



**Australian Government**  
**Cotton Research and  
Development Corporation**

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**SUMMER SCHOLARSHIP REPORT: 2016-2017 SEASON**

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| <b>1. Project Title</b>                               | : Investigating the Movement of <i>H. punctigera</i> Larvae from Pigeon Pea to Bollgard III Cotton |
| <b>2. Proposed Start Date</b>                         | : 2 <sup>nd</sup> January 2017   |
| <b>Proposed Cease Date</b>                            | : 23 <sup>rd</sup> January 2017  |
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# SUMMER SCHOLARSHIP REPORT

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## 1. Executive Summary:

The Resistance Management Plan (RMP) for Australian *Bt* cotton aims to minimise the development of resistant *Helicoverpa* moths. This report aims to assess if the legislated distance between pigeon pea and *Bt* legislated in the plan is adequate for preventing the movement of larvae between crops on commercial farms. To do this the behaviour and movement of larvae was observed. An experiment was set up to test how the movement of larvae was affected by factors such as the distance between pigeon pea and cotton (25 - 100cm), crop attractiveness (cotton either had or did not have flowers) and the presence of water. The experiments showed that larvae will move up to one metre over bare soil to reach *Bt* cotton. Furthermore, the likelihood of larvae reaching the cotton is increased by the presence of cotton flowers. Larvae were also observed swimming on water suggesting that this is a way in which young *Helicoverpa* can move within the field. This information indicates that, to improve the efficiency of the current RMP refuges should not be adjacent to *Bt* cotton within the same field but future work is needed to validate these laboratory findings under field conditions.

## 2. Background:

Potentially, the most destructive pests for the Australian cotton industry is *Helicoverpa armigera* and *Helicoverpa punctigera* larvae. Transgenic cotton was introduced to control these pests by producing “*Bt*” proteins that are toxic to the larvae. However, the use of transgenic cotton is coupled with the risk that its targeted pests could gain resistance or tolerance to the toxins present in the crop. To counter this, a key component of the Resistant Management Plan (RMP) is the use of *Bt* cotton, such as Bollgard 3, alongside refuge crops (Downes and Mahon, 2012). The aim of the refuge is to produce large numbers of susceptible moths that breed with resistant moths emerging from *Bt* cotton fields (Gould 1998). Overall, this reduces the proportion of resistant individuals in the population (Gould, 1998). For this to be successful, an attractive refuge crop, such as pigeon pea, should be used (Baker, 2008). Pigeon pea was found to be twice as attractive as conventional cotton, therefore producers only need to use half the land for the refuge crop to ensure the practice is effective (Baker, 2008). To further enhance this practice, plant breeders continually develop new pigeon pea varieties to improve the attractiveness of the refuge crops. For example, in 2016 Sunrise was trailed as a refuge crop. Sunrise has an earlier planting window than Quest, currently the most popular pigeon pea variety used in the industry. In turn, Sunrise is predicted to be more attractive than Quest because it will flower earlier in the season therefore, attracting more moths to the refuge.

However, the success of this strategy is based on several assumptions. One assumption is that susceptible and resistant moths will emerge at the same time to mate. However, Rahman et al. (2011) model showed a fitness cost associated with resistant individuals whereby larvae development is delayed when feeding on *Bt* cotton. Furthermore, the RMP assumes that the resistant allele is recessive and that the distance between the refuge crop and cotton allows moths to interact to potentially breed, but ideally limits the movement of larvae between the crops (Torres et al. 2009). This latter point is important because Holman (CRDC summer scholarship 2015) showed that if *H. punctigera* larvae are exposed to the *Bt* toxin Cry1Ac when they are older, they are more likely to survive and their offspring have a greater tolerance to this toxin. Therefore, if older larvae can move from refuges to *Bt* cotton, they could survive and damage the crop, as well as produce offspring that have a higher chance of surviving low doses of the *Bt* toxins (Rahman et al 2011).

