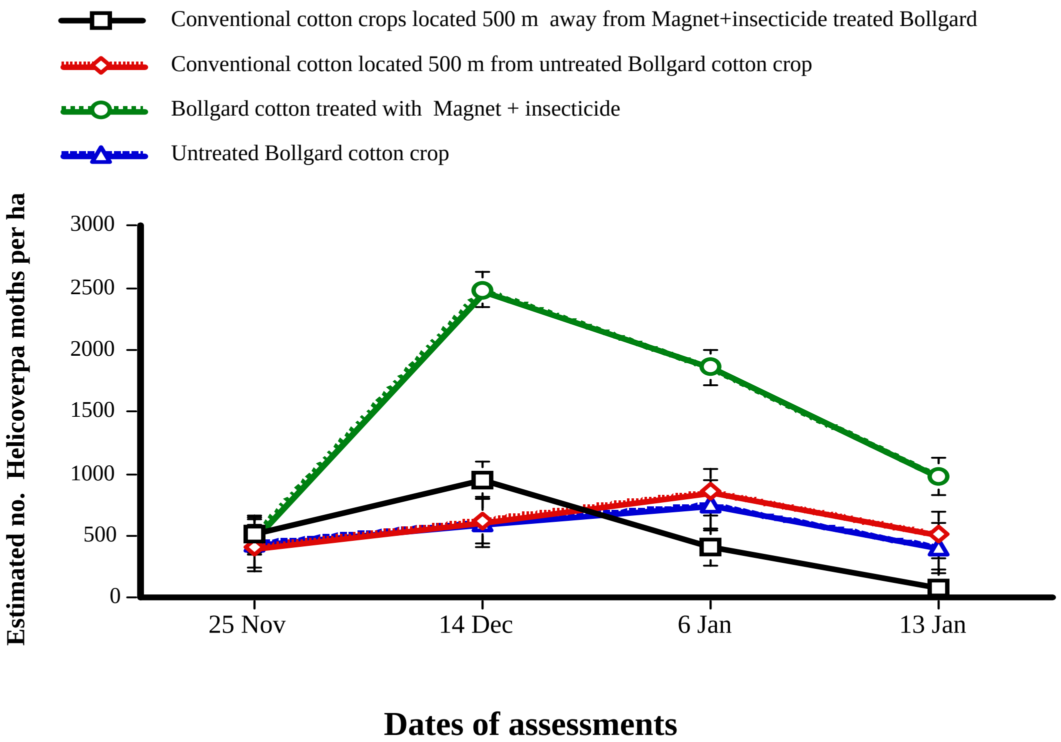


Figure 19. Number of Helicoverpa spp. moths on conventional cotton crops located 500 m from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carbucky near Goondiwindi in 2004-05 (Treatments were applied on 25 November 2004, 15 December 2004 and 7 January 2005)

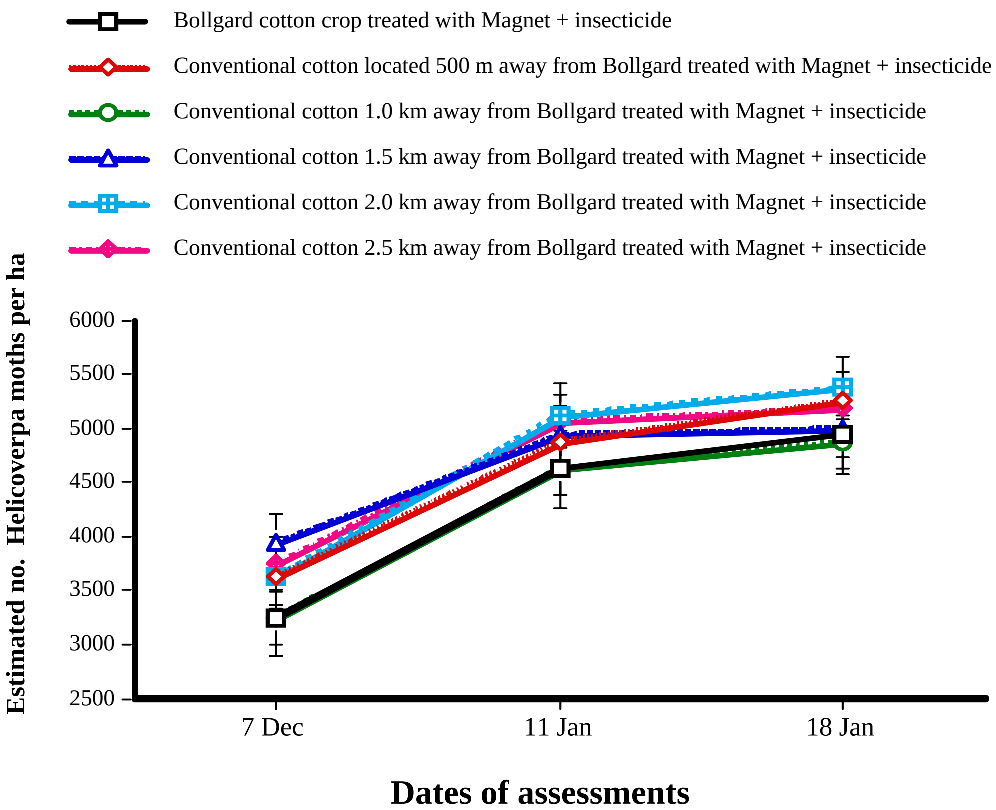


Figure 20. Number of Helicoverpa spp. on conventional cotton crops located 0.5 – 2.5 km away from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carbucky near Goondiwindi in 2005-06 (Treatments were applied on 8 December 2005 and 12 January 2006).

6.2.2 Dead moth counts

Counts of dead moths provided only a rough estimate of the actual death rate because many of the moths are either eaten by ants or fall into cracks in the soil. In 2004-05 and 2005-06, the number of dead moths per metre recorded in the "treated" BollgardII® crops was significantly higher ($P<0.0001$) than those recorded in the "treated" conventional cotton crops and the control plots (Table 13). The "treated" conventional cotton crops located 0.5 to 1.0km away from the "treated" BollgardII® cotton crop had significantly ($P<0.05$) more dead moths per metre than the "treated" conventional cotton crops located 1.5 to 3 km away from the "treated" BollgardII® crop (Table 13). No dead moths were found in the "treated" conventional cotton crops located 1.5 to 3 km away from the "treated" BollgardII® crop (Table 13). In contrast, we found 0.01 moths per metre in the conventional cotton crop located 2 km away from the "untreated (control)" BollgardII® field (Table 13). All dead moths counted were blue from the Magnet® dye indicating they had fed on the Magnet® formulation.

Overall, the number of dead moths per metre recorded in the "treated" Bollgard® cotton crop in 2005-06 was 50% higher than in 2004-05. This is the result of high moth pressure than efficacy experienced in the 2005-06 season, indicating more moths were attracted to the Magnet® formulation and killed (Table 13). Despite the high moth kill by the Magnet® formulation, the number of residual moths in the environment was too high to manage. Therefore, the study was terminated on 19 January after the third treatment application.

Table 13. Counts of dead Helicoverpa spp. adults (moths) found in 1 metre row sample strips in the Magnet® plus insecticide – treated and untreated BollgardII® cotton fields and conventional cotton fields located at varying distances away from the BollgardII® fields, Carbucky near Goondiwindi, 2004-06 (n = 4 m)

Treatments	Helicoverpa density (Dead moths/m) 3 DAT		
	2004-05 season		2005-06 season
	[1]Treated	[2]Control	[1]Treated only
BollgardII® cotton field treated or untreated with Magnet® plus insecticides	2.75 ± 0.48a	0.01± 0.01c	5.12 ± 0.88a
Conventional cotton field located 0.5 km away from BollgardII® field	0.25 ± 0.15b	0.01 ± 0.01c	0.50 ± 0.20b
Conventional cotton located 1.0 km away from BollgardII® field	0.08 ± 0.01c	0c	0.13 ± 0.13c
Conventional cotton located 1.5 km away from BollgardII® field	0 c	0c	0c
Conventional cotton located 2.0 km away from BollgardII® field	0 c	0.01± 0.01 c	0c
Conventional cotton located 2.5 km away from BollgardII® field	0 c	0c	0c
Conventional cotton located 3.0 km away from BollgardII® field	0 c	0c	–

Means within columns followed by the same letter are not significantly different ($P>0.05$), Tukey-Kramer multiple comparison test (DAT = days after treatment)

[1] *BollgardII® cotton crops treated with Magnet® mixed with insecticide (thiodicarb) and conventional cotton crops located 0.5-3.0 km from treated Bollgard® crops*

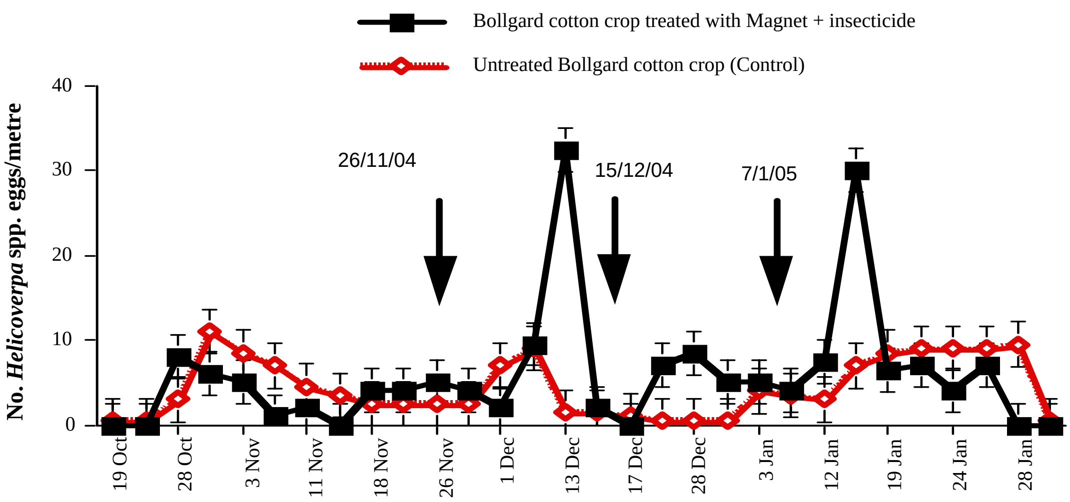
[2] *BollgardII® cotton crops untreated with Magnet® mixed with insecticides conventional cotton crops located 0.5-3.0 km from untreated BollgardII® crops*

6.2.3 Helicoverpa spp. assessments on "treated" and "untreated" BollgardII® crops

6.2.3.1 Eggs and larval counts

The study showed that the number of eggs laid on the "treated" BollgardII® cotton crop was significantly higher (P<0.005) than the "untreated (control)" Bollgard® cotton crop (Figure 21). The number of eggs per metre on the "treated" BollgardII® cotton crop ranged from 5 to 30 per metre compared with 0 to 10 per metre on the untreated BollgardII® crop (Figure 21). Although the high number of eggs recorded on the "treated" BollgardII® crop was the highest among the treatments, the number of larvae per metre was not significantly different (P>0.05) between the "treated" and "untreated (control)" BollgardII® and conventional cotton crops (Figure 21). On the "treated" and "untreated" BollgardII® cotton crops, the number of larvae per metre ranged from 0 to 0.5 per metre (Figure 21). This indicates that any eggs that hatched on the treated BollgardII® crop were killed by the BollgardII® toxin.

In 2005-06, no significant difference (P>0.05) was detected in the number of eggs and larvae on the "treated" and "untreated" BollgardII® and conventional cotton crops at the time the trial was terminated. An explanation for this was that high number of residual moths in the environment resulted in a high egg lay on both treated and control plots.



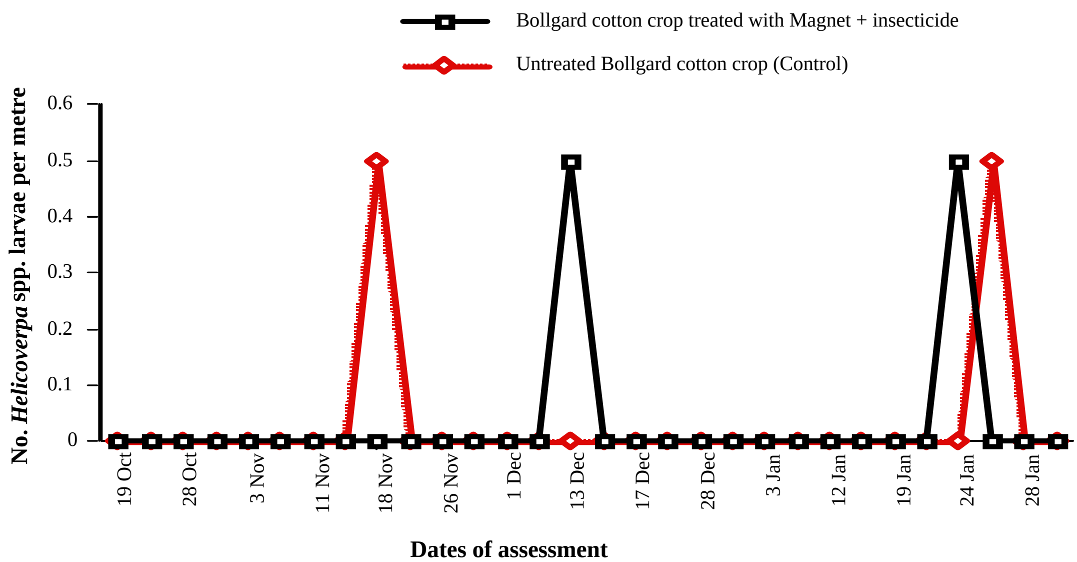


Figure 21. Effect of application of Magnet® mixed with insecticide on BollgardII® cotton crops on oviposition and larval survival of Helicoverpa spp. at Carbucky near Goondiwindi in 2004-05.

