Attracting and killing *Helicoverpa* spp. adults (moths) on Transgenic (BollgardII®) cotton crops: A strategy for *Helicoverpa* spp. management on conventional cotton crops

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1.0 Introduction

Attract and kill techniques based on an attractant and a killing agent (insecticide) have been used successful against pest insects such as cotton bollworm and native budworm, tephritis, houseflies, tsetseflies, fruit flies, pink bollworm, coddling moth, and light brown apple moth.[12] The attractants can be pheromones or other semiochemicals and are formulated with a mortality agent that can be toxins, sterilant or pathogen.

Previous attract and kill formulations against lepidopteran pests were based on pyrethroid insecticides because the pyrethroid insecticides had a rapid knockdown effect. For example an attract and kill formulation (Sirene® CM) consisting of a liquid containing pheromone, pyrethroid and a UV-absorber was developed and used against codling moth to reduce fruit damage in orchards in Switzerland. The response of *H. armigera* males to Sirene® CM in commercial cotton crops in Australia was studied but found that the contact rate of *H. armigera* males to the formulation was low. They concluded that Sirene® CM might be ineffective in suppressing *H. armigera* infestations on cotton farms.

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 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 to determine the effect of applying the attracticide to a centrally located BollgardII® (Bt) cotton crop on levels of *Helicoverpa* spp. adults, eggs and larval populations on surrounding conventional cotton crops and the cost effectiveness of the attract and kill strategy.

2.0 Materials and methods

2.1 *Attracticide formulation*

Magnet® was an attracticide product used in this study. The experiment was conducted on irrigated conventional and BollgardII® cotton fields at Carbucky near Goondiwindi in Queensland in Australia during the 2004-05 and 2005-06 cotton growing seasons. 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 (BollgardII®) 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.

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).

2.3 *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.

2.4 *Sampling*

2.4.1 *Counts of Helicoverpa eggs and larvae*

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.

2.4.2 *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.

2.4.3 *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. [23]

Foliar application of pesticides on the "treated" conventional cotton crops commenced on 7 November 2004 and "untreated (control)" on 1 December 2004 (Table 1). The last spray application on the "treated" conventional cotton crops occurred on 2 March 2005 and the "untreated" on 2 April 2005 (Table 1). 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.[3,24,25] 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.

3.0 Results

3.1 *Helicoverpa spp. assessments on “treated” and “untreated” BollgardII® crops*

3.1.1 *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 1). 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 1). 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 1). On the “treated” and “untreated” BollgardII® cotton crops, the number of larvae per metre ranged from 0 to 0.5 per metre (Figure 1) indicating that any eggs that may have 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.

