



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

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**SUMMER SCHOLARSHIP REPORT: 2015-2016 SEASON**

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<b>1. Project Title</b>	: The role of inter- and intra-specific competition in larval movement off refuges by <i>Helicoverpa armigera</i> and <i>Helicoverpa punctigera</i> .
<b>2. Proposed Start Date</b>	: 2 <sup>nd</sup> January 2017
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**1. Executive Summary:**

*Helicoverpa armigera* and *Helicoverpa punctigera* are destructive and adaptable pests that co-exist within the Australian cotton industry. There has been minimal research regarding how these two species interact within cotton crops to understand if one species dominates the other, or in which environment this dominance occurs. As part of the RMP, refuges are planted next to *Bt* cotton to produce high numbers of moths. If larval numbers are high in these refuges, then one *Helicoverpa* species could force out the other onto the neighbouring *Bt* cotton, where it would be exposed to *Bt* toxins. The aim of this work was to establish how *H. armigera* and *H. punctigera* competed for food, and whether in times of high density, one species was more likely to be pushed out of the pigeon pea into *Bt* cotton. Mixed and single species groups of 3<sup>rd</sup> to 5<sup>th</sup> instar *H. armigera* and *H. punctigera* larvae were set up on non-flowering pigeon pea or flowering non-*Bt* cotton refuges, and given the option of moving onto *Bt* cotton. *H. armigera* were likely to move off non-flowering pigeon pea irrespective of larval density. In mixed species groups, *H. armigera* and *H. punctigera* behaved as if they are one species complex and neither were more likely to move off the non-*Bt* flowering refuge. Both larvae species also seemed to be gregarious, but this needs further investigation. These results indicate that interspecific competition will not cause one species to be more exposed to *Bt* cotton than the other, but that there may be differences between the species in whether they will move onto *Bt* cotton.

## 2. Background:

*Helicoverpa armigera* and *punctigera* are common and resilient pests for the Australian cotton industry. The initial management practices within the Resistance Management Plan (RMP) for *Bt* cotton was aimed at *H. armigera* due to its rapid development of resistance to insecticides. Over time, the RMP evolved to manage both *Helicoverpa* species; as seen by the removal of maize and sorghum as refuge crops (these crops are not hosts for *H. punctigera*). The differences in *H. armigera* and *H. punctigera* origins and behaviour suggest that the RMP could be more effective if it further considers species-specific management practices.

As part of the modern RMP, pigeon pea crops are planted as refuge crops as both *Helicoverpa* species attack this crop. Presently, the most common pigeon pea variety used as a refuge crop is Quest, however there are problems with this variety because it has not been maintained as a commercial variety and often flowers later than the cotton. A new variety developed to improve the efficacy of pigeon pea refuges is Sunrise, which flowers earlier and more consistently than Quest. It is important to check that Sunrise is as effective as or more effective than Quest at performing its role as a refuge.

The aim of refuges is to produce large numbers of moths that have not been exposed to *Bt* toxins. These moths then mate with any moths emerging from *Bt* cotton that may be carrying resistant genes; thereby diluting the resistant recessive genes in the next generation and increasing the likelihood that the offspring will be susceptible to *Bt* toxins (Whitehouse et al, In Press). However, pigeon pea refuges are often planted in the same field adjacent to *Bt* cotton. Therefore, at high densities, excess larvae might be driven through competition out of the pigeon pea into the cotton.

By investigating the interspecific and intraspecific competition of *Helicoverpa* in this report, we can assess if, through competition, one species could be more exposed to *Bt* toxins than the other which would lead to resistance. There are two main types of intraspecific competition, scramble and contest competition (Berg et al 2006). Scramble competition is when a finite resource is accessible to all members of the species or population and so a single member can't monopolize the resource. Contest competition is the opposite, whereby one or a few individuals monopolize the resource (Berg et al 2006). If the larvae compete for food using scramble competition, then the population could be managed as one species group. If one species dominates the preferred food (pigeon pea refuges), as in contest competition, and force the other species onto *Bt* cotton, then the submissive species would be more exposed to *Bt*.