6.2.4 Control of Helicoverpa spp. on "treated" and "untreated" conventional cotton crops and cost effectiveness of the attract and kill strategy

6.2.4.1 Counts of Helicoverpa spp. egg and larvae on "treated" and "untreated" conventional cotton

Prior to the first treatment application, the same number of *Helicoverpa* spp. eggs and larvae per metre were recorded on "treated" and "untreated" conventional cotton crops located 0.5 and 1.0 km away from the treated and untreated BollgardII® cotton crops during the 2004-05 season (Figure 22 and 23). However, after the application of Magnet® formulation onto the BollgardII® cotton crop, the number of eggs and larvae per metre on the "treated" conventional cotton crops located 0.5 km away from the "treated" BollgardII® crop were significantly lower (P<0.005) than the "untreated" conventional cotton crops located same distance from the "untreated" BollgardII® crop (Figure 22 and 23). The number of eggs and larvae per metre on the "treated" conventional cotton crops located 0.5 km away was significantly lower (P<0.001) than the "treated" conventional cotton crops located 1.0 and 1.5 km away (Figure 24). In contrast, no significant difference (P>0.05) was detected in the number of eggs and larvae per metre on the "treated" BollgardII® and conventional cotton crops located 0.50 and 1.0 km away (Figure 25). In addition, no significant difference (P>0.05) was detected in the "untreated" conventional cotton crops located 0.5 and 1 km away from the "untreated" BollgardII® cotton crops.

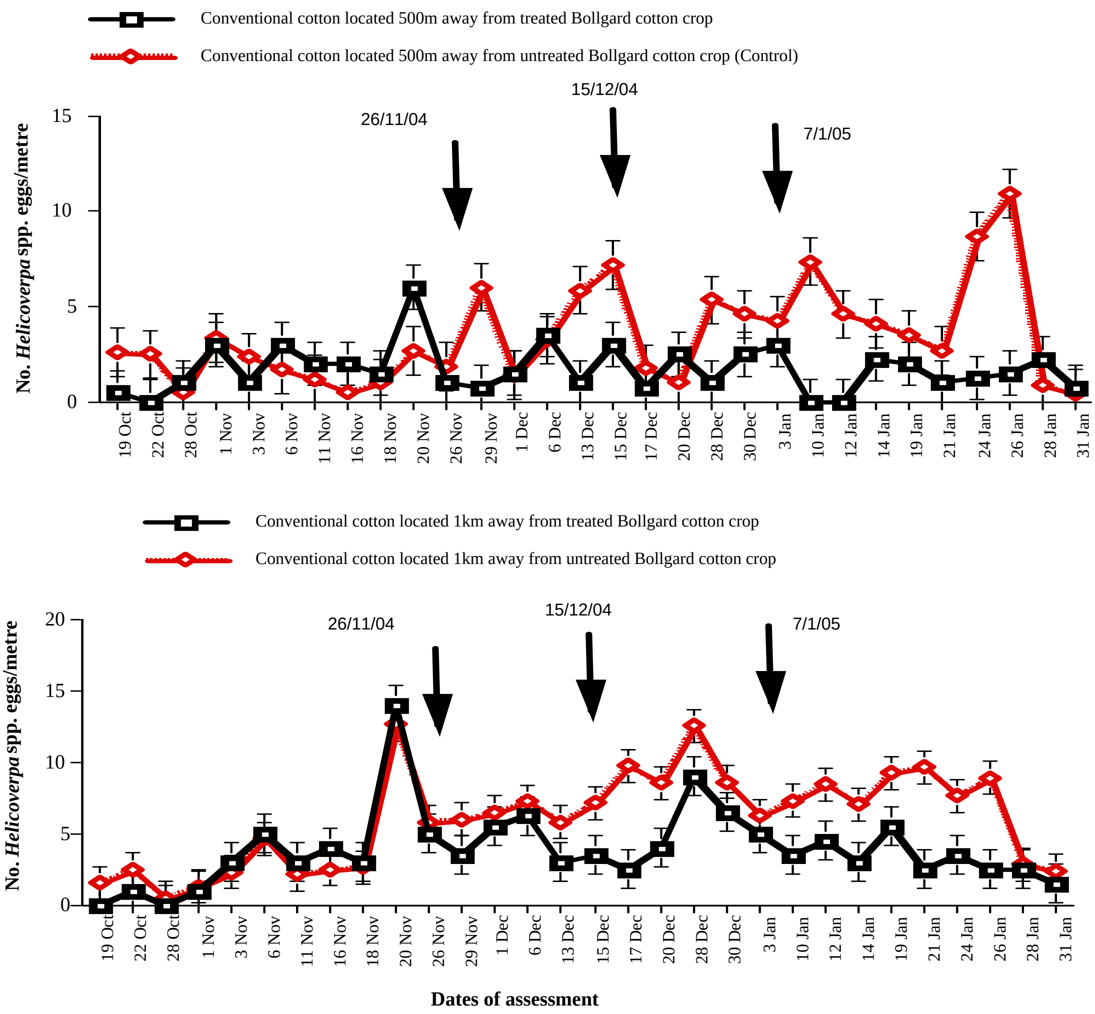


Figure 22. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of Helicoverpa spp. eggs per metre on conventional cotton crops located 0.5 and 1 km away from "treated" and "untreated" Bollgard® (transgenic) cotton crops at Carbucky in Goondiwindi in 2004-05.

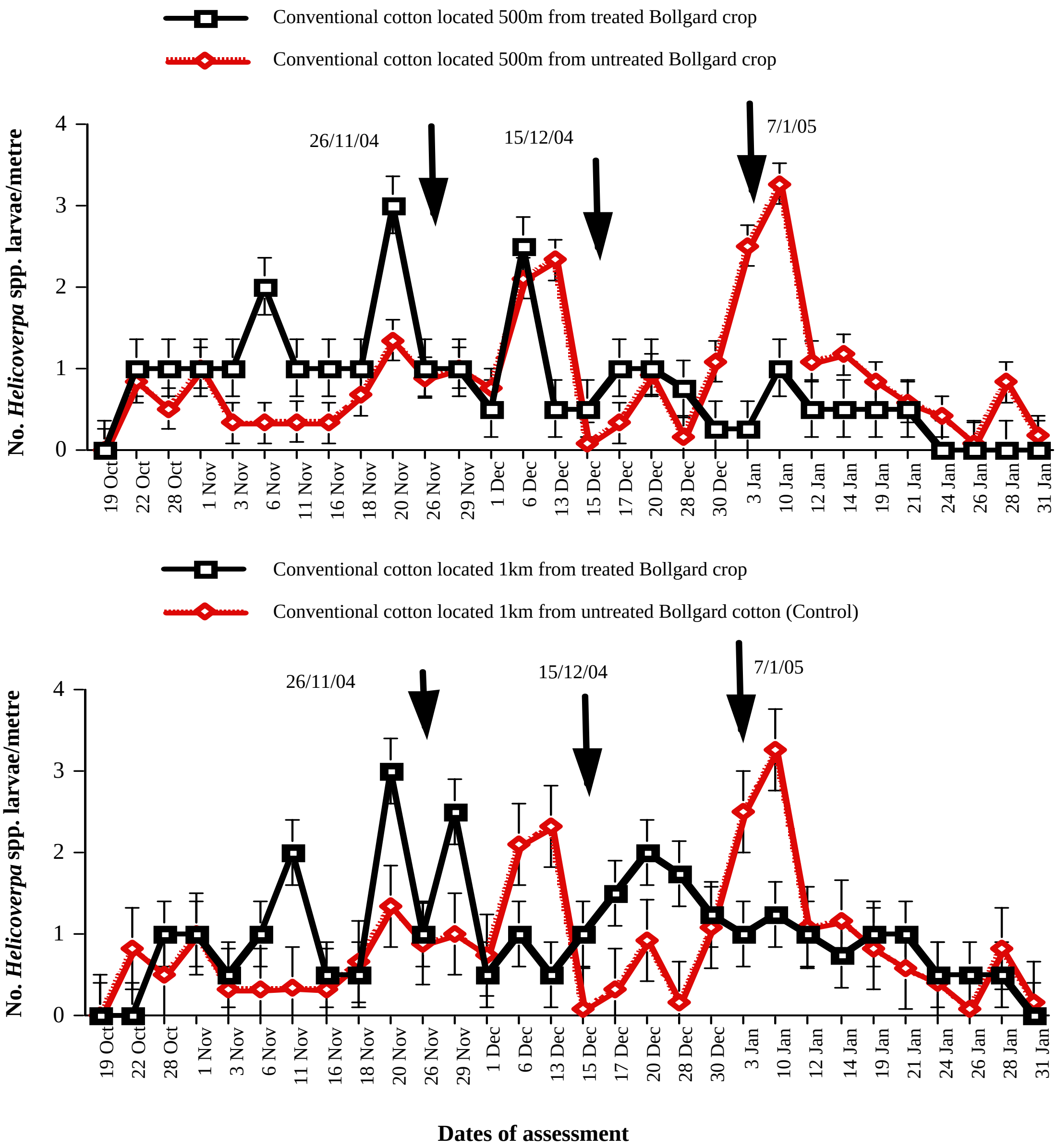


Figure 23. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of Helicoverpa spp. larvae per metre on conventional cotton crops located 0.5 and 1 km away from "treated" and "untreated" BollgardII® (transgenic) cotton crops at Carbucky in Goondiwindi in 2004-05.

6.2.4.2 Control of Helicoverpa spp. on "treated" and "untreated" conventional cotton crops

The total number of products (biological and synthetic insecticides) applied to the "treated" and "untreated" conventional cotton crops located 0.5 km away is given in (Table 12). The overall number of larvae per metre recorded in 2004-05 season on the "treated" conventional cotton crops was significantly lower ($P<0.05$) than the "untreated" conventional cotton crops (see Figures 22 and 23). Control of *Helicoverpa* spp. larvae on the "treated" conventional cotton crops commenced on 7 November 2004 whereas the "untreated" conventional crops commenced on 1 December 2004 (Table 12). An explanation for this was some moths attracted to the Magnet® formulation on the "treated" Bollgard® crops initially land on the adjacent conventional crops and lay some eggs before moving on to the Magnet source on the BollgardII® crops. In all, we applied seven pesticide sprays to manage *Helicoverpa* spp.

on the "treated" and nine on the "untreated" conventional cotton crops in the 2004-05 season (Table 12). There was no insecticide savings on the "treated" conventional cotton crops relative to the "untreated" conventional cotton crops in 2005-06 season.

The benefit to the grower in terms of pest control was $11.40 per hectare on the "treated" over the "untreated" conventional crops in 2004-05 season (Table 12). However, in 2005-06 season, no benefit was achieved by the grower in terms of pest control in relation to application of Magnet® formulation on the BollgardII® cotton crops. This was due to high *Helicoverpa* spp. pressure in that season and the fact that the grower terminated the trial early (19 January 2006) when each treatment has received 6 pesticide sprays against *Helicoverpa* spp.