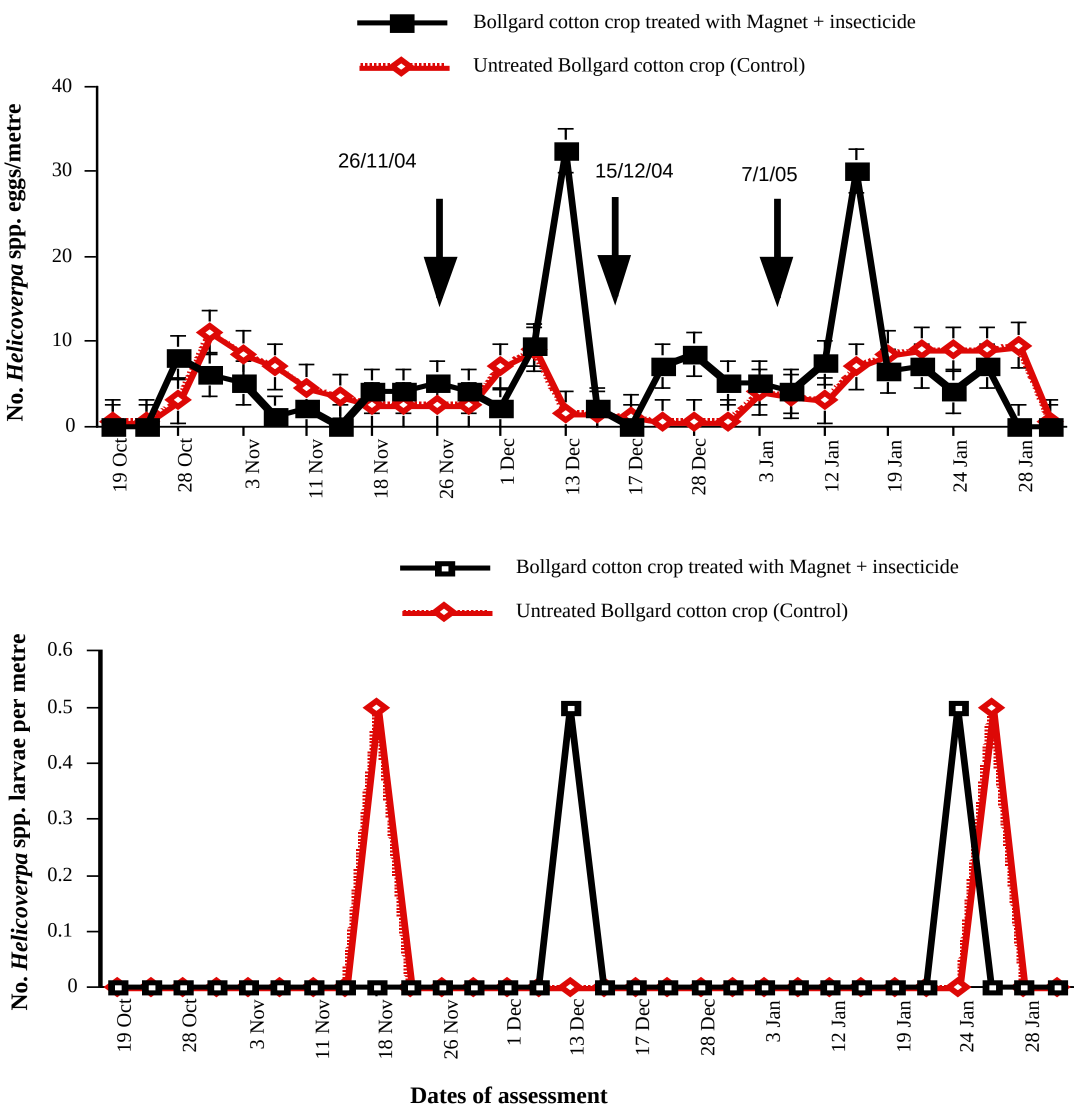

Figure 1. Effect of application of Magnet® mixed with insecticide on BollgardII® cotton crops on oviposition and larval survival of *Helicoverpa* spp. at Carbucky near Goondiwindi in 2004-05.

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

3.2.1 *Assessment of Helicoverpa spp. egg and larvae on "treated" and "untreated" conventional cotton crops*

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 2 and 3). 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 2 and 3). 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 4). 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 5). 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.