*H. armigera* are known to be cannibalistic (Bentivenha et al 2016) and are thought to be more aggressive than *H. punctigera* and therefore are assumed to be dominant to *H. punctigera* (Dial and Adler 1990). Consequently, when larval densities are high, *H. punctigera* could be pushed out of the pigeon pea by *H. armigera* and on to *Bt* cotton. Consequently, *H. punctigera* would have more exposure to *Bt* toxins than *H. armigera* larvae, and be subject to more environmental pressure to develop tolerance or resistance to *Bt* toxins.

## 3. Aims and Objectives:

To understand the inter- and intra-specific competition in *Helicoverpa*.

- 1) Do either *H. armigera* or *H. punctigera* prefer either Quest or Sunrise pigeon pea varieties?

This is to ensure that I select the most attractive pigeon pea variety, thereby gaining the strongest response in further experiments.

- 2) At what density will *H. armigera* move off non-flowering pigeon pea/ flowering non-*Bt* cotton and onto non-flowering/ flowering *Bt* cotton?

Before investigating intraspecific competition, I needed to establish the density at which intraspecific competition occurred to cause *H. armigera* to move off pigeon pea. However, initial results indicated that pigeon pea was not a strongly attractive resource for *H. armigera*, so I repeated this experiment with flowering non-*Bt* and *Bt* cotton.

- 3) Does the species composition of *Helicoverpa* larval groups on an attractive cotton refuge affect displacement from the refuge crop?
  - i) Are *H. punctigera* more likely to be excluded from an attractive refuge than *H. armigera*?
  - ii) Are *H. punctigera* more likely to feed on *Bt* cotton?
  - iii) Are individuals in a single species group less likely to be displaced than those in mixed species groups?

## 4. Materials and Methods:

**Materials:**

- Cat litter trays (44cm x 33cm x 9.5cm or 42.5x29.5x5 or 39cm x 29cm x 8.5cm)
- Baking Trays (31cm x 20 cm x 5cm)
- 100ml specimen jars

- Soil
- Non-*Bt* cotton cuttings
- Quest Pigeon Pea cuttings and leaves
- Bollgard 3 cotton cuttings
- Lab raised *H. armigera* larvae, third to fifth instars
- Lab raised *H. punctigera* larvae, third to fifth instars
- Petri dishes
- Filter paper

## Methods

### Experiment 1 – Paired Pigeon Pea Variety Choice Test

This was a preliminary test to identify if *Helicoverpa armigera* or *H. punctigera* prefer either Quest or Sunrise pigeon pea varieties, to determine which variety I should use in the following experiments.

In each petri dish I placed a Sunrise and a Quest pigeon pea leaf following one of four possible arrangements (Figure 1). These leaves were placed onto a moistened filter paper sheet, with the underside of the leaf contacting the paper. All leaves were marked with a small dot, with Quest marked on the top left and Sunrise on the bottom right of the leaf.

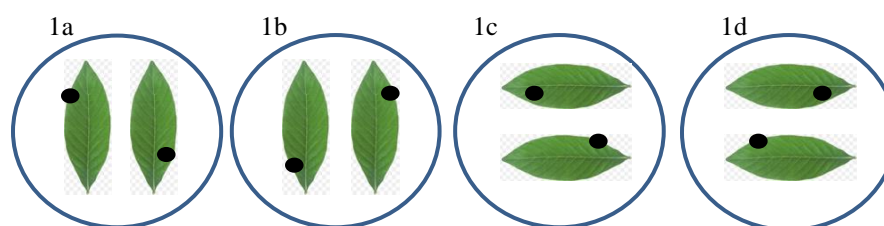


Fig. 1 The different leaf arrangements used to identify *Helicoverpa* larval preference for pigeon pea variety Sunrise or Quest. 1a illustrates Quest being on the left beside Sunrise, 1b shows Quest on the right of Sunrise. 1c shows Quest above Sunrise horizontally, then 1d shows Quest below Sunrise horizontally. Quest is illustrated with a mark on the top left of the leaf and Sunrise with a mark on the bottom right of the leaf, as was undertaken in the experiment.

In replicate 1, we placed a single third instar *H. armigera* larva between the two leaves in each of the 10 petri dishes. In replicate 2, a *H. armigera* or *H. punctigera* was placed in 12 petri dishes (3 replicates of each of the 4 arrangements described above; n=24 petri dishes). The petri dishes were randomly placed in a tray (44cm x 33cm x 9.5cm) and placed in the experiment room for 22hrs. Interim results were recorded after 12hrs with the final results after 22hrs.