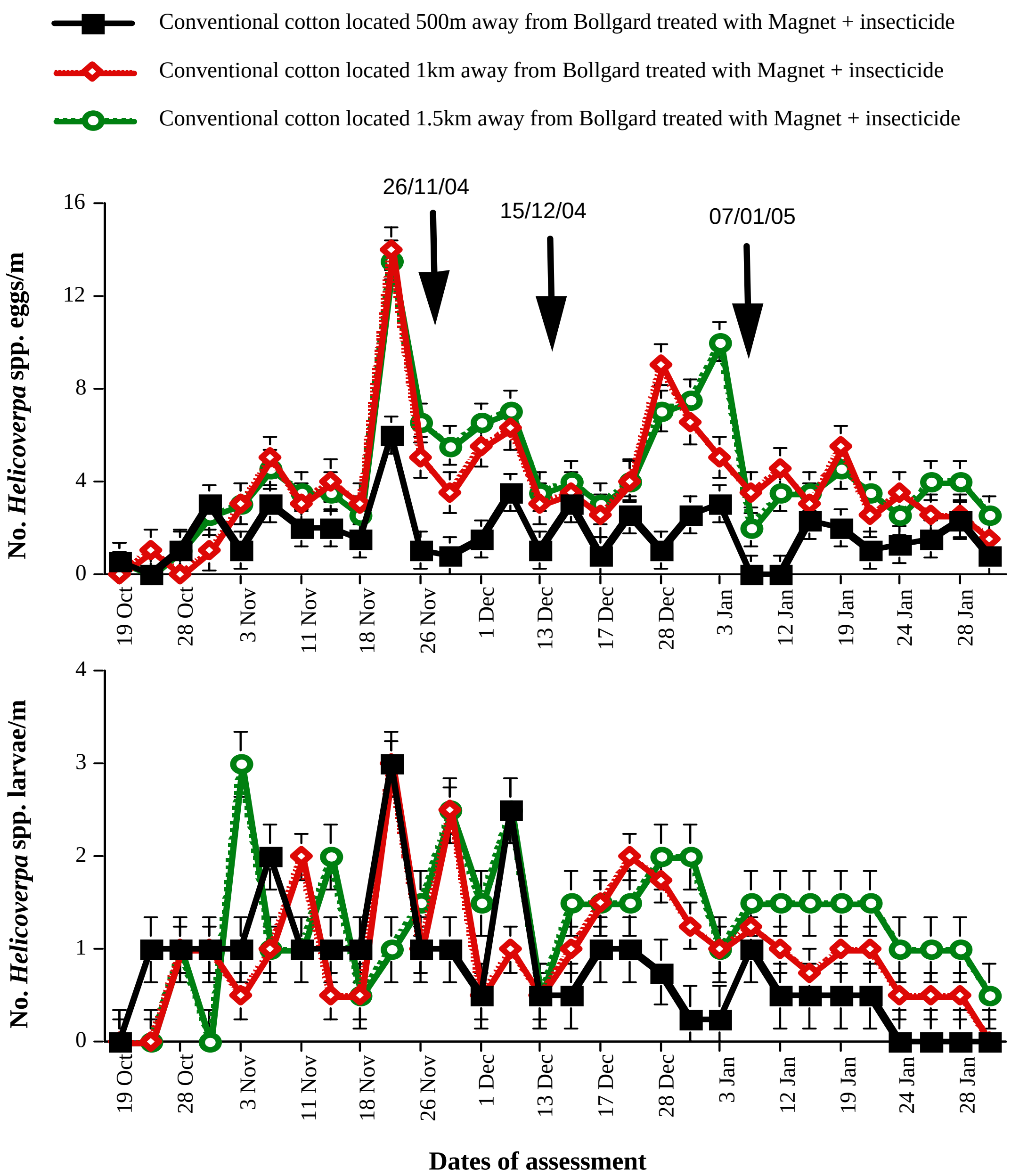


Figure 24. Comparison of numbers of Helicoverpa spp. eggs and larvae per metre on conventional cotton crops located 0.5, 1.0 and 1.5 km away from Bollgard® (transgenic) cotton crops treated with Magnet® mixed with insecticide (thiodicarb) at Carbucky in Goondiwindi in 2004-05.

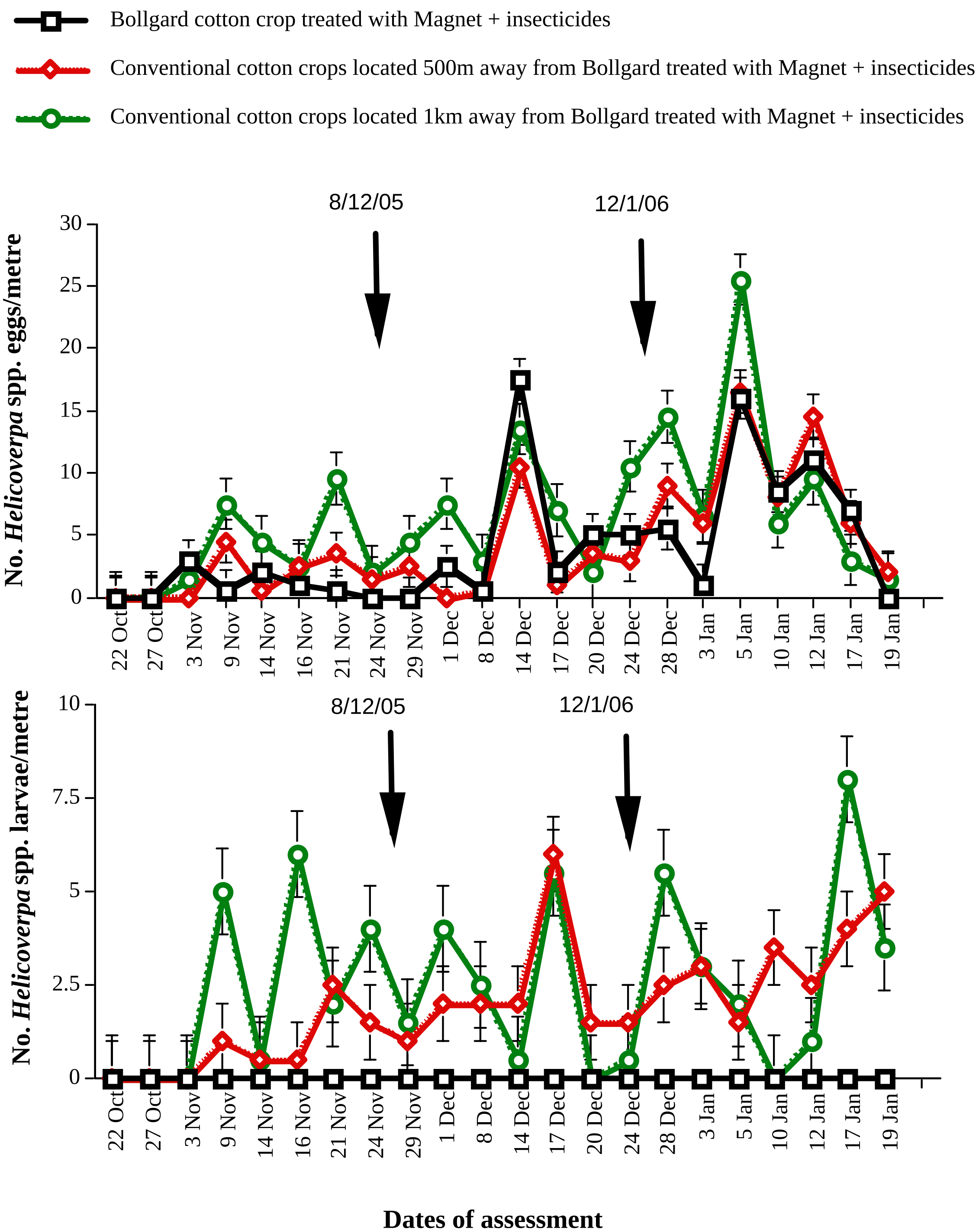


Figure 25. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of Helicoverpa spp. eggs and larvae per metre on conventional cotton crops located 0.5 and 1 km away from "treated" Bollgard® (transgenic) cotton crops at Carbucky in Goondiwindi in 2005-06.

6.2.4.3 Conclusion

- Three applications of Magnet (attracticide) to BollgardII® cotton crops reduced Helicoverpa moth populations on conventional cotton crops located 0.5 km away from 950 to 85 moths per ha.
- In addition, it reduced the number of Helicoverpa eggs and larvae on the “treated” conventional cotton crops.
- In terms of *Helicoverpa* control, the grower saved $11.40 per ha on the “treated” over the “untreated” conventional cotton crops.

7.0 Determine strategic use of Magnet (moth) attractant on Bollgard II cotton crops and PSOs on conventional cotton crops on the level of *Helicoverpa* spp. production and management of Helicoverpa spp. on the conventional crops

Application of Magnet® mixed with toxicant on BollgardII® cotton crops can reduce *Helicoverpa* spp. infestation and synthetic insecticide use on adjacent conventional cotton crops. The Magnet® formulation applied to the transgenic cotton crops attracted moths from the environment to the treated area and those residual moths that could not reach the Magnet® treated zone before the Magnet® odour dissipated stayed and laid on the conventional cotton crop thereby reducing the total benefit of the attract and kill strategy.

Many studies have reported that petroleum spray oils (PSOs) similar to summer spray oils or nC19-27 oils can be used in cotton to reduce *Helicoverpa* spp. egg lay and also cause mortality to larvae. Hence there is the need to undertake studies whereby nC27 PSO is applied to conventional cotton crops located near Bollgard II crops treated with Magnet® mixed with toxicant. By doing this, residual moths that may be attracted by the Magnet® formulation to the treated zone but could not reach the Magnet® before the Magnet® odour dissipated may be deterred by the PSO from ovipositing on the conventional cotton crop.

In this study, Magnet® formulated with insecticide (attracticide) in combination with PSO was used in field trials to determine the effect of applying the attracticide to a centrally located BollgardII® (Bt) cotton crop and the PSO on adjacent conventional cotton crops on levels of *Helicoverpa* spp. adults, eggs and larval populations on the conventional cotton crops.

7.1 Methodology

The experiment was conducted on irrigated conventional and BollgardII® cotton fields at Carrington near Goondiwindi (28° 30'S, 150° 21'W) in Queensland in Australia during the 2004-05 cotton growing season. The layout of the study site is shown in Figure 26. The cotton crops were planted on 12 October 2004. In each year of the study, two transgenic (BollgardII®) cotton fields each measuring 50 ha and 12 conventional cotton fields each measuring 80 ha were selected for the study (Figure 26). The Bollgard II cotton crops (50 ha) was located perpendicular to the 12 conventional cotton fields. The 12 conventional cotton fields were selected so that 6 fields (each measuring approximately 80 ha) and located at 0.1, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 km away from, and perpendicular to the "treated" BollgardII® cotton field were selected to assess *Helicoverpa* spp. population (Figure 26).

Magnet mixed with Larvin was applied to the Bollgard II cotton crops and six of the conventional cotton fields were treated with Canopy® oil (PSO) and the other six fields left untreated (Figure 26). The PSO is known to suppress volatiles released by the cotton plants, and this may assist in deterring egg lay on the cotton crops treated with the PSO.

The BollgardII® cotton field that was not treated with Magnet® formulation (Magnet mixed with insecticide) was referred to as "treated Bollgard II crop", the 6 conventional cotton fields treated with Canopy® oil (PSO-treated) were referred to as ("PSO-treated") and the untreated conventional cotton fields were referred to as "untreated (control). The "treated" BollgardII® and the conventional cotton fields (PSO- treated and untreated) were each divided into four subplots and each subplot (16 ha cotton) was considered as a replicate. Each subplot or replicate was separated by a buffer of 4 ha cotton.

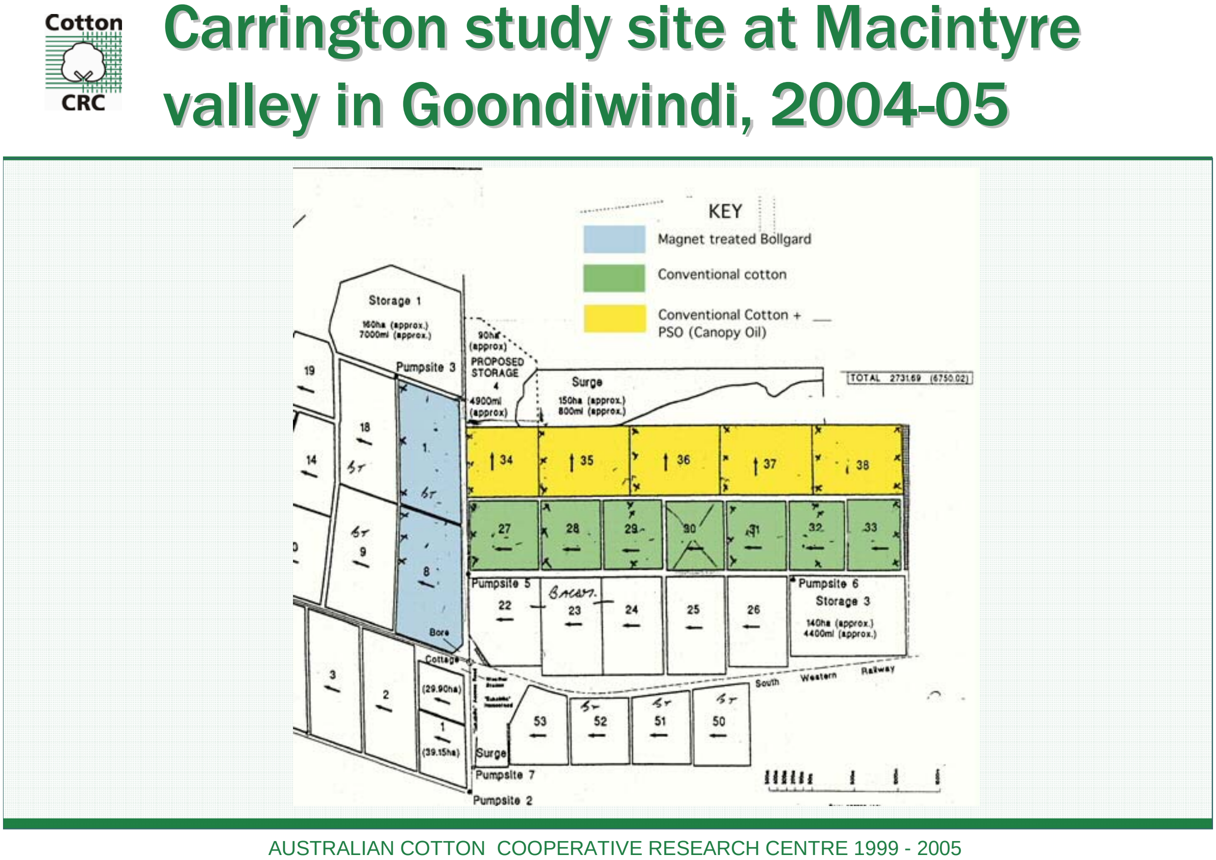


Figure 26. Moth attractant study site at Carrington near Goondiwindi, 2004-05.