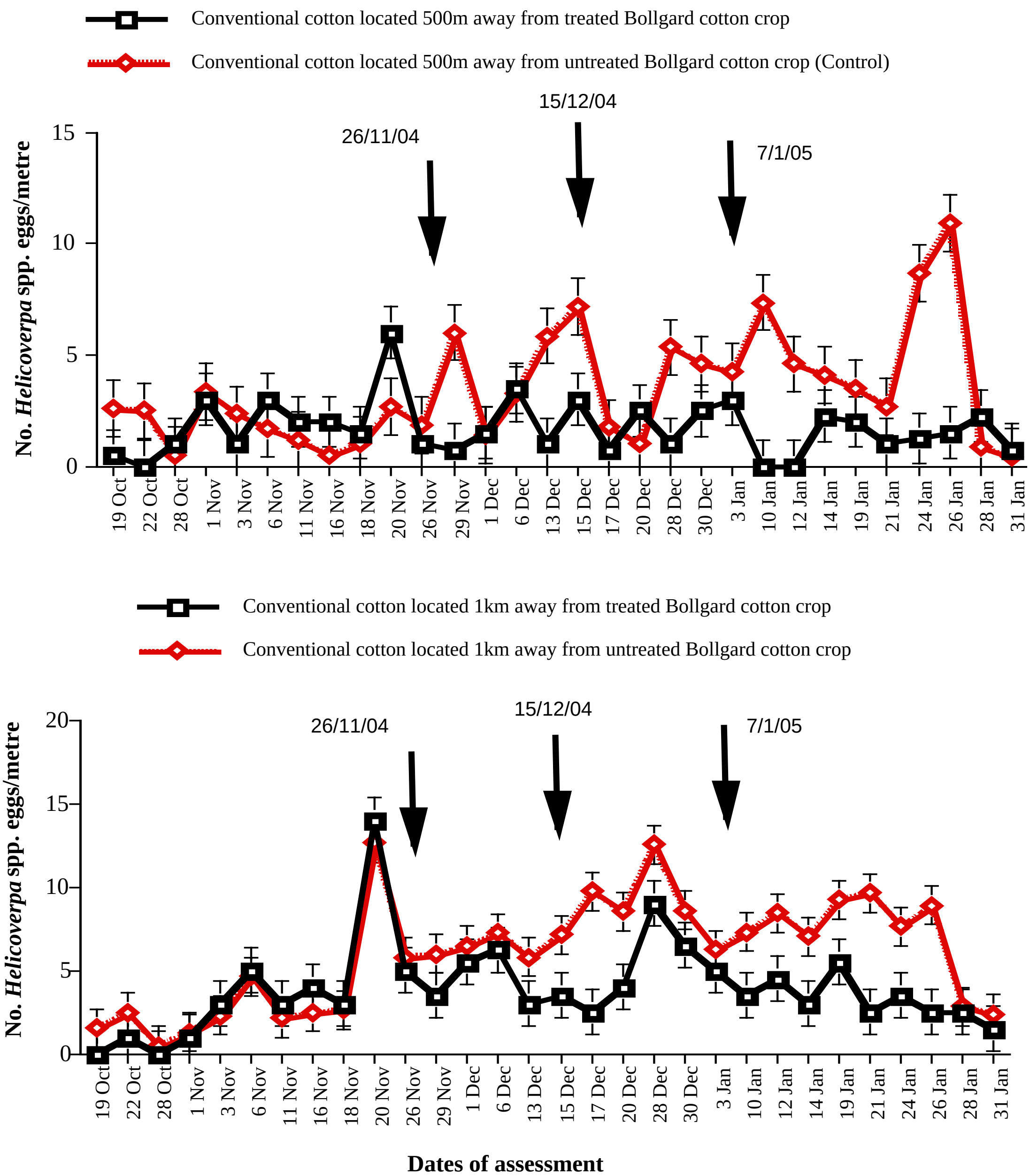

Figure 2. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of *Helicoverpa* spp. eggs per metre on conventional cotton crops located 0.5 and 1 km away from “treated” and “untreated” Bollgard® (transgenic) cotton crops at Carbucky in Goondiwindi in 2004-05.