I scored the degree of insect damage into 4 categories:

- 1) No damage
- 2) Slight – some small holes (up to 3mm wide) and/or marginal damage.
- 3) Moderate – large holes (3mm to 8mm) covering up to 50% of the leaf and/or marginal damage.
- 4) High – large holes or chunks removed from leaf (>8mm) covering >50% leaf damage.

The final results were analysed with a simple Chi-squared test.

### Experiment 2 – At what density will *H. armigera* move off non-flowering pigeon pea and onto non-flowering *Bt* cotton?

#### Standardised Method:

The top 25cm of pigeon pea Quest variety and Bollgard 3 cotton, with 4-5 branches with leaves, were used in these tests. Pigeon pea was not flowering at the time of the experiment, unlike the *Bt* cotton, so cotton branches without a flower were selected for this experiment.

Specimen jars (100ml) were filled with water and approximately 1cm was cut off the base of the plants stem before securing the branch within the water in the jar. These jars were then buried in a baking foil tray filled with soil, so that the top of the jar was flush with the soil surface. The plants were placed 12cm apart (stem to stem), ensuring no branches overhung the baking tray, and that none of the *Bt* cotton or pigeon pea branches touched. The foil tray was then placed within a cat litter tray containing a moat of water, 1.5cm deep, to prevent larvae from escaping. This tray set-up acts as one treatment unit.

The experiment was undertaken in a temperature and light controlled room (Temperature range: 24-28 °C, 16:8 day:light). The order of treatments (densities 1-4 of larvae) on the shelf, from the door to the window of the experiment room, was randomised using dice. The positioning of the cotton and pigeon pea within the tray was randomised, so that either plant could be next to the wall or on the edge of the shelf closer to the window and light. The temperature and humidity was monitored continuously throughout the experimental period with an AZ 8829 datalogger.

Individuals from a well-established laboratory colony of *Helicoverpa armigera* were taken as third or fourth instar larvae and randomly placed on the pigeon pea in accordance with treatment densities. The 2015-16 CRDC Summer Scholarship Report

treatments were of one larvae (T1), two larvae (T2), three larvae (T3) and four larvae (T4) on the pigeon pea. The larvae were randomly placed on an individual leaf and/or branch when applicable. Interim results were taken approximately 12hrs after set-up, followed by the final results after 22hrs.

The location of larvae on either pigeon pea, *Bt* cotton or 'other' ('other' = soil, water or missing), was recorded at set-up, interim (12hrs) and final results (22hrs). Notes on plant health status was also recorded at set-up and final results. The head sizes and instar of larvae was recorded after the completion of the experiment. A Kruskal-Wallis ANOVA analysis was performed to analyse the movement of larvae off the pigeon pea.

*Adapted Method:*

The initial results of Experiment 2 (10 replicates), indicated that non-flowering pigeon pea was not very attractive and therefore not a good basis for experiments on the behaviour of larvae at high densities, in order to observe movement off the refuge crop and onto *Bt* cotton. Therefore, a preliminary experiment was conducted by replacing pigeon pea with non-*Bt* cotton, with neither cotton plants containing a flower.

From these results, this method was then adapted to include a flowering non-*Bt* cotton branch and a flowering *Bt*-cotton branch. As such, both the cotton branches contained a single white flower on one branch. The larvae were then placed on non-*Bt* cotton leaves initially. The remainder of the standardised method, as described above, was followed.

**Experiment 3** – Does the species composition of *Helicoverpa* larval groups on an attractive cotton refuge affect displacement from the refuge crop?

- i) Are *H. punctigera* more likely to be excluded from an attractive refuge than *H. armigera*?
- ii) Are *H. punctigera* more likely to be found on *Bt* cotton?
- iii) Are individuals in a single species group less likely to be displaced than those in mixed species groups?