In Australia refuges and *Bt* cotton are commonly adjacent within the same field, only separated by a one metre row of soil between main stems. However, the fields are usually flood-irrigated, separating the rows of pigeon pea and cotton by a body of water for short periods. It is unclear whether the soil or the water restricts the movement of larvae between the refuge and *Bt* cotton. With this in mind I tested the ability of *H. punctigera* and to a lesser extent *H. armigera*, to move between pigeon pea refuges and *Bt* cotton crops over both soil and water.

### 3. Aims and Objectives:

Aim: Is there sufficient distance between pigeon pea refuges and *Bt* cotton to prevent larval movement?

Objectives:

- Do larvae prefer Sunrise or Quest pigeon pea varieties?
- Will *Helicoverpa punctigera* move from pigeon pea to *Bt* cotton via exposed soil at distance xx?
- Does the presence of cotton flowers affect the movement of larvae from pigeon pea to cotton?
- Are *Helicoverpa punctigera* more likely to move from a refuge onto *Bt* cotton than *Helicoverpa armigera*?
- Can either *Helicoverpa* species overcome a water barrier that mimics inter-row irrigation?

### 4. Materials and Methods:

Experiment 1: What is the attractiveness of Sunrise and Quest pigeon pea leaves to *Helicoverpa* spp.

#### Materials

- 24 Petri dishes containing moist filter paper
- 12 *Helicoverpa punctigera* (late 3<sup>rd</sup> instar; first generation field caught larvae)
- 12 *Helicoverpa armigera* (early 4<sup>th</sup> instar; first generation field caught larvae)
- 24 Quest and Sunrise leaves

#### Method

Leaves were first labelled with a small dot of permanent marker at the base or tip of the leaves, for Quest and Sunrise respectively, to ensure varieties could be distinguished during the experiment. They were then placed on a moist piece of filter paper fitted inside a petri dish. One larva was then placed in the centre of the leaves. The location and orientation of each petri dish was then randomised. The trial was run for twenty-four hours and once time had elapsed the percentage of leaf area eaten was calculated. Once the data was collected, it was analysed with a Chi squared test.

Experiment 2: Are both *Helicoverpa* spp. equally likely to move from Pigeon Pea to Cotton?

#### Materials

- Plastic trays (45cm x 35cm x 10cm) filled with soil.
- Plant samples with the stem of the plant in specimen jars (100mL) filled with water: Pigeon pea (25cm tall) and Bollgard 3 cotton with flowers (10cm).
- Twenty *H. punctigera* and twenty *H. Armigera* (all 3<sup>rd</sup> instar larvae).
- Masking tape and tangle-foot (used to trap larvae).

#### Method

This experiment was undertaken to test the assumption that both *H. armigera* and *H. punctigera* displayed similar behaviour. The experiment was undertaken in a temperature and light controlled room (16/8 day/night hours; Temperature range 24-28 °C). The setup of this experiment is shown in Figure 1, showing how trays were filled with soil, lined with tape and tangle-foot to prevent larvae from escaping and the plants (whose stems were in water-filled specimen jars) which were placed 12cm apart. The treatment (first generation field caught *H. armigera* and *H. punctigera* larvae) and location of plants were randomly allocated to each tray and shelf location. To commence the trial one larva was placed on the pigeon pea, then its location was recorded after 14 and 22 hours. This was repeated until 20 replicates of each species was acquired. This data was assessed with a Chi Squared test.



**Figure 1:** The arrangement of the pigeon pea and cotton plants in Experiment 2. Plants were placed 12cm apart and specimen jars were covered with soil, however lids were visible during the trials. The masking tape is used to cover the tangle foot to prevent larvae from escaping the tray.