7.1.1 Application of treatments

A 20 L Magnet®) formulation was applied at 500ml per 100 m row of cotton plants in a narrow (50 cm wide) band on the foliage of single rows of the "treated" Bollgard II® cotton crop at 72 m spacing using a rig fitted to a motor bike. The method of application was similar to experiment 6.0 above. In 2004-05 season, three applications were made on 30 November, 16 December 2004 and 7 January 2005.

The application dates of the Magnet® formulation on the Bollgard II cotton crops coincided with the pre and peak squaring periods of the cotton plants when *Helicoverpa* moths were abundant in the study area. The decision to apply the Magnet® was based on consultant and grower observations of moths flying around in the farm.

7.1.2 Sampling

7.1.2.1 Flush counts of Helicoverpa adult moths, eggs and larvae

In assessing *Helicoverpa* moth numbers in the BollgardII® and conventional cotton fields, 4 m (rows) wide by 10 m long plots were marked with pegs in each subplot or replicate of the BollgardII® and conventional cotton fields. Flushing moths from each of the 4 by 10 m subplots (replicates) assessed the *Helicoverpa* adult population in the BollgardII® and conventional cotton crops. The number of moths per ha was estimated from these counts.

In 2004-05, *Helicoverpa* moth counts were taken on 29 November, 15 December 2004 and 6 January 2005. Moth assessment was done by walking 50 m into the field and throwing one handful of dry gravely soil across each plot and counting the number of *Helicoverpa* moths that were disturbed and emerged from the canopy.

Visual counts of *Helicoverpa* spp. eggs and larvae on whole cotton plants in the Bollgard and the PSO treated and untreated conventional cotton fields was made twice a week commencing at 24 hours after each treatment in four randomly selected 1m lengths of row of each treatment replicate, i.e. a total of 4m per row of cotton in each treatment. Counts were separated into *Helicoverpa* spp. eggs and larvae. Data were expressed as numbers per metre for each treatment.

7.1.2.2 Dead moth counts

Dead moths in the Bollgard II and conventional cotton fields were assessed 3 days after each application of the Magnet® formulation. In the case of the BollgardII cotton fields, assessment was done by walking 50 m into the crop in the furrows beside the rows where the Magnet® formulation was applied and at 72 m spacing in the "PSO-treated" and untreated conventional cotton crops. A metre stick was placed in the furrow and all dead moths in the one metre length of furrow were counted. This was repeated in each of the 4 plots in the treated and control plots.

7.1.3 Control of Helicoverpa spp. on the PSO-treated and "untreated" conventional cotton crops and cost effectiveness of the attract and kill strategy

The aim of the study was to determine whether *Helicoverpa* spp. infestation and the cost of controlling *Helicoverpa* spp. on the PSO-treated conventional cotton crops located near BollgardII® cotton field treated with attracticide was lower than non-PSO treated conventional cotton crops located at similar position from treated BollgardII crops. The pest management and agronomic practises used on the conventional cotton crops were the same.

Application of pesticides to the PSO-treated and untreated conventional cotton crops was commenced on 5 November 2004. The decision to apply pesticides to control *Helicoverpa* spp. on each treatment was based on a predator-to- *Helicoverpa* spp. eggs and larvae (pest) ratio of 0.5. The grower did all pesticide applications. In addition, all farm management or agronomic inputs in each treatment were the same and applied by the farmer.

7.1.4 Analysis of data

All data was analysed using repeated measures ANOVA (Graphpad Instat Software, Inc., Version 2.03, San Diego, CA, USA). Treatment and sample dates were the independent variables. Tukey-Kramer multiple comparisons test was used to separate the means.
In the analysis of dead moths, all data collected 3 days after treatment was transformed by (X + 0.5) before analysis. Arithmetic, rather than transformed means are given in the results.

7.2 Results

7.2.1 Flush counts of adult moths

The number of *Helicoverpa* moths per ha recorded on the "PSO-treated" and "untreated" conventional cotton crops is given in Figure 27. The number of moths recorded on the conventional cotton crops treated with PSO and those untreated were not significantly different ($P>0.05$) on the 30 November 2004 prior to the first application of the attracticide on the Bollgard cotton crops and PSO on the conventional cotton crops (exception was conventional crops located 4 km away) (Figure 27). However, after the first application of the attracticide and the PSO, the number of *Helicoverpa* spp. moths on the conventional crops receiving PSO treatment decreased significantly whereas the number of moths increased significantly on the untreated conventional crops (Figure 27).

After the second treatment application, the number of moths recorded on both "PSO-treated" and untreated conventional cotton crops declined significantly to approximately 2000-3000 moths per ha. No significant difference was detected between the treated and untreated conventional cotton crops after the third application of the attracticide. The third treatment application resulted in the decline of moths in all treatments (Figure 27). Overall, the lowest number of moths per ha per sample date was recorded on the conventional crops that received PSO treatment and the highest on conventional crops that did not receive any PSO sprays (Figure 27).

7.2.2 Dead moth counts

The number of dead moths per metre recorded in the BollgardII® cotton crops treated with the attracticide was significantly higher ($P<0.01$) than those recorded in the PSO- treated and "untreated" conventional cotton crops located 0.10 to 4.0 km away (Table 14). The PSO-treated and untreated conventional cotton crops located 0.1 km away had 0.25 and 0.42 dead moth/m respectively compared to zero dead moths in the conventional crops located at 1.0 to 4.0 km away (Table 14). No significant differences were detected in the number of dead moths recorded on the conventional cotton crops treated with PSO and those that received no PSO sprays (Table 14). All dead moths counted were blue from the Magnet® dye indicating they had fed on the Magnet® formulation.

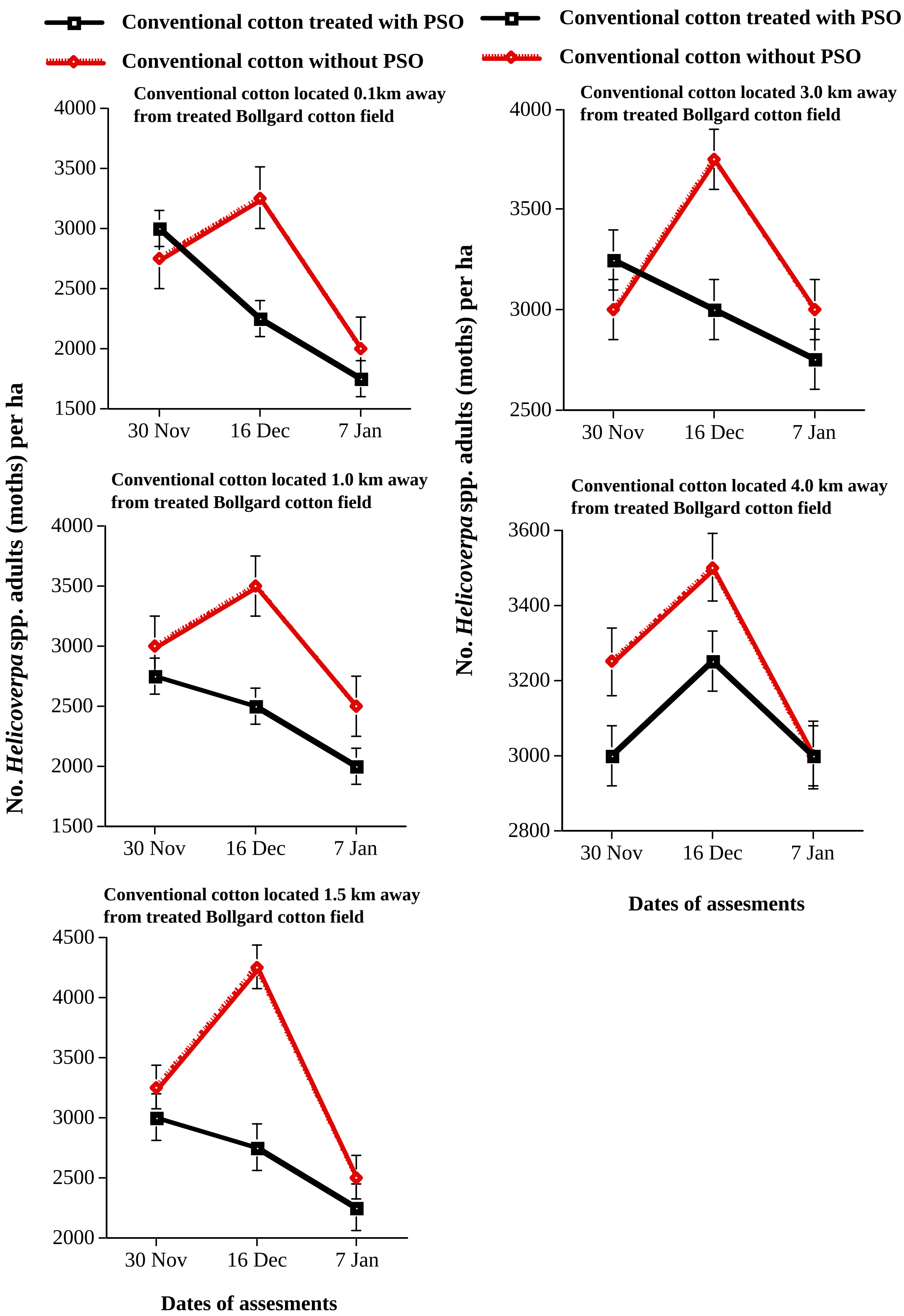


Figure 27. Comparison of *Helicoverpa* spp. moths on PSO treated and untreated conventional cotton crops located near Bollgard® cotton crops treated with Magnet® mixed with insecticides at Carrington near Goondiwindi, 2004-05 (Treatments were applied on 30 November, 16 December 2004 and 7 January 2005)

Table 14. *Counts of dead Helicoverpa spp. adults (moths) found in 1 metre row sample strips in the Magnet® plus insecticide – treated and untreated BollgardII® cotton fields and conventional cotton fields located at varying distances away from the BollgardII® fields, Carbucky near Goondiwindi, 2004-06 (n = 4 m)*

Treatments	PSO-treated	Non-PSO treated (Control)
BollgardII® cotton field treated with Magnet mixed with insecticide (Thiodicarb)	1.50 ± 0.08a	1.50 ± 0.01a
Conventional cotton field located 0.10 km away from BollgardII® field	0.25 ± 0.05b	0.42 ± 0.01b
Conventional cotton located 1.0 km away from BollgardII® field	0 b	0 b
Conventional cotton located 1.5 km away from BollgardII® field	0 b	0 b
Conventional cotton located 3.0 km away from BollgardII® field	0 b	0 b
Conventional cotton located 4.0 km away from BollgardII® field	0 c	0 b

Means within columns followed by the same letter are not significantly different (P>0.05), Tukey-Kramer multiple comparison test (DAT = days after treatment)

7.2.3 Helicoverpa spp. assessments on "treated" and "untreated" BollgardII® crops

7.2.3.1 Eggs and larval counts

The study showed that the number of eggs laid on the "treated" BollgardII® cotton crop was significantly higher (P<0.01) than the conventional cotton crops (both PSO treated and untreated) located 100 metres away (Figure 28). The conventional cotton crops that was treated with PSO and located 100 metres away from the BollgardII crop had significantly lower (P<0.01) number of eggs and larvae per metre than the conventional cotton crops located at 100 metres away from the Bollgard crop but did not receive PSO sprays (Figure 28).

At 1.0 km away from the BollgardII cotton crop, the conventional cotton crop treated with PSO had significantly lower (P<0.01) number of eggs than the conventional crops not treated with PSO (Figure 29). In contrast no significant differences (P>0.05) was detected between the number of larvae per metre on the PSO-treated and untreated conventional cotton crops (Figure 29).

Additionally, no significant differences were detected in the number of eggs and larvae on the PSO-treated and non-PSO treated conventional cotton crops located 1.5 to 4 km away from the Bollgard II cotton crops (Figures 30, 31 and 32).