The adapted method from Experiment 2 was followed, whereby each cotton branch had a single flower and a minimum of 2 leaves. See Figure 2 to see the experimental set-up of this adapted method. The treatments were also expanded and enhanced to give 7 treatments, as seen below:

- Treatment 1 (intraspecific interactions) – four *H. armigera* larvae
- Treatment 2 (intraspecific interactions) – four *H. punctigera* larvae
- Treatment 3 (interspecific interaction)– two *H. armigera* with two *H. punctigera* larvae
- Treatment 4 (control) – two *H. armigera* larvae
- Treatment 5 (control) – two *H. punctigera* larvae
- Treatment 6 (interspecific interactions)– three *H. armigera* with one *H. punctigera* larvae
- Treatment 7 (interspecific interactions)– one *H. armigera* with three *H. punctigera* larvae

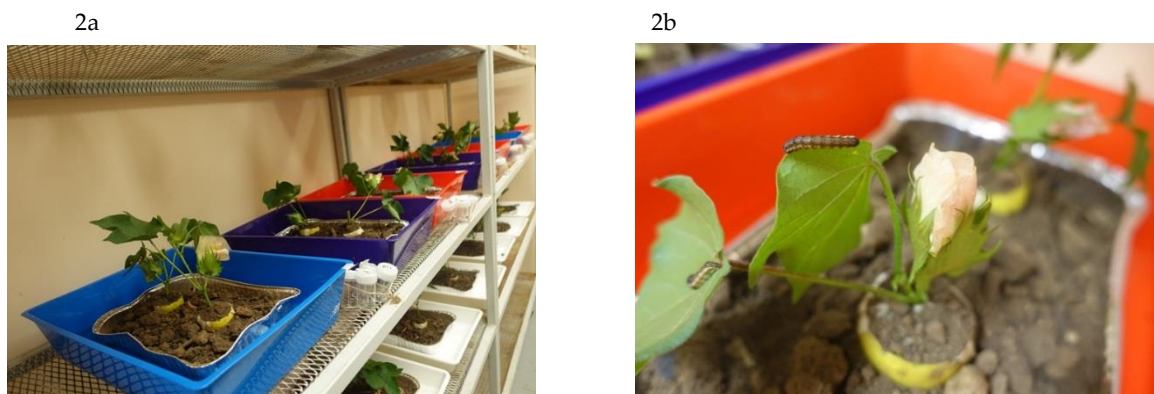


Fig. 2. The experimental setup illustrating the cotton branches with a single white flower and two leaves. 2a shows the overall set-up within the experimental room, whilst 2b shows *H. armigera* on the leaves of the non-*Bt* cotton plant of treatment 1 (four *H. armigera* larvae).

*H. armigera* and *H. punctigera* instars ranged from third to fifth instars, yet these were standardised within replicates by matching the instar, size and moult stage of the larvae.

The interim (12hrs) and final (22hrs) location of each larvae was recorded and categorised into either 'non-*Bt*', '*Bt*' or 'other' which included soil, water or missing. The head size, weight and instar of each larvae was also recorded. Other observations recorded, included plant health status and growth stage (e.g. presence of bolls).

After the final results were taken, a microscope was used to identify the *H. armigera* and *H. punctigera* larvae within treatments 3, 6 and 7. Finally, a Kruskal-Wallis ANOVA analysis was performed to analyse the

movement of larvae off the non-*Bt* cotton, as well as the interactions within and between the *Helicoverpa* species.

## 5. Results:

### Experiment 1 – Paired Pigeon Pea Variety Choice Test

The amount of leaf eaten was ranked on a scale of 1 to 4, where 1= the leaf remained un-eaten and 4 = most of the leaf was eaten (>50%; see methods). A larvae was classified as preferring one variety over the other, if it ate more of that variety (as indicated by the ranking system). This analysis indicated that both *H. armigera* and *H. punctigera* larvae preferred Quest leaves over Sunrise leaves. 15 *H. armigera* larvae and 8 *H. punctigera* larvae, out of 22 and 12 respectively, preferred Quest leaves (Table 1; Chi square Goodness of fit; *H. armigera*; Chi sq=3.98, df=1, P=0.046; *H. punctigera*; Chi sq = 6.2, df=1, P=0.013; Statistical package= GenStat 16.1).

To identify whether the two species had different preferences, a Mann-Whitney U test on the ranked preferences revealed that *H. armigera* and *H. punctigera*, consumed similar amounts of Quest (H= 0.39, df=1, P=0.53 NS) and Sunrise (H=2.9, df=1, P=0.088), although there was a trend for *H. armigera* to eat more Sunrise.

Variety Preferred	Quest	Sunrise	Neither
<i>H. armigera</i>	15	6	0
<i>H. punctigera</i>	8	1	3

Table 1. *Helicoverpa* leaf preference for Quest or Sunrise variety.