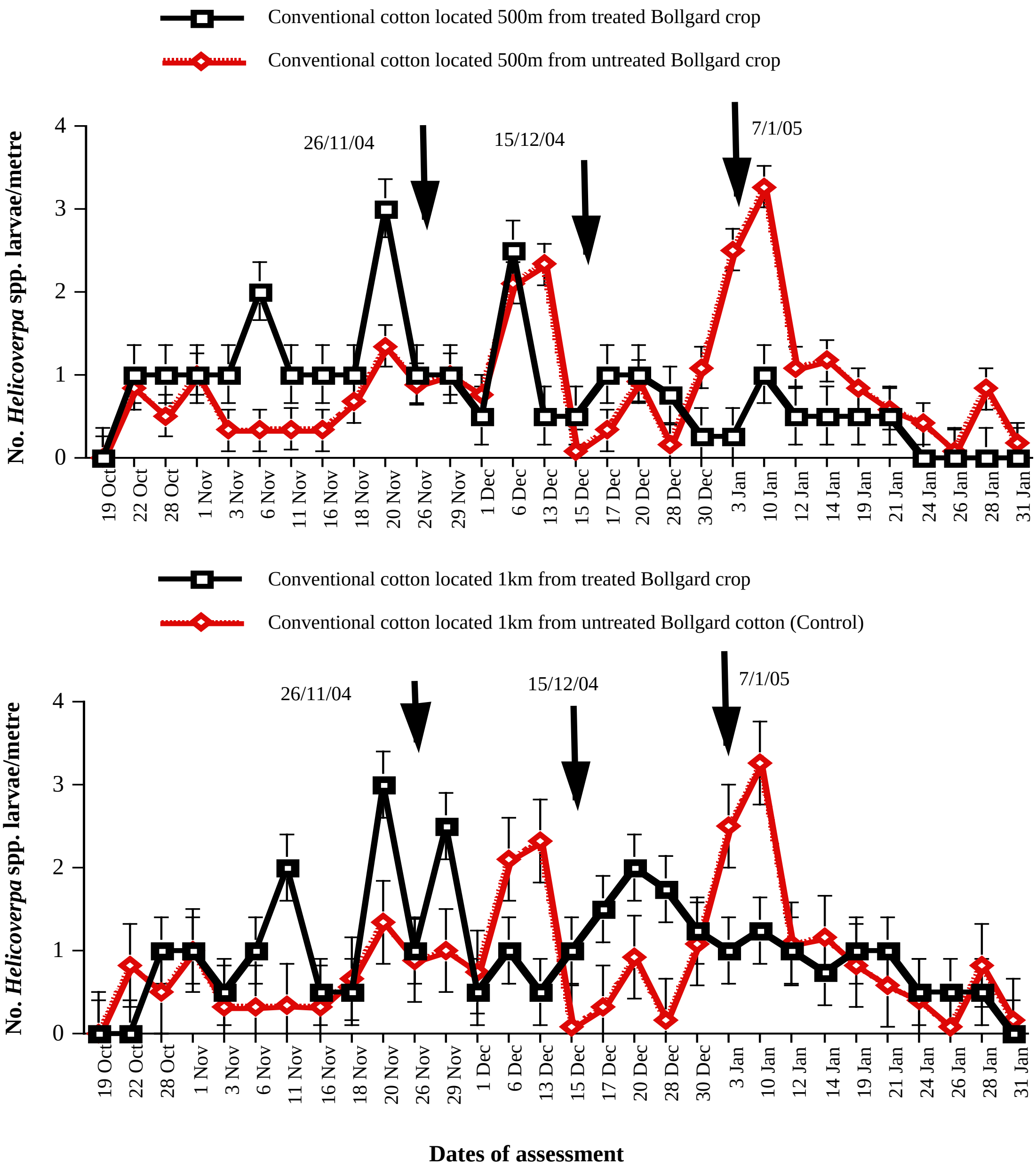

Figure 3. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of *Helicoverpa* spp. larvae per metre on conventional cotton crops located 0.5 and 1 km away from "treated" and "untreated" BollgardII® (transgenic) cotton crops at Carbucky in Goondiwindi in 2004-05.

3.3 *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 1). 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 2 and 3). 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 1). 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 1). There was no insecticide savings on the "treated" conventional cotton crops relative to the "untreated" conventional cotton crops in 2005-06 season.

Table 1. 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*	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	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

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 1). 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.