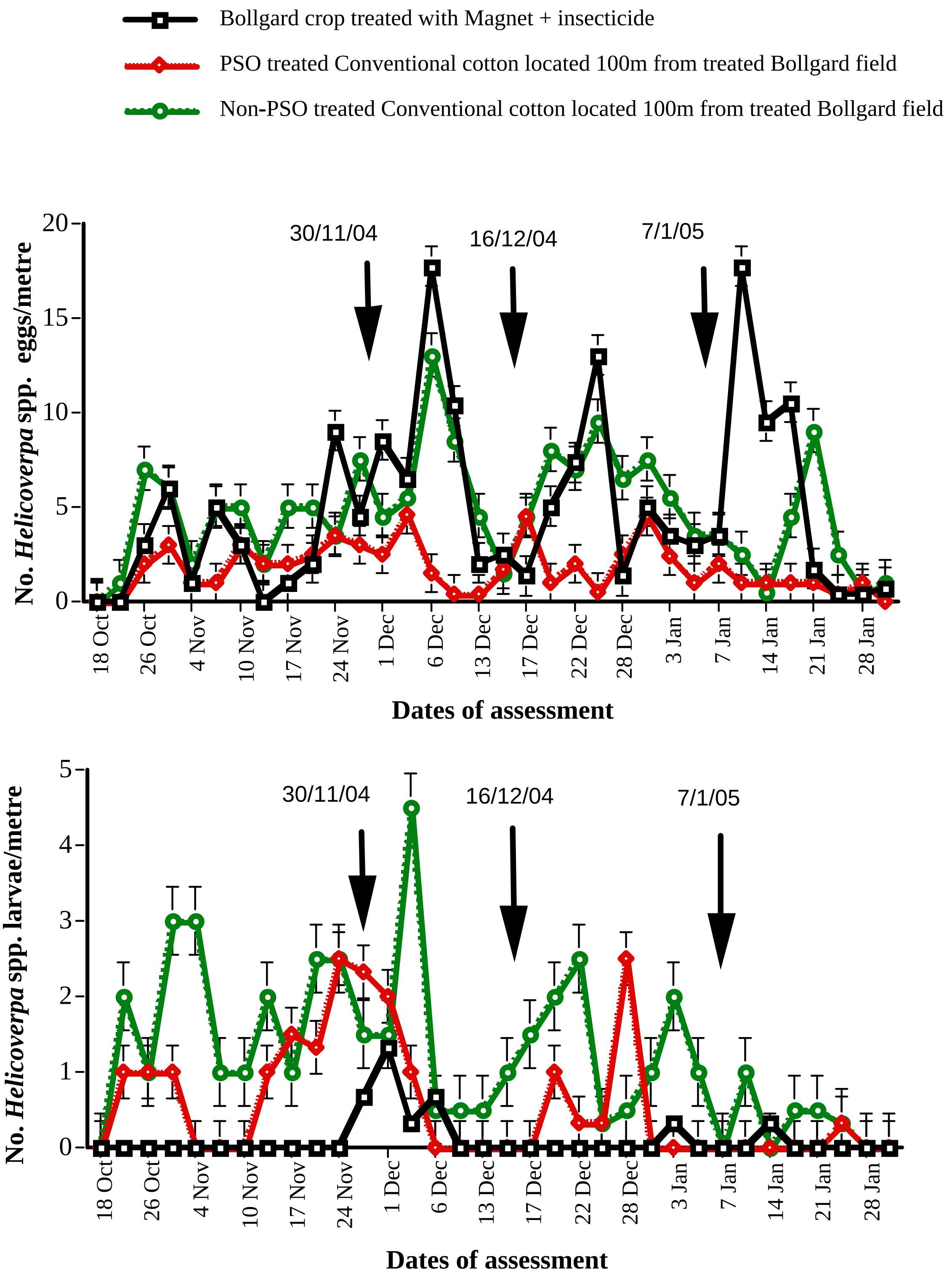


Figure 28. Comparison of numbers of *Helicoverpa* spp. eggs and larvae per metre on Bollgard® cotton crops treated with Magnet® mixed with insecticide and conventional cotton crops located 100 metres away and treated with and without PSO at Carrington near Goondiwindi in 2004-05.

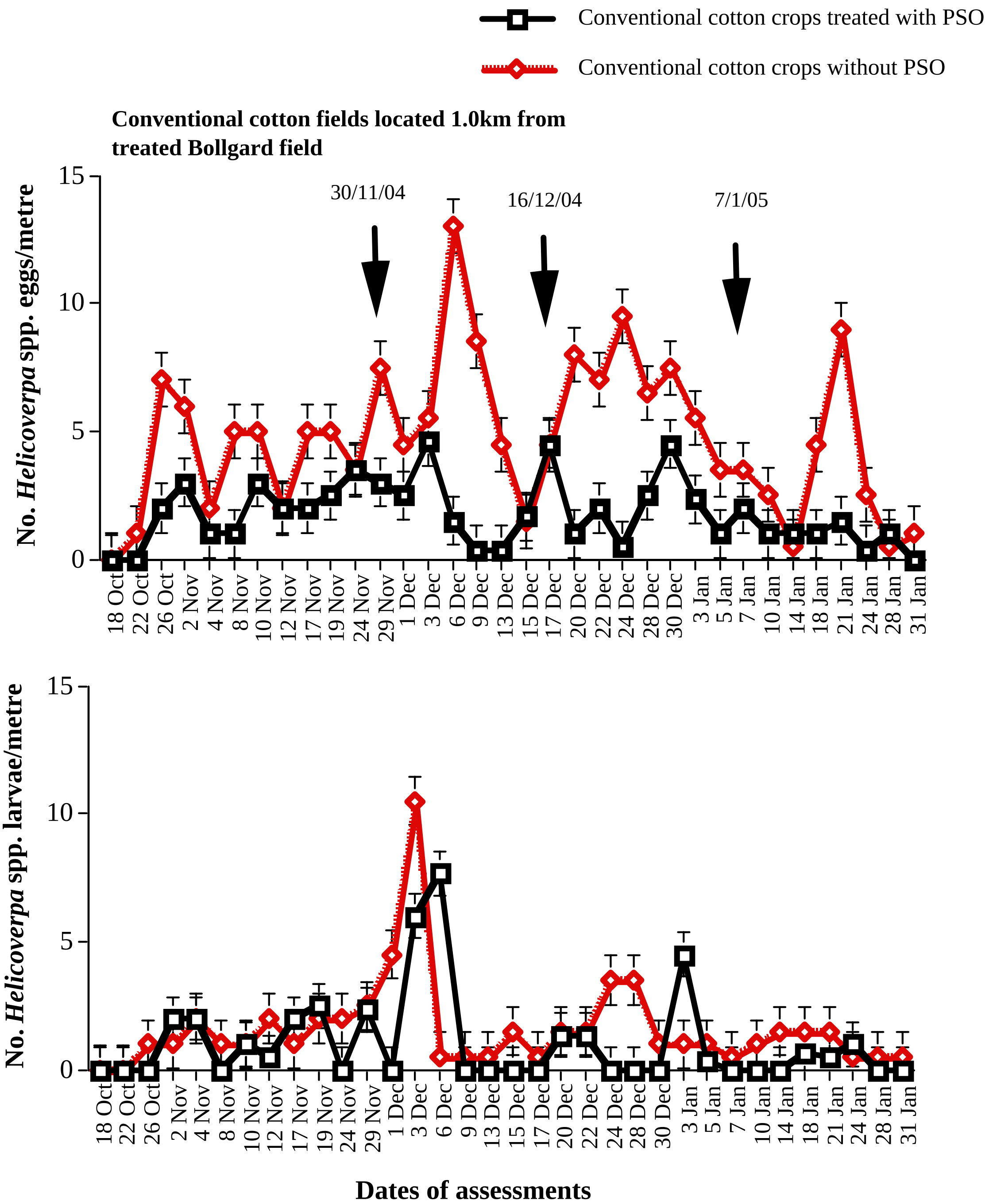


Figure 29. Comparison of numbers of Helicoverpa spp. eggs and larvae per metre on a PSO – treated and non- PSO treated conventional cotton crops located 1.0 km away from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carrington near Goondiwindi in 2004-05.

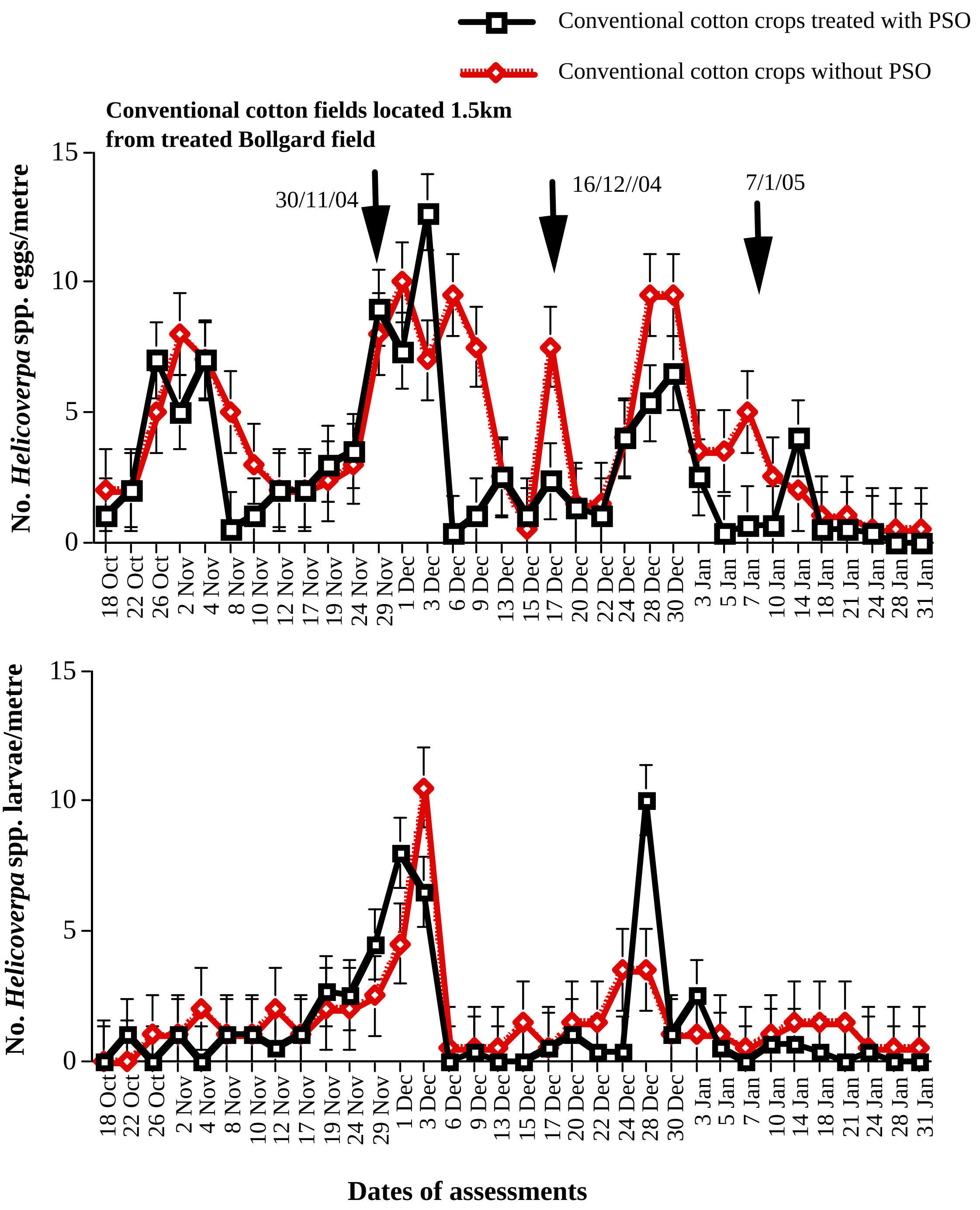


Figure 30. Comparison of numbers of Helicoverpa spp. eggs and larvae per metre on a PSO – treated and non- PSO treated conventional cotton crops located 1.5 km away from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carrington near Goondiwindi in 2004-05.