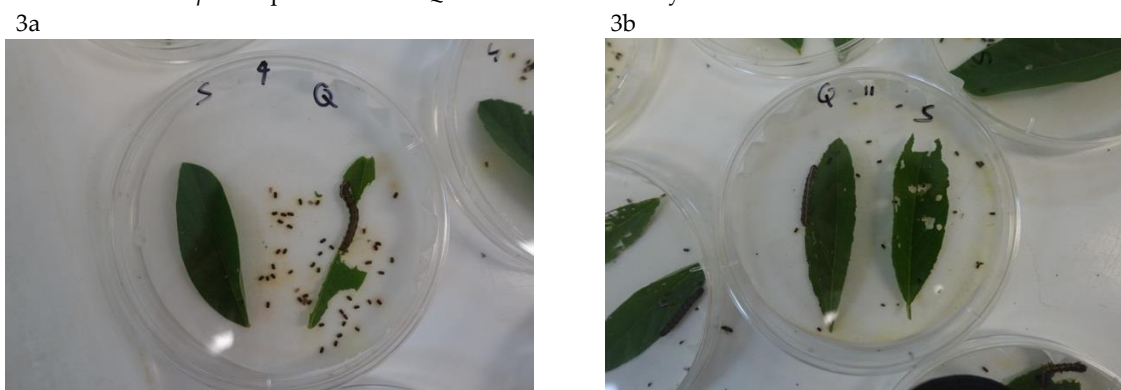


Fig. 3. The above images show the results of *H. armigera* eating behaviour on Quest (Q) and Sunrise (S) pigeon pea varieties after 22hrs. The Sunrise leaf in 3a (left leaf) shows no damage, whilst the Quest leaf in this same petri dish (3a) shows high damage, illustrating the larvae's preference for Quest pigeon pea. 3b shows slight damage on the margins of the Quest leaf (left) and moderate damage to the Sunrise (left) leaf. 3b reflects a preference for Sunrise pigeon pea variety over Quest, unlike 3a.

### Experiment 2 – At what density will *H. armigera* move off the refuge onto *Bt* cotton?

#### Moving from: non-flowering pigeon pea to non-flowering *Bt* cotton

As *H. armigera* larval density increased, the number of larvae remaining on the pigeon pea after 22hrs increased (GenStat 16.1: Kruskal-Wallis one-way ANOVA; H=8.9, df=3, p=0.031, n= 40 with 10 reps per treatment). However, there was no clear density at which larvae left the pigeon pea, as on average, about 50% of all the larvae in the 4 treatments stayed on the pigeon pea plant (average number staying: groups of 1 larvae = 0.5, 2 larvae=1.1, 3 larvae=1.3, 4 larvae=2; Fig. 4). Conversely, higher densities did not indicate that more larvae moved onto *Bt* cotton (GenStat 16.1:Kruskal-Wallis one-way ANOVA; H=1.2, df=3, p=0.66 NS, 10 reps per treatment) as, irrespective of larval density, a total of 3-7 individuals moved onto the *Bt* cotton for each treatment.

There was no difference between treatments in the proportion of larvae that moved onto *Bt* cotton (GenStat 16.1: Kruskal-Wallis one-way ANOVA; H=2.1, df=3, p=0.56 NS, 10 reps per treatment, Fig. 4). As there was no significant difference between treatments, *H. armigera* movement wasn't dictated by density. However, there may be a trend of increased density increasing the gregarious behaviour of the larvae, as there is more larval movement in densities 1-3 as opposed to 4. But this argument needs further analysis to be verified.