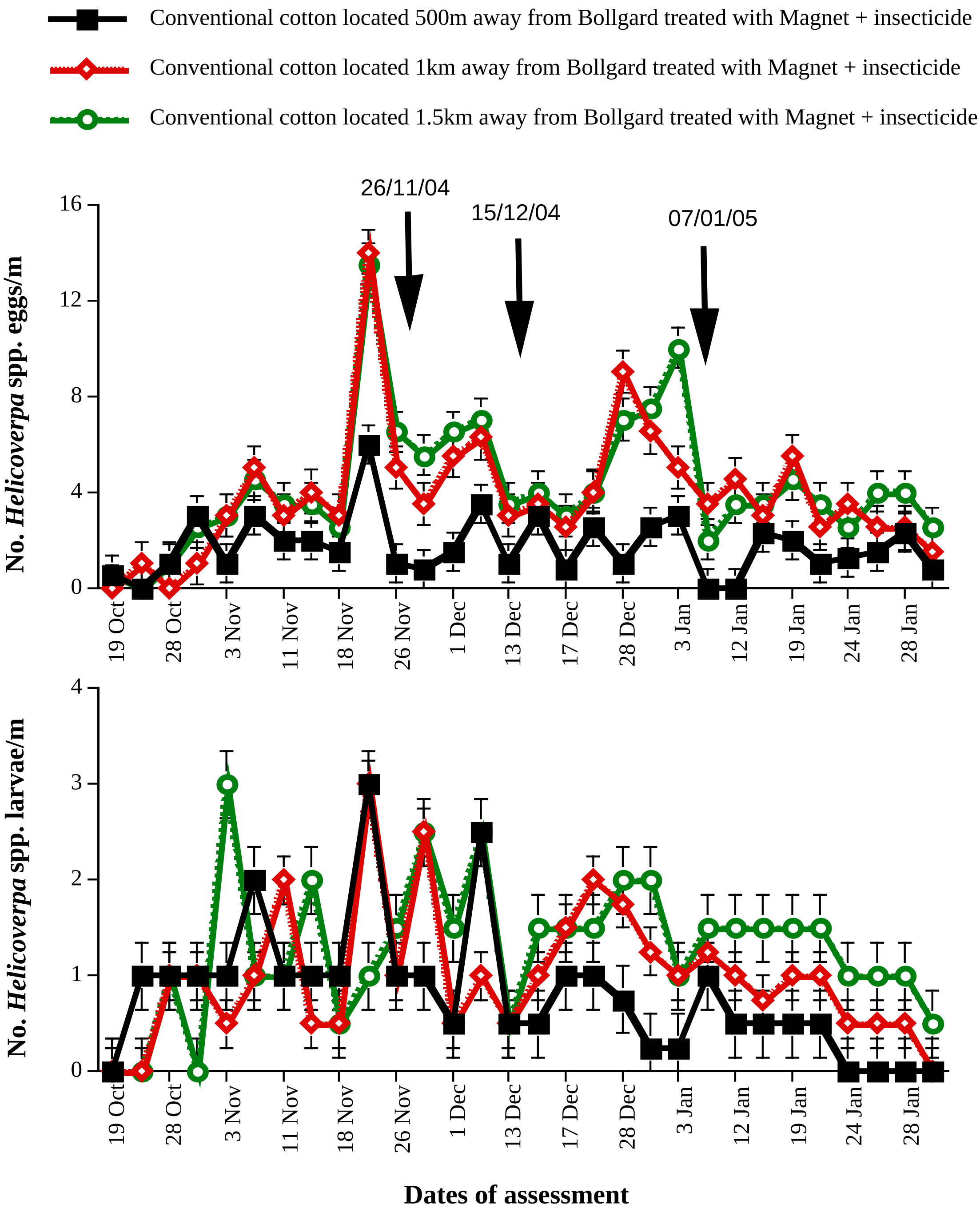

Figure 4. Comparison of numbers of *Helicoverpa* spp. eggs and larvae per metre on conventional cotton crops located 0.5, 1.0 and 1.5 km away from Bollgard® (transgenic) cotton crops treated with Magnet® mixed with insecticide (thiodicarb) at Carbucky in Goondiwindi in 2004-05.