### Experiment 3: How is the Movement of *H. punctigera* from Pigeon Pea to Cotton Affected by Distance and the Flowering of Bollgard 3 Cotton

#### Materials

- Runs: Rectangular containers (100cm x 30cm x 50cm) filled with soil and smeared with tangle-foot around the top (Figure 2). These were situated in a shed with a 16/8 day/night cycle and a temperature range of 24-28 °C
- Plant samples with the stem of the plant in specimen jars [100mL] filled with water: Quest pigeon pea (35cm tall) & Bollgard 3 cotton (6 nodes approximately 35cm tall) either with or without flowers.
- Larvae: first generation field caught *H. punctigera* were used once they reached their third instar. Three larvae were used for each replicate.

#### Method

The aim of this experiment was to establish if *H. punctigera* would move from pigeon pea to cotton on exposed soil and whether larval movement was affected by the presence of *Bt* cotton flowers. Prior to each trial larvae were exposed to pigeon pea leaves for twelve hours and plant samples were cut to size and their stems were placed in specimen jars filled with water. Plants were left for about an hour to enable them to adjust to the specimen jars to minimise wilting. Treatments, as outlined below, were then randomly allocated to each of the eight runs whilst the location of pigeon pea and cotton was randomly assigned to either end of the run (Figure 2).

**Treatment 1:** Control. Cotton was placed 25cm away from pigeon pea, allowing leaves to touch.

**Treatment 2:** 'Close'. Plants were 25cm apart, leaves of different plants did not touch (plants used in this experiment were larger than those in Experiment 2).

**Treatment 3:** 'Distant'. Plants were 100cm apart and cotton flowers were removed from the sample.

**Treatment 4:** 'Flower'. Plants were placed 100cm apart and flowers were present on the cotton.

Experiments were initiated at around 3pm each weekday for three weeks. For each replicate, the specimen jars containing plants were set at the required distance and covered with soil. Next, three larvae were placed on the pigeon pea. An intermediate observation was made after fourteen hours, then final observations were undergone at twenty-two hours. At both stages the location of each larva was noted, to obtain a count of how many animals were in each location i.e. plant, soil, wall or lost. Once 22 hours had elapsed, topsoil was removed and replaced for the next replicate. Then the average head size from each larvae was measured for each trial because this is indicative of age (instar). This was repeated until 19 replicates of each treatment were acquired. To determine the effect of treatment on larvae location, a Krustal-Wallace One-way ANOVA was undertaken, with 3 degrees of freedom.



**Figure 2:** Illustrates the set up for Experiment 3. The first image (A) shows the runs, whilst B shows the setup of each replicate including the positioning of plants for 'Close' (bottom run) and 'Flower' (top run) treatments.

### Experiment 4: When confronted with a body of water, what behaviour do larvae display and how long can they remain in the water?

#### Materials

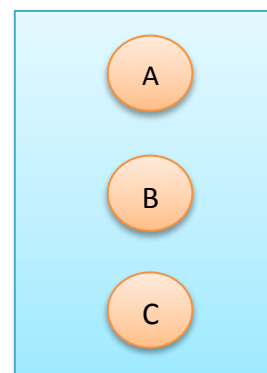
- Plastic tray (45cm x 35cm x 10cm) filled with water
- 3x Specimen jars (100mL) with white lids that were covered with soil.
- 15x Conical flasks (12x 250mL, 3x 150mL) each filled with water and coated with Fluon (a white fluid used to prevent caterpillars from escaping).

- 30 *Helicoverpa punctigera* (15 used for 4A and 15 used for 4B). All were first generation field caught larvae when they reached their 3<sup>rd</sup> instar.
- 15 first generation, field caught *Helicoverpa armigera* larvae (3<sup>rd</sup> – 4<sup>th</sup> instar).

### Method

The aim of this experiment was to see if larvae could overcome a water barrier. As such, two experiments were designed to test this. Experiment 4A involved placing larva on an island (island B in Figure 3) and monitoring its' behaviour over 15 minutes. For each replicate the location of the flower (island A or C) was randomised as well as the species of *Helicoverpa* used. Prior to using each larva, its' head size and weight was recorded. At the end of the trial, the larva's final position was noted and new soil was placed on each island.