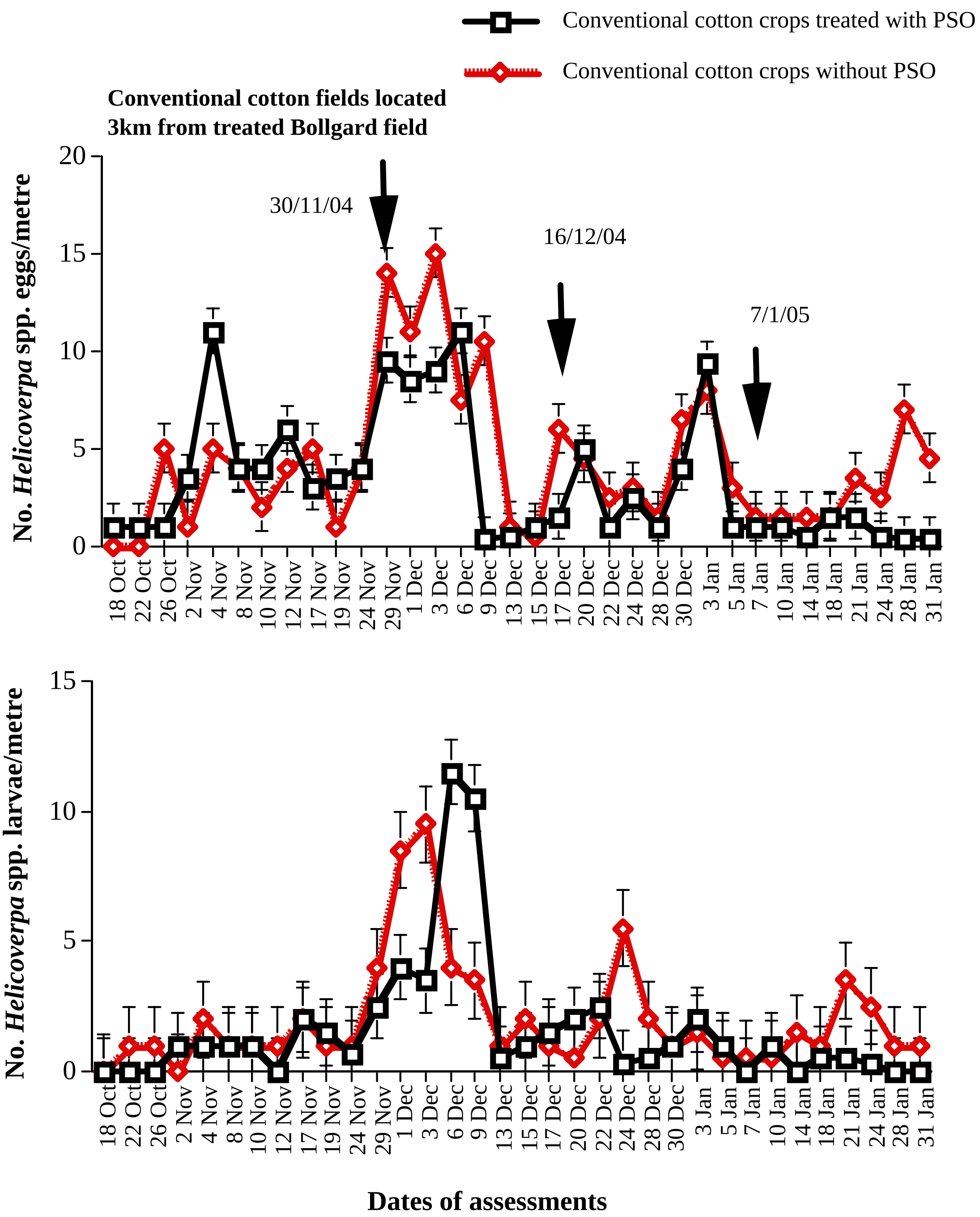


Figure 31. Comparison of numbers of Helicoverpa spp. eggs and larvae per metre on a PSO – treated and non- PSO treated conventional cotton crops located 3.0 km away from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carrington near Goondiwindi in 2004-05.

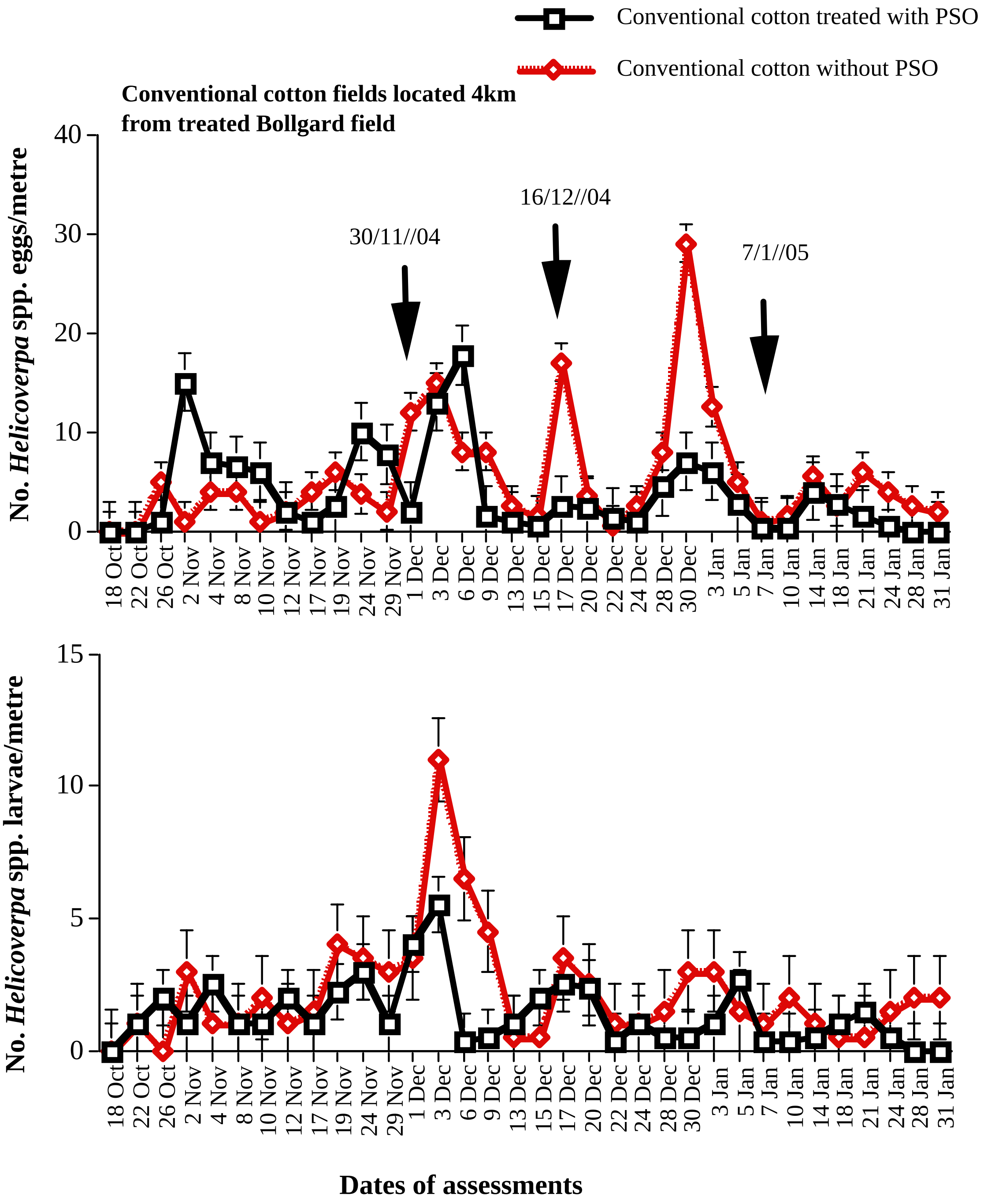


Figure 32. Comparison of numbers of Helicoverpa spp. eggs and larvae per metre on a PSO – treated and untreated conventional cotton crops located 4.0 km away from Bollgard® cotton crops treated with Magnet® mixed with insecticide at Carrington near Goondiwindi in 2004-05.

7.3 Conclusion

- Helicoverpa spp. moths recorded on PSO- treated conventional cotton crops located 0.10 to 4 km away from BollgardII cotton crops treated with Magnet mixed with insecticide was significantly lower than untreated conventional cotton crops.
- The number of Helicoverpa eggs and larvae per metre recorded on PSO-treated conventional cotton crops located 0.10 and 1.0 km away from the BollgardII crop treated with Magnet mixed with insecticides was significantly than untreated conventional cotton crops located at the same distance.
- In the conventional cotton crops located 1.5 to 4 km away from treated BollgardII cotton crops, the same number of and larvae were recorded on the PSO-treated and untreated conventional cotton crops.
- Application of PSO on conventional cotton crops close to BollgardII cotton crops treated with Magnet mixed with insecticides can reduce *Helicoverpa* spp. eggs and larvae.

8.0 DISCUSSION: RESEARCH OUTCOMES VERSUS OBJECTIVES

8.1 Determine the efficacy of PSO as stand alone and as adjuvants of reduced label rates of synthetic insecticides against *Helicoverpa* spp. and green mirids

There has been extensive research on conventional cotton crops in Australia which has unequivocally demonstrated the effectiveness of PSOs against *Helicoverpa* spp. but little studies have focussed on the use of PSOs as a stand alone or in mixtures with synthetic insecticides against green mirids in cotton. The project determined the feasibility of using petroleum spray oils (PSOs) as a stand alone product and as adjuvants of full and reduced label rates of synthetic insecticides against green mirids in Bollgard cotton crops. The study was undertaken to enable growers gain knowledge in the optimum rate, timing, and method of application and use patterns of PSOs in Bollgard cotton crops.

The results of the studies emphatically illustrated the importance of PSOs as adjuvants of synthetic insecticides in cotton pest control. PSOs by themselves can cause direct mortality to *Helicoverpa* spp. larvae particularly 1st -3rd instar stages. The addition of PSOs to either full or reduced rate insecticides may assist in increasing efficacy against the pests. Another likely contributor to the enhanced efficacy of the synthetic insecticides when applied in combination with the PSOs could be the increased persistence of the insecticides. This effect may be dependent on the average molecular weight of the molecules within the base oils. The higher the molecular weight of the oil, the slower its likely rate of dissipation from the leaf surface. This is consistent with the observation that the 1% Canopy® oil mixed with full label rate of insecticides and 2% Canopy oil mixed with ½ label rate of insecticides tended to show fewer green mirid numbers per count than the Biopest®/insecticide mixtures at Norwood in 2005-06.

The study also found that the addition of 2% v/v of either Biopest or Canopy oil to ½ rate insecticides achieved similar control of *Helicoverpa* spp and green mirids as 1% PSO + full label rate insecticide and also the full label rate insecticides alone. In a *Helicoverpa* spp. high pressure situations with egg lay of over 20 eggs per metre, the use of PSO + ½ label rates of insecticides cannot control all the larvae effectively. In such situations, growers are advised to mix 1 or 2% PSO with a full label rate of the insecticides for effective control.

In terms of conservation of predatory insects in the cotton cropping system, the application of PSO mixed with ½ label rate of insecticides minimised the impact of the insecticides on beneficial insects compared to the full label rate or 1% plus the full label rate of the insecticides. In this study, the number of predatory beetles, bugs, lacewings and spiders recorded in plots treated with full label rate insecticides were significantly lower than plots treated with ½ label rate insecticides mixed with 2% PSOs and the untreated control plots. However, no significant difference was detected on plots treated with full label rate insecticides alone and full label rate insecticides mixed with 1% PSOs indicating that the addition of PSO to a full label rate of insecticides does not make the insecticide softer on predatory insects.

The cotton yields achieved by adding 2% v/v PSO to ½ rate insecticides were the same as those achieved with 1% plus the full label rate or full label rate insecticides alone. Thus the study showed clear synergies from the combined use of PSOs such as Canopy® and Biopest, and synthetic insecticides, in the control of *Helicoverpa* spp. and green mirids in cotton. The main advantages of reduced rate insecticides/ PSO mixtures over full rate insecticides alone, are (1) reduction of the quantity of insecticides growers use to manage pests (2) the presence of oil in the spray mixture means that the insecticide actives reaches their target on the crop, (3) improved persistence of the insecticides (4) reduced disruption to the natural enemies of pests and (5) improved efficacy without sacrificing cotton yields and gross margin. Such an insecticide combination could therefore form a useful tool to complement existing IPM programs in cotton.

8.2 Effect of PSO residues on the survival and consumption rate of key predators on *Helicoverpa* spp.

Several researchers have reported that PSOs have minimal effect on natural enemies of pests in terms of survival, occurrence and abundance. What is not known is the effect PSOs have on the consumption rate of natural enemies that survived the PSO spray. For example, a predator that survives a PSO spray, might be so much affected by the spray that their appetite to consume prey might have reduced resulting in reduced consumption rate of the particular natural enemy. Under field situation, and after a PSO application on cotton crops, numbers of natural enemies on the crop may still be very high but still cannot bring the pest numbers under threshold. This might be due to the reduction in the consumption rate of the natural enemies as a result of the spray.

In this project, our research showed that *C. transversalis* adults treated with PSO and exposed to *Helicoverpa* spp. eggs one hour after treatment (1 HAT) have had their consumption rate reduced by 13.30 eggs per day. However, the consumption rate that was reduced one hour after PSO treatment increased 1 to 3 days after treatment. The result of this study has implications to "soft" conventional insecticides used in the cotton industry. Some of the commercial synthetic insecticides used in the IPM programs in the cotton industry are classified as "soft" or "selective" to beneficial insects. The selectivity of these insecticides to beneficial insects is based on survival and abundance of these insects after insecticide treatment. PSOs that are regarded as "softer" or more selective to beneficial insects than these "soft" synthetic insecticides can reduce the consumption rate of these beneficial insects after treatment. Therefore, it is more likely that these "soft" synthetic insecticides may be reducing the consumption rate of the surviving beneficial insects making them ineffective in controlling the pests after insecticide application. The result of the study have significant implication to pest control in conventional or Bollgard cotton fields where "soft" synthetic insecticides are used by growers to complement beneficial insects in IPM programs.

8.3 Evaluation of the utility of locally grown alternative crops as trap for green mirids in cotton

The study clearly showed that green mirids prefer sunflower and lucerne to other crops such as soybean, sorghum, mungbean, cotton and pigeon pea. However, sunflower usually dries off in January and green mirids can move onto cotton crops which are at a peak squaring, flowering and boll setting during that period. Therefore, sunflower cannot be used as a trap crop in cotton in terms of green mired management. In contrast, lucerne crop grows throughout the year and can hold onto the mirids during the peak squaring stage of the cotton crops. Therefore, lucerne is the best crop to use as a trap crop for green mirids in commercial cotton farms.

In an experiment where lucerne was planted as a block adjacent to Bollgard cotton crops, the densities of green mirids was significantly lower on the Bollgard® crops than another Bollgard® crops without adjacent lucerne block. The number of green mirids in the Bollgard® cotton crop was lower from 10 to 250 metres away from the lucerne crop but numbers increased significantly from 400 m away. This means that the lucerne block was acting as a sink and trap for the green mirids in the cotton crop. The lucerne could trap green mirids from about 250-300 metres away from the lucerne crop. The use of lucerne to trap green mirids from adjacent cotton fields is very important for the management of green mirids in cotton because it can assist in reducing synthetic insecticide use by growers in cotton.