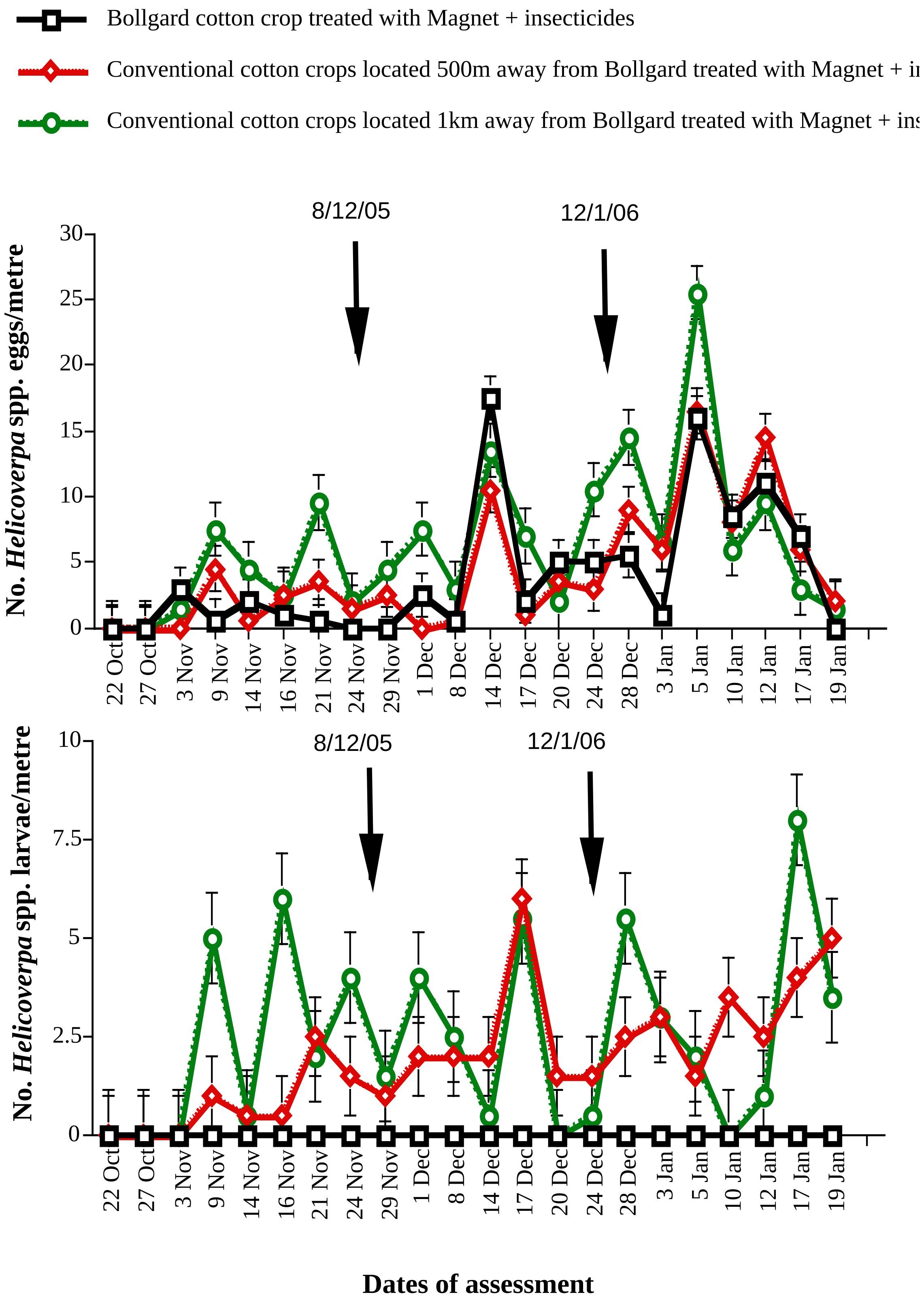

Figure 5. Effect of application of Magnet® mixed with insecticide (thiodicarb) on numbers of *Helicoverpa* spp. eggs and larvae per metre on conventional cotton crops located 0.5 and 1 km away from "treated" Bollgard® (transgenic) cotton crops at Carbucky in Goondiwindi in 2005-06.

4.0 Discussion

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 an 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.

One may argue as to why we did not apply the Magnet® formulation to a refuge or conventional crop. The advantage of applying the formulation to the BollgardII® rather than a refuge or conventional cotton crop was because *Helicoverpa* spp. females attracted to the Magnet® formulation lay eggs before ingesting the product and dying. Thus, if the product was applied to conventional cotton crops, then growers would need to spend extra money to control the artificially created *Helicoverpa* larval population in their farm. Similarly, if the formulation was applied to a refuge crop in a period of high moth pressure, it would result in a high Helicoverpa population build up early in the cotton season in the refuge crops. This would result in high moth numbers generated from the refuge crops in the second generation to attack the conventional cotton crops in the middle and late cotton seasons causing considerable crop damage and increase pest control costs to the grower.

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,[26] 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 Australia, the risk of cotton growers having to spray conventional cotton crops many times with synthetic insecticides to control *Helicoverpa* spp. and the implication on gross margin and the environment has forced growers to grow more (about 70 per cent) BollgardII® (transgenic) cotton crops on their field. However, the advantage of this study is that growers can choose to grow a small proportion of their field to BollgardII® cotton crops with a high proportion of conventional cotton crops. The grower can then apply a Magnet® formulation on the BollgardII® crops to reduce *Helicoverpa* population on the conventional crops. This will be cost effective to the grower because BollgardII® cotton crops is effective against Helicoverpa larvae not sucking pests. If the cost of controlling these sucking pests is added to the cost of the BollgardII® license, grower's gross margin is significantly reduced. Therefore, by strategically placing BollgardII® cotton crop in the field and using it as part of attract and kill strategy using Magnet® formulation will help to manage *Helicoverpa* spp. on the surrounding conventional cotton crop.

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.

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