In experiment 4B, flasks were filled with 100 or 150mL of water, depending on the containers size. Then one *H. punctigera* larva was placed in each flask and observed for either 10, 30, 60 or 120 minutes. Once the allocated time had elapsed the larvae were removed from the water and their behaviour was observed for 30 minutes to determine if they were dead or alive. Five replicates of each treatment (time) were undertaken. For both experiment 4A and 4B, a chi squared test was undertaken to highlight any significant relationships.



**Figure 3:** Demonstrates the set-up of Experiment 4A, whereby the blue square shows a body of water in the tray and the orange circles represent 'islands'. The middle island B was covered with soil and 7cm from both A and C. Islands A and C was either an island covered with soil or an island with a Bollgard 3 cotton flower on soil.

## 5. Results:

### Experiment 1

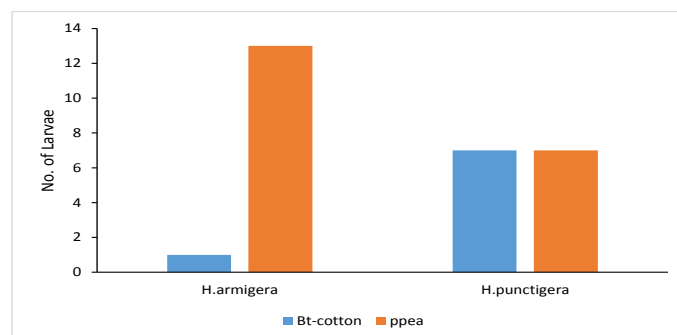
From this experiment, there was no significant difference in variety preference between the two *Helicoverpa* species ( $P > 0.05$ ; Table 1). However, Table 1 suggests that *H. armigera* ate one leaf at a time, rather than moving from one leaf to the other because *H. punctigera* had a higher 'neither' count.

**Table 1:** Displays the raw data from Experiment 1, showing that neither *Helicoverpa* species had a clear preference for Quest or Sunrise pigeon pea varieties.

	Sunrise	Quest	Neither
<i>H. armigera</i>	6	5	1
<i>H. punctigera</i>	3	4	5

### Experiment 2

In regards to the movement of each *Helicoverpa* species, *H. punctigera* was shown to be more mobile as they were more likely to reach flowering cotton after 22 hours than *H. armigera* (Fisher's exact test;  $df = 1$   $P = 0.033$ ; Figure 4). This is clear as one of fifteen *H. armigera* larvae was found on the cotton, whilst seven of the fifteen *H. punctigera* were found on cotton (Figure 4). Therefore, showing that *H. punctigera* were more likely to find cotton than *H. armigera*.



**Figure 4:** Shows the number of larvae on pigeon pea and cotton. This graph shows that *H. punctigera* were more likely to reach cotton than *H. armigera*.

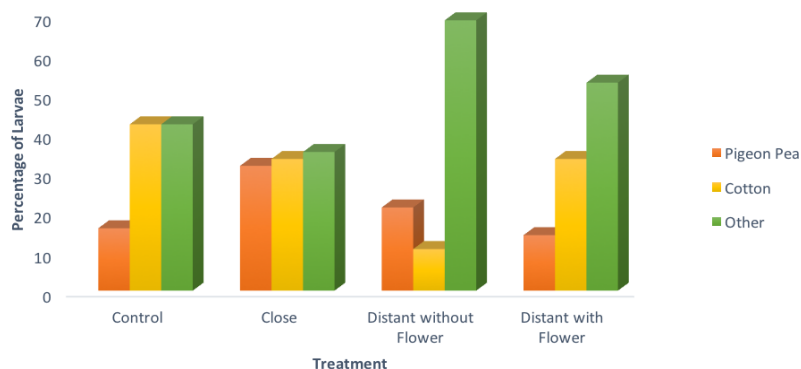
### Experiment 3

The distance between the pigeon pea and the *Bt* cotton affected the likelihood of *H. punctigera* larvae reaching the cotton plants (Figure 5). Furthermore, the presence of flowers on cotton plants significantly affected the chances of larvae reaching cotton (Kruskal-Wallis ANOVA:  $H = 9.9$ ,  $df = 3$ ,  $P = 0.01$ ). However, the number of larvae leaving pigeon pea did not change with treatment (Kruskal-Wallis ANOVA:  $H = 3.7$ ,  $df = 3$ ,  $P = 0.20$ , NS).