8.4 Evaluation of the efficacy of a new myco-insecticide for activity against green mirids on Bollgard® cotton crops

The two new myco-insecticides used in the studies were BC 639 and BC 667. These fungi are Metarhizium spp. and Beauveria spp. which are known to have significant potential for the development as commercial mycoinsecticides. Strains of these two fungal species have been isolated from many parts of the world, therefore their exploitation does not involve introducing foreign agents into new environments. There was high mortality (over 70%) of green mirids adults and nymphs at a rate of 0.5 – 1.0 L/ha of BC 639 and BC 667. In the trials, application of 0.5-1.0 L/ha of either BC 667 or BC 639 fungal insecticide can reduce densities of green mirids adults and nymphs from about 3.5 per metre to zero within 14 days after treatment. The efficacy of the fungal insecticides applied at these rates against green mirids adults and nymphs was the same as Fipronil applied at ½ the label rate.

The application of the fungi killed green mirids within 3-4 days. The fungi can cause secondary infection to green mirids whereby the death of infected mirid or insect can produce more spores which will continue to infect and kill insects. In some of the trials in commercial cotton crops, dead insects were found 28 days after treatment application. The fungi are effective against soft body insects so that hard body insects which comprise most of our key predatory insects in cotton are saved. In our studies we found no significant differences between the number of predatory beetles, bugs, lacewings and spiders per metre in plots treated with fungal insecticides and unsprayed (control) plots. In contrast, the number of predators recorded on the plots treated with the fungal insecticides was significantly higher than the commercial insecticide (Fipronil). This indicates that the fungal insecticide is “softer” than Fipronil which is regarded as a “soft” insecticide in the cotton industry.

The average cotton yield harvested from commercial cotton fields at Norwood and ACRI from 2004-2007 showed that yields from fungal insecticide treated plots were not significantly different ($P>0.05$) from the conventional cotton fields where green mirids were controlled with Fipronil insecticide.

In addition to green mirids, the fungi were found to cause some mortality to Apple Dimpling bug, pollen beetles and green vegetable bug nymphs. It was also found to cause mortality to Helicoverpa spp. very small and small larvae when sprays were targeted to *Helicoverpa* spp. egg hatch. The fungal spores are formulated in oil to enable the spores stick onto the target insects and crops and also protect the spores from drying. In 2004-05, the fungi were formulated in Canopy® oil and Horticultural oil and their efficacies against green mirids were compared. The result showed that the efficacy of the fungi in the two different formulations were similar and not significantly different from Fipronil. However, since the cost of the Canopy® oil per litre was higher than the Horticultural oil, the fungi was formulated in Horticultural oil in 2005-06 and the efficacy of the two formulations evaluated at 1L/ha and 1.5L/ha. The result showed that both rates have similar efficacy against green mirids and have no effect on beneficial insects.

In trials in 2006-07, the results showed that the fungal insecticides applied at 500ml/ha caused the same efficacy as 1L/ha and half rate of Fipronil insecticide. In the same trials, BC 667 was tested only at 500ml/ha upon the advice of the commercial partner. The Commercial partner advised that sporulation of BC 667 is far lower than BC 639 indicating and until technique that allows BC 667 to be mass produced effectively in the laboratory is developed it will be uneconomical to produce BC 667, hence unnecessary to focus research effort on BC 667. As a result research effort concentrated on BC 639. The results in 2006-07 showed that efficacy of the different rates of the fungi against green mirid adults and nymphs were the same and not significantly different from Fipronil insecticide. In addition the effect on beneficial insects was not significantly different from the unsprayed treatment. Furthermore, the yield and quality of the cotton crop harvested from the fungal insecticides and the Fipronil treated plants was not different.

The application of the fungal insecticides to the cotton crops occurred in the mornings and sometimes in the evenings when the sun goes down. In cotton cropping systems, application of pesticides to manage pests is often required in summer, when maximum temperatures are 35-40^{o}C. However, application of the fungal insecticides have controlled the green mirids effectively because spores have been screened over 6 years for pathogenicity in temeperatures between 30-40^{o}C. When products are applied in the mornings when temperatures are below 30^{o}C, the high temperatures in the middle of the day may be inhibitory, the warm temperatures in the rest of the day and in the night may be ideal for fungal growth and development, leading to relatively rapid mortality of the green mirid. Sometimes, green mirid control may be required in mid and late December where temperatures are less than 30^{o}C. In this period, the fungus can develop well during the day. Furthermore, to enhance spore development, it is advisable for growers to apply the fungus to coincide with furrow irrigation. In this way, artificial humidity is created in the cotton crop and this could enhance development of the spores. Therefore it can be said that the fungus can develop well at night when it is very hot, and well in the day when temperatures are mild.

The fungal insecticides BC 667 and BC 639 have shown to have good efficacy against the green mirid adults and nymphs but has minimal effect on beneficial insects. The use of the fungal insecticides against the green mirid so far is confined in New South Wales and Queensland. In the coming season, it is expected that the fungus will be tested at 250ml/ha on cotton and grain crops against *Helicoverpa* spp., green mirids and Rutherglen bugs in NSW and Queensland. It is anticipated that the optimum rate of application will be confirmed and become more firmly established. If registration is obtained in Australia, and area of cotton planted in Australia continue to be low, collaborative research will be undertaken on other crops in Australia and cotton in overseas and this may lead to the use of BC639 commercially on many crops in Australia and overseas.

8.5 Determine strategic use of Magnet attractant on Bollgard cotton crops on the level of Helicoverpa spp. production and management on conventional cotton crops

Magnet®) is an insect attractant technology that has been developed to manage *Helicoverpa* spp. and other lepidopteran pests in a wide range of crops. The product contains a blend of synthetic plant volatiles that can attract *Helicoverpa* spp. moths from a distance of three kilometres radius towards the application site. Application of Magnet® to the foliage of commercial cotton crops, can mediate changes in the populations of lepidopteran pests in the environment by attracting moths from a distance to feed on the formulation.

In this study, we added thiodicarb (toxicant) to the Magnet® product and applied the formulation to BollgardII® (transgenic) cotton crops surrounded with conventional cotton crops. The study showed that application of the Magnet® formulation to the BollgardII® cotton crop attracted and killed *Helicoverpa* spp. (moths) resulting in reduction of Helicoverpa moth populations on the conventional cotton crops located 0.5 to 1 km away from the "treated" BollgardII® field. However, after the first treatment application, moth numbers on the conventional cotton crops located 0.5 km away from the "treated" BollgardII® cotton crop increased by 86 per cent. An explanation for this was that the Magnet® formulation attracted moths from the environment to the treated area and those residual moths that could not reach the Magnet® treated zone before the Magnet® odour dissipated stayed in the conventional cotton crop that was closest to the "treated" BollgardII® crops. After the second and third application of the Magnet® formulation, the number of moths on this plot declined significantly from a peak of 950 to 85 moths per ha indicating most of the residual moths might have moved to the treated zone in the BollgardII® field. In contrast, the number of moths in the "untreated" conventional cotton crop remained high after the first treatment application and only declined to 510 per ha after the second and third applications. This could mean that the residual moths in the "untreated" conventional cotton crop, which was located 6 km away, may not have moved to the "treated" BollgardII® cotton crops but remained and established in the crop.

The study also showed that despite the high initial increase in moth numbers on the conventional cotton crop that was closest to the "treated" BollgardII® crop, it did not translate into higher number of *Helicoverpa* spp. eggs and larvae in the plot. Rather, the number of eggs and larvae were significantly lower in the "treated" conventional cotton crops than the "untreated" conventional cotton crops. In terms of *Helicoverpa* spp. management in the conventional cotton crops, the "untreated" conventional cotton crops received nine pesticide sprays compared to seven on the "treated" conventional crops. This resulted in a pest control saving of $11.40 per ha in the "treated" over the "untreated" conventional cotton crops.

The study also showed that the "treated" BollgardII® crop had 3 to 5 times more *Helicoverpa* spp. eggs per metre than the "untreated" BollgardII® cotton crop. The higher number of eggs recorded on the "treated" BollgardII® cotton crop could mean that the *Helicoverpa* spp. moths that were attracted to the Magnet® formulation on the BollgardII® crop, laid on the crop before ingesting the formulation and dying. Despite the high number of eggs laid on the "treated" BollgardII® cotton crop, the number of larvae per metre was not significantly different from the "untreated" BollgardII® cotton crop (control) indicating the Bt toxins in the BollgardII® cotton crop killed any larvae that hatched from the eggs. Thus, the use of the "attract and kill" strategy on the BollgardII® cotton crop may not in any way put undue resistance development pressure on the BollgardII® technology.

In this study, *Helicoverpa* spp. pressure in the study area during the 2005-06 season was significantly higher than the 2004-05 season. As a result, there was a high population of residual moths in both “treated” and “untreated” BollgardII® and conventional cotton crops in the study area despite high number of dead moths killed on the treated BollgardII® cotton crops. In fact, 3.04 *Helicoverpa* larvae per metre per sample date, which was above the recommended larvae threshold of 2 per metre, were recorded during the mid and late season in both “treated” and “untreated” conventional cotton crops. As a result, there was no pest control savings made on conventional cotton crops by using the Magnet® formulation on Bollgard® crops. Therefore, the benefit to be gained in pest management on conventional cotton crops using the “attract and kill” strategy on BollgardII® cotton crops, may vary between seasons based on moth pressure and that the strategy may be used successfully only at low to medium *Helicoverpa* pressure.

The “attract and kill” strategy used in this study offers a number of advantages over foliar application of conventional insecticides on conventional cotton crops. The application of the Magnet® formulation uses comparatively a small amount of synthetic insecticides compared with foliar application of insecticides when used to manage conventional cotton crops. The formulation is target specific and there is no spray drift to contaminate the environment. Furthermore, growers could save approximately $11.40 per hectare on their conventional crops by using the strategy under similar pest pressure conditions.

In conclusion, the study showed that application of Magnet® mixed with toxicant in an “attract and kill” strategy on BollgardII® cotton crops as a component of an IPM program can minimise *Helicoverpa* spp. infestation and synthetic insecticide use on adjacent conventional cotton crops, resulting in an insecticide cost saving to the grower.

8.6 Determine strategic use of Magnet (moth) attractant on Bollgard II cotton crops and PSOs on conventional cotton crops on the level of Helicoverpa spp. production and management of Helicoverpa spp. on the conventional crops

The results of the study showed that application of Magnet® formulation onto Bollgard cotton crops attracted moths from the environment to the treated area and those residual moths that could not reach the Magnet® treated zone before the Magnet® odour dissipated stayed on the conventional cotton crop that was closest to the “treated” BollgardII® crops and laid eggs. By applying PSO on the conventional cotton crops, the number of Helicoverpa moths per hectare in the conventional cotton crops treated with PSO was significantly reduced compared with conventional crops that did not receive PSO sprays. In addition, the number of Helicoverpa spp eggs and larvae per metre recorded on the conventional cotton crops treated with PSO was significantly lower than the conventional cotton crops not treated with PSO.

The explanation given is that cotton plants release airborne volatiles and this may play a role in the ability of cotton pests especially *Helicoverpa* spp. to choose between good and bad host plants. The quantity of airborne volatiles released by the plants may assist the female moths to detect and select appropriate plants for oviposition. The application of PSO on the cotton plants suppressed the quantity of airborne volatiles released by the plants resulting to lower egg lay and larvae on the PSO-treated plants compared to the non-PSO treated plants. The effect of this suppression activity may last for 5 to 7 days after a single application of the PSO.

The use of airborne volatiles by moths to detect their host plants has been reported by many researchers. For example, Mitchell *et al*. (1991) reported that many moths use airborne volatiles emitted from plants to locate their host in contrast to visual cues such as colour, shape and size of the host plant. *Helicoverpa* spp. female adults migrate and lay most of their eggs at night, it is possible that they may utilise airborne volatiles to locate cotton plants. Hence, the quantity of volatiles released by the plants may influence host selection and the amount of eggs the moths lay on the selected host plant.

In this study, application of PSO on conventional cotton crops located 100 to 1km away from the Magnet treated Bollgard cotton crops, reduced the number of Helicoverpa moths, eggs and larvae per metre compared to the conventional cotton crops located at the same distance but not treated with PSO. In contrast no significant difference was detected in the PSO and non-PSO treated cotton plants located at 1.5 to 4km away from the Bollgard crop treated with the Magnet formulation. The reason given was the high number of residual moths in the PSO treated and non-PSO treated plots. As a result, of the high population of residual moths in both "PSO-treated" and "untreated" conventional cotton crops in the study area and despite the suppression effect of the PSO on the plant volatiles, no significant difference in egg and larval numbers were achieved. Therefore, the benefit to be gained in pest management on conventional cotton crops using the "attract and kill" strategy combined with PSO sprays may vary between seasons based on moth pressure and that the strategy may be used successfully only at low to medium *Helicoverpa* pressure.