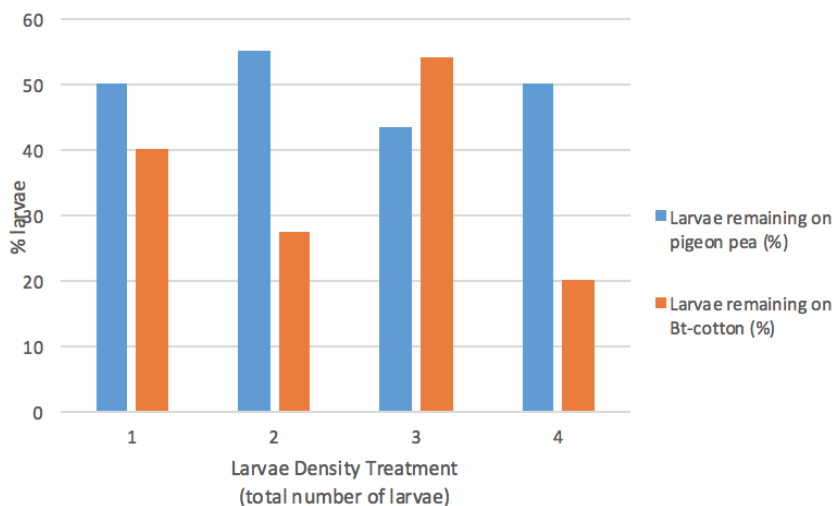


Fig. 4. The larval position after 22hrs of the density experiment, as a percentage of the total larvae tested within each treatment (number of larvae within the treatment). The positions were either pigeon pea or *Bt*-cotton (other locations are not displayed).

Moving from: flowering non-*Bt* cotton to flowering *Bt* cotton

This experiment, which was undertaken to test if Experiment 3 should use pigeon pea or flowering non-*Bt* cotton as the refuge, had similar results to the first Experiment 2. By combining the results for *H. armigera* and *H. punctigera* (n=16, 4 reps/treatment, Fig. 5) it appeared that the cotton plants were more likely to have a consistent carrying capacity of 2 larvae (Fig. 5). This suggested that the optimum group size on these branches is two individuals, and that non *Bt* cotton with flowers should be used in Experiment 3.

There may be a trend for gregarious behaviour to increase as larval densities increase, as larvae in densities lower than four, especially with a single larvae, appeared to move between locations more than those in groups of four. However, this possibility needs further investigation.

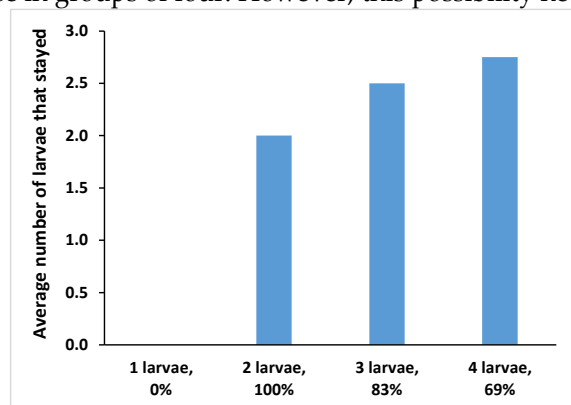


Fig. 5. The number of larvae after 22hrs still on the flowering non-*Bt* cotton in each treatment. The percentages of larvae that stayed are indicated under the histogram.

Experiment 3 – Does the species composition of *Helicoverpa* larval groups on an attractive cotton refuge affect displacement from the refuge crop?

i) Are *H. punctigera* more likely to be excluded from an attractive refuge than *H. armigera*?

Twenty replicates of the seven treatments (total n=140) were used in this analysis. To test if *H. punctigera* were more likely to be excluded, I compared the percentage of *H. armigera* and *H. punctigera* located on non-*Bt*-cotton after 22 hours. By comparing all *H. armigera* treatments, there was no significant effect of treatment either in terms of density or group composition, on the proportion of *H. armigera* remaining on non-*Bt* cotton (Kruskal-Wallis one-way ANOVA; H=5.6; P= 0.232 NS, df=4). Similarly, when all *H. punctigera* treatments were compared, there was no significant effect of density or group composition on the proportion of *H. punctigera* remaining on the non-*Bt* cotton (Kruskal-Wallis one-way ANOVA; H=4.8; P= 0.305 NS, df=4). When treatments 6 and 7 (1. *H. armigera* with 3 *H. punctigera*; 1*H. punctigera* with 3 *H. armigera*) were directly compared, *H. punctigera* was not more likely to be excluded from an attractive refuge than *H. armigera* (Fisher's exact test, P=0.12, NS).

ii) Are *H. punctigera* or *H. armigera* more likely to be found on *Bt* cotton?

There was no effect of group composition on the proportion of *H. armigera* found on *Bt*-cotton (Kruskal – Wallis one-way ANOVA; H=4.8; P=0.31 NS; df=4; all treatments excluding 2 *H. punctigera* and 4 *H. punctigera*;

Figure 6) with only 5-15% of larvae found on *Bt* cotton in any treatment. However, there is a trend for a higher proportion of *H. punctigera* to move onto *Bt* cotton when there are more *H. punctigera* present (Kruskal –Wallis one-way ANOVA;  $H=8.6$ ;  $P=0.072$  NS;  $df=4$ ; all treatments excluding 2 *H. armigera* and 4 *H. armigera*), again with 5-15% of larvae found on *Bt* cotton in any treatment.