### Experiment 4

Results suggest that *H. armigera* were more likely to enter the water than *H. punctigera* (Fisher's exact test:  $df = 1$ ,  $P = 0.042$ ; Table 2) but that there was no significant difference in final location, between either species (Chi squared test:  $X^2 = 2.22$ ,  $df = 3$ ,  $P = 0.53$ ; Table 3). Furthermore, it was unlikely that larvae reached another island rather, the animals usually remained in the water or reached the wall of the tray (Table 3).

### Final Location of Larvae



**Figure 5:** Illustrates the effect of treatment on the final position of larvae. The percentage shows that larvae are less likely to reach cotton as distance increases, especially if there are no flowers present.

Note: The ‘other’ column includes larvae that were found on the soil, walls or those that escaped the run.

When observing the behaviour of larvae when in the water, it was found that larvae exhibit a variety of movements. These movements were classified as swimming if the larvae were moving in a particular direction by paddling or moving its abdomen horizontally or vertically (the latter appeared to help with orientation). On the other hand, animals were classified as floating if they were lethargic and appeared to be resting on the water surface.

Usually the larvae used the surface tension to their advantage by elevating their head above the water and floating or paddling with their legs to move (17 observations). Furthermore, the surface tension was seen to be advantageous when larvae neared an object. Once close, the larvae did not have to paddle or wiggle in the water to be pulled towards the edge of the island or tray (9 observations). The larvae either used this motion to crawl up the surface, or to re-orientate itself and push off from the wall.

Larvae also used a wavelike motion, moving their abdomen up and down to move forward or backward (18 observations). The head remained stable while the front legs usually paddled to aid in movement. Sometimes floating larvae formed a ‘C’ shape; bending their abdomen around so their head could rest on the dorsal segments of their abdomen (16 observations). This allowed them to move in the water with minimal energy expenditure.

Experiment 2B suggests that larvae can survive in water for prolonged periods of time. Larvae were left in water for 10, 30, 60 and 120 minutes with 5, 10, 10 and 5 replicates respectively (total larvae = 30). Of these only one larvae died in the trial (one of the 120 minute replicates).

**Table 2:** Shows the number of *H. armigera* and *H. punctigera* that entered or fell during Experiment 4A. This raw data suggests that *H. armigera* is more likely to enter the water than *H. punctigera*.

	Entered	Fell
<i>H. armigera</i>	5	10
<i>H. punctigera</i>	0	15

**Table 3:** Displays the final locations of each larva used. It is clear that once the larvae enter the water it is most likely that they will remain in the water or reach the edge of the pond (wall). This is a similar trend for both species, however *H. armigera* are more likely to remain or swim back to the middle island from where they entered the water.

	Middle Island (B)	Island with Flower	Second Soil Island	Other (Water or Wall)
<i>H. armigera</i>	4	2	1	8
<i>H. punctigera</i>	1	3	1	10

## 6. Discussion and Conclusions:

These laboratory experiments showed that the distance between pigeon pea and cotton did affect the likelihood of larvae reaching the transgenic crop. As the distance between plants increased so did the chance of larvae not locating the cotton. This means, that if pigeon pea is sown in a row adjacent to cotton then the refuge crop may become less effective as larvae can move between crops (Gould, 1998). As such, this research indicates that refuge crops might be better planted further than one metre from *Bt* cotton. Further research is required to quantify the ideal planting distance between pigeon pea refuges and *Bt* cotton fields by investigating the maximum distance larvae will move between plants, and repeating experiments in field conditions.