8.7 Assist in the review of the cotton industry IPM guidelines.

The IPM guidelines is a tool to help improve pest management in cotton. The Cotton Industry IPM guidelines was reviewed in 2005 with contributions from Dr Robert Mensah, Dr Lewis Wilson and Ms Sandra Deutscher. The guidelines was launched by the Hon. John Anderson MP, Deputy Prime Minister of Australia in 18th February 2005. New information on new IPM tools such as PSOs and predator to beneficial insect ratio has been incorporated into the guidelines. The IPM guidelines has been distributed to growers and the reviewers anticipate that growers may provide feedback on problems they encounter as users. As new pest management information becomes available the reviewers will review the guidelines and incorporate all current information. This will ensure that Australian Cotton Growers are current with pest management techniques, tools and strategies.

9.0 Technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

The project identified two fungal isolates BC 639 and BC 667 for commercialization in the cotton industry to control green mirids, Helicoverpa and possibly aphids (work on aphids is on-going in collaboration with Dr Lewis Wilson). Each of the 2 products have been formulated in Horticultural oil. Both BC 639 and BC 667 are efficacious against green mirids and *Helicoverpa* spp. Lower rates of 0.25L/ha are now being tested in a new project funded by CRDC, Becker Underwood. Both BC 639 and BC 667 are efficacious against green mirids and Helicoverpa, BC 639 will be commercialized first because mass production of the spores of these fungus is more economical than BC667 (Beauveria spp). BC639 produce more spores than BC667 which can grow vegetatively during winter and spring months where mass production of spores for formulation is required. As a result research effort for 2007-08 has been concentrated on BC639.

The commercial partner identified for the products is Becker Underwood Pty Ltd. Becker Underwood Pty Ltd is not new to the biopesticide market. They have produced and commercialized a wide range of fungal insecticides and granules in the agricultural industries in the USA, Canada, Australia and New Zealand.

The Intellectual Property is owned by Becker Underwood Pty Ltd (40%), CRDC (30%) and NSW Department of Primary Industries (30%). Product registration will commence in April 2008. A 40 ha trial permit has been issued by APVMA.

10.0 Extension Opportunities

10.1 *Detail a plan for the activities or other steps that may be taken:*
(a) to further develop or to exploit the project technology.

Dr Robert Mensah will collaborate with the Commercial partners to assist in the registration of the fungal insecticide product. In addition, Dr Mensah and the Commercial partner will link with NSWDPI molecular biologists to develop a genetic bar-coding for the fungal strains. This will enable the fungal strains to be patented. After the product registration, selected growers and consultants will have access to the fungal products for large scale trials in Bollgard and conventional cotton crops to generate more data to develop product use pattern and registration.

10.2 *for the future presentation and dissemination of the project outcomes.*

Research data of the project outcomes will be compiled in tabular and graphical format and published in the Australian Cotton Grower and also Spotlight for growers to access information. All project information will be given to the extension focus group for pest management. Dr Robert Mensah plans to go on "Pest Management Road show" in June and September 2008 to communicate the results of the project to cotton growers.

10.3 *For future research.*

The future research will concentrate on the impact of the new fungal insecticide products on prey consumption rate non-target organisms such as key predators. So far studies of the effect of the fungal insecticide products have focussed on green mirids, Helicoverpa and some aspects of cotton aphids. Future research will focus on the effect on the product on other cotton pests such as green vegetable bugs, whiteflies, cotton stainers. In collaboration with the commercial partner, research will concentrate on lower rates of the fungal insecticides as a stand alone and as mixtures with reduced rates of synthetic insecticides on cotton and other crop pests. It is important that the products are evaluated for efficacy against a whole range of agricultural pests both broadacre, horticultural crops etc.

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

11.1 Refereed Journal papers

1. ALDABEL F., MENSAH, R. K. and FREROT, B. (2008). Effects of nC24 and nC27 petroleum spray oils on oviposition and egg survival of Ostrinia nubilalis Hubner

(Lepidoptera, Pyralidae) and Trichogramma brassicae Bezdenko (Hymenoptera, Trichogrammatidae) adults on maize. International Journal of Pest Management. 54(1):5-11, 2008.

2. ANDERSON, C. M. T., McGEE, P. A. , NEHL, D. B. and MENSAH, R. K. (2007). The fungus *Lecanicillium lecanii* colonises the plant *Gossypium hirsutum* and the Aphid, *Aphis gossypii* Australasian Mycologist 26 (2-3), 65 – 70.
3. MENSAH, R. K. MACPHERSON, I. and MAY, K. (2007). Attracting and killing Helicoverpa spp. adults (moths) on Transgenic (Bollgard®) cotton crops: A strategy for Helicoverpa spp. management on conventional cotton crops. Pest Management Science (submitted)
4. MENSAH, R. K. and PYKE, B. A. (2007). Beneficial insects in cotton. In: Pests of Field Crops and Pastures – Identifications and control; (Editor) P. T. Bailey, CSIRO Publishing, Canberra, Australia page 102-121.
5. NAJAR, R. A. J., WALTER, G. H. and MENSAH, R. K. (2007). "The efficacy of a petroleum spray oil against *Aphis gossypii* Glover on cotton: (Part 2) Indirect effects of oil deposits". Pest Management Science 63; 596-607.
6. NAJAR, R. A. J., WALTER, G. H. and MENSAH, R. K. (2007). The efficacy of petroleum spray oils against *Aphis gossypii* Glover on cotton: (Part I) Mortality rates and sources of variation" Pest Management Science 63; 586-595.
7. Al DABEL, F., MENSAH, R. K. and FREROT, B. (2007). Effects of nC24 and nC27 petroleum spray oils on oviposition and egg survival of *Ostrinia nubilalis* Hubner (Lepidoptera, Pyralidae) and *Trichogramma brassicae* Bezdenko, Trichogrammatidae) adults on maize plants. International Journal of Pest Management (In press).
8. MENSAH, R. K., LIANG, W., GIBB, D., COATES, R. and JOHNSON, D. (2005). Improving the efficacy of nuclear polyhedrosis virus and *Bacillus thuringiensis* against *Helicoverpa* spp. with ultra-violet light protected petroleum spray oils on cotton crops in Australia. International Journal of Pest Management 51 (2) 101-109.
9. MENSAH, R. K., FREROT, B. and AL DABEL, F. (2005). Effects of Petroleum Spray oils on oviposition behaviour and larval survival of Helicoverpa armigera (Lepidoptera: Noctuidae) and Ostrinia nubilalis (Lepidoptera:Pyralidae). International Journal of Pest Management 51 (2) 111-119.
10. MENSAH, R. K., LIANG, W., GIBB, D., COATES, R. and JOHNSON, D. (2005). Evaluation of *n*C27 Petroleum spray oil for activity against *Helicoverpa* spp. on commercial cotton fields in Australia. International Journal of Pest Management 51 (1) 63-70.
11. MENSAH, R. K. and SEQUIERA, R. (2004). Habitat manipulation for insect management in cotton systems in Australia. In: Pest Suppression: Habitat Manipulation Approaches (Editors) Steve Wratten and Geoff Gurr, CSIRO Publications, Canberra, Australia ; pages 187-199
12. WILSON, L. J., MENSAH, R. K. and FITT, G. (2004). Implementing IPM in Australian cotton. In: "Novel Approaches to insect Pest Management in Field and Protected Crops" (Editors) Rami Horowitz and Isaac Ishaaya, Spinger Press, Netherlands; pages 87-106.

11.2 Technical conferences and Bulletins

1. MENSAH, R. K. and LEAH, A. (2008). Managing *Helicoverpa* spp. and *Creontiades dilutus* on cotton crops: role of entomopathogenic fungus, semiochemicals and trap crops. International Congress of Entomology, Durban South Africa 6-12 July 2008.

2. MENSAH, R. K., VODOUHE, D. S. and SANFILIPPO, D. (2008). Habitat manipulation and supplementary food spray for enhanced pest management in Organic cotton: implications to cotton IPM. Int. Congress of Entomology, Durban, South Africa, 6-12 July 2008.
3. ANDERSON, C. M. T., McGEE, P. A. , NEHL, D. B. and MENSAH, R. K. (2006). Lecanicillium lecanii colonises cotton and the cottn aphid. Int. Mycology Congress, Cairns, Queensland, Australia, 21-25 August 2006
4. MENSAH, R. K. and MACPHERSON, I. (2006). Attracting and Killing Helicoverpa moths on Bollgard cotton crops: A new strategy for Managing Helicoverpa spp. on conventional cotton crops. Proceedings 13th Australian Cotton Conference, 8-10 August 2006, Broadbeach, Gold Coast, Australia
5. MENSAH, R. K. and LEAH, A. (2006). A New Fungal insecticides for Managing Creontiades dilutus (green mirids) on Bollgard and conventional cotton crops. Proceedings 13th Australian Cotton Conference, 8-10 August 2006, Broadbeach, Gold Coast, Australia.
6. NAJAR, R. A. J., WALTER, G. H. and MENSAH, R. K. (2006). Factors affecting aphid tolerance to Petroleum spray oils. Proceedings 13th Australian Cotton Conference, 8-10 August 2006, Broadbeach, Gold Coast, Australia
7. NAJAR, R. A. J., WALTER, G. H. and MENSAH, R. K. (2005). Cotton aphids: Can petroleum oils (PSO's) replace toxic insecticides? presented at the 7th International Symposium of Aphids, Perth, West Australia, Australia.
8. MENSAH, R. and SEQUEIRA, R. (2004). Habitat manipulation and conservation of beneficial insects in cotton systems. Proceedings of the International Congress of Entomology, 15-22 August, Brisbane, Australia. Proceedings of the International Congress of Entomology, 15-22 August, Brisbane, Australia
9. MENSAH, R. K., LIANG, W. and COATES, R. (2004). Petroleum spray oils- Lubricating the path to IPM: Part 1. Use of Petroleum spray oil as insecticide to control Helicoverpa spp. on commercial cotton fields. Proceedings 12th Australian Cotton Conference, Broadbeach, Gold Coast, Australia. Pages 675-681
10. MENSAH, R. K., FREROT, B. and ALDABEL, F. (2004). Petroleum spray oils- Lubricating the path to IPM: Part 2. How do PSOs deter oviposition of Helicoverpa spp. on cotton plants. Proceedings 12 Australian Cotton Conference, Broadbeach, Gold Coast, Australia. Pages 683-689
11. MENSAH, R. K., LIANG, W. and COATES, R. (2004). Petroleum spray oils- Lubricating the path to IPM: Part 3. Use of Biological insecticides with Petroleum spray oil to improve persistence and efficacy against *Helicoverpa* spp. on cotton crops. Proceedings 12 Australian Cotton Conference, Broadbeach, GoldCoast, Australia. Pages 691-698.
12. MENSAH, R. K., COATES, R. and HOQUE, Z. (2004). Petroleum spray oils- Lubricating the path to IPM: Part 4. Use of synthetic insecticides and Petroleum spray oil combinations for improved efficacy against Helicoverpa spp. and green mirids on cotton crops. Proceedings 12 Australian Cotton Conference, Broadbeach, Gold Coast, Australia.Page 699-710.
13. SINGLETON, A., WANG, E., MOORE, C. and MENSAH, R. K. (2004). Managing Helicoverpa spp. on cotton with semio (signalling) chemicals. Proceedings 12th Australian Cotton Conference, Broadbeach, Gold Coast, Australia. Pages 711-720

14. SCHOLZ, B and MENSAH, R. K. (2004) Effect of PSOs on Trichogramma spp. in cotton fields. Proceedings 12th Australian Cotton Conference, Broadbeach, Gold Coast, Australia pages 667-674.

11.3 Grower Articles

1. MENSAH, R. K. (2007). Use of Petroleum spray oils and Fungal insecticides in Cotton Pest Management. Gwydir Valley Cotton Field Book. Compiled by Julie O'Halloran (In press).
2. MENSAH, R. K. (2007). Bio-warfare on sucking pests. Cotton Spotlight Spring 2007, page 21
3. MENSAH, R. K. (2006). Biopesticides for Emerging cotton pests. The Land Magazine (Agriculture Today) April 2006 Edition, page 8.
4. SINGLETON, A., RASIKARI, H., MENSAH, R. K. and LEACH, D. (2006). Role of plant extracts in managing pests in cotton. In: Lower Namoi Growers Association Field Day book (T. Farrell editor), pages 57-58.
5. MENSAH, R K, KAUTER, G., MACPHERSON, I., LARSEN, D. and GIBB, D. (2005). Use of Petroleum Spray Oils to Manage Cotton pests in IPM Programs. Australian Cotton CRC Research Review 16: 4pp.
6. Moazzem, K., Wilson, L., Mensah, R., Hickman, M., Brier, H. (2004). Mirid Ecology in Australian cotton, Australian Cotton CRC Research Review 14. 4pp.
7. Moazzem, K., Wilson, L., Mensah, R., Hickman, M., Brier, H. (2004). Mirid Management in Australian cotton, Australian Cotton CRC Research Review 15. 4pp.

12.0. Have you developed any online resources and what is the website address? **NO**

13.0 BUDGET

Total funds contributed to DAN 179C by CRDC

Year	DAN 179C
2004-05	$140,000
2005-06	$140,000
2006-07	$140,000

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