When only treatments 2, 3 and 5 (2 *H. punctigera*; 2 *H. punctigera* and 2 *H. armigera*; 4 *H. punctigera*) are compared, *H. punctigera* are more likely to move onto *Bt* cotton as larval densities increase (Kruskal –Wallis one-way ANOVA;  $H=8.8$ ;  $P=0.012$ ;  $df=4$ ). This suggests that *H. punctigera* may be more sensitive to *H. punctigera* numbers than overall *Helicoverpa* numbers. However, as the first analysis was only a trend, and the second analysis was *a posteriori*, further research is required to investigate if *H. punctigera* are more likely to move onto *Bt* cotton when the number of their larvae increases on refuges.

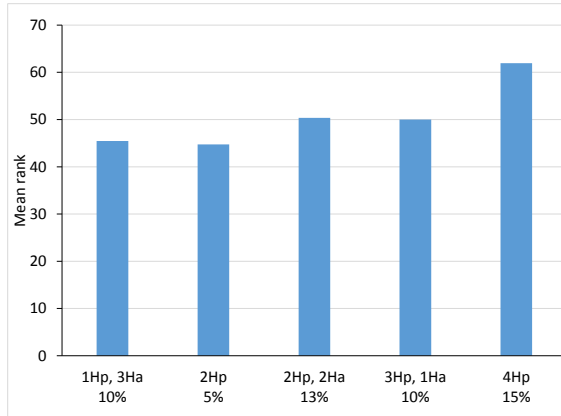


Fig. 6. The mean rank of the proportion of *H. punctigera* found on *Bt* cotton in treatments which vary in total number, and the number of *H. punctigera* present. Percentages under the histogram indicate the proportion of all *H. punctigera* larvae in that treatment that were found on *Bt* cotton

iii) Are individuals in a single species group less likely to be displaced than those in mixed species groups?

When single species and mixed species groups were compared, there was no statistically significant effect of density or group composition on the proportion of *H. armigera* moving off non-*Bt* cotton. Therefore, when group size is constant, the group composition had no effect on the number of larvae remaining on non-*Bt* cotton (Kruskal –Wallis one way ANOVA;  $H=5.1$   $P=0.28$  NS;  $df=4$ ) or moving to *Bt* cotton; (Kruskal –Wallis one way ANOVA;  $H= 2.3$   $P=0.683$  NS;  $df=4$ ). Furthermore, when a Fishers exact test was undertaken, *H. punctigera* was excluded from the non-*Bt* cotton no less than *H. armigera*.

### Discussion and Conclusions:

As a refuge crop, pigeon pea ideally needs to be flowering in-conjunction with the cotton in order to remain attractive to *Helicoverpa* (Firempong and Zalucki 1990; Zalucki et al 1994 and Downes et al 2010, Whitehouse et al in press). For this reason, Sunrise was developed as it flowers earlier and is more likely to be flowering in sync with the *Bt* cotton. However, during this experiment neither Sunrise nor Quest were flowering, even though the cotton was flowering. As Sunrise was not flowering, the leaves were tested, but the leaves of Sunrise were less attractive than those of Quest, a less reliable pigeon pea variety. This could be problematic, as producers are likely to switch to Sunrise from Quest, and so the attractiveness of refuge crops when not flowering could be reduced. However, the larvae used in this study were from a laboratory colony, and may have been more sensitive to plant defence chemicals, such as tannins, than field caught larvae. Larvae from field caught moths used in another study showed no preference between pigeon pea varieties (L Paisley pers com). Nevertheless, as *H. armigera* larvae were equally likely to vacate the pigeon pea at any density (Experiment 2), the results indicate that non-flowering pigeon pea was not highly valued as a food resource for *Helicoverpa* larvae.

It is important to compare lab and field caught larvae because these two groups could behave differently due to the environment to which they have been previously exposed. For example lab raised *Plodia interpunctella* (Hubner) larvae have a greater ability to consume food and gain weight than wild caught larvae of the same species (Sambaraju and Phillips 2008). Evidently, lab and wild larvae exhibit different behaviour, but depending on the experiment, lab larvae can be advantageous.