When cotton flowers were present and pigeon pea flowers were absent the larvae were more likely to move to the cotton. This shows that cotton flowers are an attractive food source that affects larval movement. Work undergone by Torres et al. (2009) showed that larvae prefer to feed on flowers rather than stems or leaves, likely due to lower concentration of toxins. Consequently, when larvae consume flowers they are more likely to survive the lower levels of toxin and may be able to pass on an improved ability to tolerate toxins onto their offspring (Holman 2015, Rahman et al. 2011). *H. punctigera* larvae were more likely to move onto flowering *Bt* cotton than *H. armigera* suggesting that *H. punctigera* may be more likely to gain and pass on tolerance to *Bt* toxins, but the sample sizes used here are small and this finding needs further investigation. Other behaviours, such as migration or diapause, are likely to play a part in the development of resistance and induced tolerance in either species (Fitt, 1989 & Heuberger et al. 2011) but this was not investigated in this study.

Results here showed that non-flowering pigeon pea was unattractive to larvae when compared with flowering cotton (Whitehouse et al in press). New pigeon pea varieties are being developed by the industry to flower earlier in the season to increase the crops attractiveness when cotton is also flowering. Thereby the likelihood of larvae moving into *Bt* cotton would be reduced, to ultimately increase the number of susceptible animals in the pest population.

The results from the swimming trials suggest that irrigation is unlikely to inhibit larval movement. However, the distribution of larvae may change, with an increase in animal numbers downstream. The experiments presented here suggest that larvae cannot change the direction in which they are swimming. As such, even if there was a favourable food source on the other side of the water it is unlikely that caterpillars will reach it. With this in mind, it can be said that water is a semi-permeable barrier because *Helicoverpa* larvae can cross it, however it is unfavourable due to the difficulty and unpredictability of crossing the water (Heuberger et al. 2011). As such, irrigation of cotton is unlikely to affect the movement of larvae between a refuge adjacent to a *Bt* cotton crop.

Due to the conditions in which this experiment was conducted we were able to achieve fairly conclusive results. This is due to the degree of control over several factors such as temperature, light exposure and plant sample quality. However, in regards to the latter, the time to prepare plant samples usually resulted in pigeon pea wilting before the experiment commenced. Although this was reduced by storing plants in jars filled with water, wilting continued to occur over the duration of the experiments. This may have affected the results and encouraged larvae to move to another food sources, such as cotton. As such, it is recommended to repeat these experiment in the field to evaluate how other conditions such as rain, wind, temperature and crop health affects larvae movement. Furthermore, field experiments would enable larvae to move in any direction, rather than in a relatively straight line to one food source as shown in Experiments 2 and 3. Therefore, if these trials were repeated in the field a more holistic understanding of *Helicoverpa* movement could be gained.

First generation field caught larvae were used in this experiment to simulate the movement of wild *Helicoverpa* larvae. However, the number of available larvae was limited and their age varied over the three week trial period. Although, the mean age of larvae was third instar larvae and larval instar and size was held constant within replicates, individual larva age ranged from 2<sup>nd</sup> to early 5<sup>th</sup> instars. To counteract this we would have needed to establish much higher numbers of larvae, or used large numbers of laboratory larvae; but it was assumed that these animals would display different behaviours to field caught *Helicoverpa*. In turn it was decided that selecting larvae from the same population, i.e. field caught, was more important than keeping larvae age consistent between all experiments and replicates.

## 7. Highlights from these laboratory experiments include:

- *Helicoverpa* did not have a preference for the leaves of either tested pigeon pea variety.
- *H. punctigera* was shown to be more likely to move off pigeon pea and reach *Bt* cotton, compared to *H. armigera*.
- Distance and presence of flowers on Bollgard 3 cotton affects the likelihood of larvae reaching the cotton plant when moving from pigeon pea.
  - *H. punctigera* were more likely to move onto flowering *Bt* cotton plants than *H. armigera* from pigeon pea.
  - The distance between pigeon pea and cotton does not affect the number of larvae moving off pigeon pea
  - *H. punctigera* larvae can move onto *Bt* cotton if it is 1m from pigeon pea.
- Both species of *Helicoverpa* could float and survive in bodies of water for extended periods of time. However, *H. armigera* appear more willing to enter the water than *H. punctigera*.

## 8. Future Research:

- Undertake more data analysis to assess if factors such as temperature and humidity affected the behaviour of larvae and therefore the results of this experiment.
- Confirm if the same results occur later in the season when pigeon pea is flowering and a more attractive crop.
- Repeat experiment 1 when new refuge crop varieties are released to investigate its attraction compared to the current refuge crops being used. The process would need to be standardised, for example with Quest as the control leaf, so the results are comparable. With this information, producers can make an informed decision when choosing which pigeon pea variety to plant, to maximise its effectiveness as a refuge crop.
- Repeat the swimming trials with a camera so movement can be tracked and software can be used to determine if larvae swim or float in a deliberate direction and if there is a pattern in the way they move.
- Repeat the key experiments in fields to see if the laboratory findings translate to a field situation.

## 9. Presentations and Public Relations:

There is a SOWFI (Step Off With Fresh Ideas) conference on the 1<sup>st</sup> of November, where I will present the results of this project at the University of Sydney.

## 10. Reference List:

Baker, HG, Tann, CR, Fitt, GP. (2008) Production of *Helicoverpa* spp. (Lepidoptera, Noctuidae) from different refuge crops to accompany transgenic cotton plantings in eastern Australia, *Australian Journal of Agricultural Research*, 59, 723-732, DOI: 10.1071/AR07421

Downes, S, Mahon, R. (2012) Evolution, ecology and management of resistance in *Helicoverpa* spp. To Bt cotton in Australia, *Journal of Invertebrate Pathology*, 110(3), 281-286, DOI: <http://dx.doi.org/ezproxy1.library.usyd.edu.au/10.1016/j.jip.2012.04.005>

Downes, S, Walsh, T, Tay, WT. (2016) Bt Resistance in Australian Insect Pest Species, *Insect Science*, 15, 78-83, DOI: <http://dx.doi.org/10.1016/j.cois.2016.04.002>

Fitt, GP. (1989) The Ecology of *Heliothis* species in Relation to Agroecosystems, *Annual Review of Entomology*, 34, 17-52

Gould, F. (1998) Sustainability of Transgenic Insecticidal Cultivars: Integrating Pest Genetics and Ecology, *Annual Review of Entomology*, 43, 701-26, DOI: 0066-4170/98/0101-0701\$08.00

Heuberger, S, Crowder, DW, Brevault, T, Tabashnik, BE, Carriere, Y. (2011) Modelling the Effects of Plant-to-Plant Gene Flow, Larval Behaviour, and Refuge Size on Pest Resistance to Bt Cotton, *Environmental Entomology*, 40(2), 484-495, DOI: <http://dx.doi.org/10.1603/EN10247>

Rahman, M, Glatz, R, Roush, R, Schmidt, O. (2011) Developmental Penalties Associated with Inducible Tolerance in *Helicoverpa armigera* to Insecticidal Toxins from *Bacillus thuringiensis*, *Applied and Environmental Microbiology*, 77 (4), 1443-1448.

Torres, JB, Ruberson, JR, Whitehouse, M. (2009) Transgenic Cotton for Sustainable Pest Management: A Review, *Organic Farming, Pest Control and Remediation 15 of Soil Pollutants*, Sustainable Agriculture Reviews 1, DOI 10.1007/978-1-4020-9654-9 4.

Whitehouse, M.E.A., Cross, D., Mansfield, S., Harden, S., Johnson, A., Harris, D., Tan, W., Downes, S. IN PRESS Do pigeon pea refuges pull their weight as "genetic diluters" to counter *Bt* resistance in *Helicoverpa* moths? *Crop Protection*