For ‘bulk’ experiments, where large numbers of larvae are required, lab larvae are preferred as wild caught moths are difficult to catch and induce to produce offspring. Nevertheless, as the type of larvae used for the experiment could influence the results, the origin of the larvae must be considered when assessing the experimental results. Lab larvae were used in this experiment as there were sufficient numbers of lab larvae while wild larvae were scarce, and the lab larvae, due to their ravenous feeding habits, were more

likely to compete for favourable food resources after placement on a less favourable food resource (Sambaraju and Phillips 2008).

Regardless, as larvae remained on pigeon pea and non-*Bt* cotton even though the densities increased (Experiments 2 and 3), *Helicoverpa* larvae were acting as one large species group competing for food using scramble competition. There was no evidence that *H. armigera* excluded *H. punctigera* from refuges at higher densities, hence they did not display any contest competition.

However, there was some evidence that *H. punctigera* was more likely to move off the refuge onto *Bt* cotton as the density of its own species increased. However, the statistics supporting this are not strong, so further investigation is required.

The gregarious behaviour of the larvae suggests that they stay in groups to overcome the plant defences that take place once herbivorous eating occurs. This could be due to *Helicoverpa* larvae overcoming the plants defence mechanisms by effectively metabolising host-plant toxins. These toxins are primarily the plants P450's (Despres 2007). Plants have difficulty producing sufficient toxins to target multiple larvae, and so it is important for cotton growers to manage *Helicoverpa* larval numbers at the genus, not the species level, as they will act as one species to cause crop damage by overcoming plant defences.

This suggests that the observed movement of larvae at a density of 1, is larva moving to find another larva to overcome the plants defences together. Alternatively, this movement may have been due to the unattractiveness of the pigeon pea seen in Experiment 1. In the experiments where flowering non-*Bt* cotton and flowering *Bt* cotton were used to ensure both plants were equally attractive, increased density had less of an effect on the movement off non-*Bt* cotton. When non-flowering pigeon pea was used, larvae continued to move off the pigeon pea even in larger groups that could overcome plant defences, reflecting its lower attraction to larvae compared to flowering cotton.

It seems that *H. armigera* and *H. punctigera* do not distinguish between species, as exclusion and aggression was not increased within mixed species. Stanley 1978 found similar results when *H. armigera* was raised with *H. punctigera* on diet. In these studies, *H. punctigera* survival rate was lower in mixed species groups compared to single species groups, yet the survival of *H. armigera* was not significantly altered by interspecific competition.

Overall despite the expectation that *H. armigera* are more aggressive than *H. punctigera*, *H. armigera* and *H. punctigera* acted as one species in mixed species groups and so *H. armigera* not display this dominance. Therefore, *H. punctigera* may not be more likely to be excluded from refuges and driven onto *Bt* cotton. Further research into this interaction between *H. armigera* and *H. punctigera* could be undertaken to identify any forms of dominance or hierarchy structure within mixed group species. This research could be used also to understand *Helicoverpa* interactions and how these influence the acquisition of *Bt* tolerance.

## 6. Highlights:

Mixed species groups seem to be acting as one species with gregarious behaviour, presumably to overcome the plant's defence mechanism. In these mixed species groups scramble competition not contest competition was observed.

There was no evidence that *H. armigera* were likely to exclude *H. punctigera* from refuges when larval densities are high.

## 7. Future Research:

*H. punctigera* seem more tolerant to *Bt* cotton than *H. armigera*, as they tend to move towards *Bt* cotton when pushed off pigeon pea. This needs further investigation.

The observation that the density of its own species may affect *H. punctigera* needs further investigation.

Research to investigate the effect of larval size, weight and instar age on interspecies interactions between *H. armigera* and *H. punctigera* needs to be undertaken.

Similarly, how mixed species groups interact in a competitive environment could be studied to identify if there is a hierarchical system between *H. armigera* and *H. punctigera*.

Further research needs to be undertaken regarding the effect of using laboratory raised larvae, as opposed to wild caught larvae, in these experiments.

## 8. Presentations and Public Relations:

SOWFI Conference (Final presentation for university) on the 1<sup>st</sup> of November, 2017.

## 9. Reference List:

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