Cotton CRC Summer Scholarship Program
CSIRO Ecosystem Sciences Narrabri

Correlating Refuge Attractiveness with Productivity

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SUMMER SCHOLARSHIP

Final Report

Part 1 - Summary Details

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Correlating refuge attractiveness with productivity

Background

With the introduction of cotton containing the two Bollgard II® genes (Cry1Ac and CryIIAc), the need for cotton producers to spray their crops with pesticides to control *Helicoverpa* spp. has been greatly reduced (Fitt, 2000). This has made cotton more suitable to Integrated Pest Management (IPM) strategies that are an ever increasing economically and environmentally sound approach to the control of pests (Fitt et al 2004). IPM strategies have provided the grower with many benefits in terms of costs saved, and to the community in terms of lower levels of insecticide in the environment (Fitt, 2000). Most notably, there has been a marked reduction in pesticide use and a shift to using softer (target-specific) pesticides, as well as a greater interest in the management of beneficial invertebrates such as predators and parasitoids (Hoque et al 2000, Mansfield et al 2006).

However the major challenge to the sustainable use of Bt cotton is the risk of *Helicoverpa* spp. developing resistance to the *Cry* toxins (Fitt, 2000). Resistance to conventional Bt sprays has evolved in field populations of another moth (*Plutella xylostella*), Bt resistant strains of *H. armigera* have been generated in the laboratory, and alleles that confer resistance have been isolated from field populations (Downes et al 2010; Mahon et al 2007; Baker et al 2008). Concerns for Bt resistance are well founded, and much effort is being applied to developing and implementing resistance management strategies.

The most notable strategy is the mandatory use of refuge crops to maintain sources of susceptible moths in the field. The rationale is that susceptible moths from the refuges will mate with potentially resistant individuals produced in the Bt crops, thus dampening the development of resistance (Roush et al 1998; Baker et al 2008). Previous work has indicated that refuges of pigeon pea are more attractive to *Helicoverpa* spp. moths, and produce twice the amount of pupae and moths than cotton refuges (Baker et al 2008). Because of this productivity, growers only need to plant half as much pigeon pea refuge as cotton refuge.

There is further pressure to reduce refuges from the development of Bollgard III® cotton, in which another insecticidal toxin, coded by the *vip3A* gene (derived from the vegetative stage of *Bacillus thuringiensis*) is added to Bollgard II® cotton, This is because the added *vip3A* gene is expected to significantly reduce the chance of *Helicoverpa* spp. pests surviving on Bt cotton (as it now has to be resistant to three toxins, rather than just two). The combination of *vip3A* and Bollgard II® referred to as Bollgard III® is expected to protect cotton against important lepidopteran pests of cotton including *Helicoverpa armigera* and *H. punctigera* (Monsanto Australia Limited, 2010). Refuge crops will still be essential in dampening the development resistance to the Bt genes in *Helicoverpa* spp., but most likely there will be room for a reduction in the area of refuge needed to accompany the transgenic cotton varieties.

An aim of the Cotton CRC is to deliver sustainable ecosystems with reduced impacts on catchments. Transgenic Bt cotton reduces cotton's impact on surrounding catchments by drastically reducing insecticide use. Refuges maintain the viability of Bt cotton by delaying resistance by *Helicoverpa* spp. to the Bt toxin. With the advent of Bollgard® III there will be pressure to reduce refuges. To ensure that we have the best resistant management strategies in place for Bollgard III®, we needed to confirm refuge assumptions. These include that refuge attractiveness is correlated with refuge productivity, and that pigeon pea refuges are more attractive and productive than cotton refuges. The aim of this project was to test these assumptions by measuring the relationship between crop attractiveness and pupae productivity in both cotton and pigeon pea refuges. This information will enable us to quantify refuge productivity and to better estimate refuge requirements for Bollgard III®.

This project involved the sampling of 22 different fields from 20 farms in the Namoi/Gwydir area of NSW, in the months of January and February 2011. My supervisors and I are very grateful to the growers of these fields for allowing us to use the fields in this study. Their direct involvement with this project will hopefully give them a sense of ownership and receptiveness to the results. Thus the project supports the Cotton CRC's mission to both provide high quality collaborative research and improve adoption of the findings by the cotton industry.

Methodology

In this project 11 Pigeon Pea fields and 11 Conventional Cotton fields were sampled to compare the survivorship of *Helicoverpa* spp. These fields were matched as closely as possible for crop management and growing conditions in the Namoi/Gwydir region (Fig. 1).

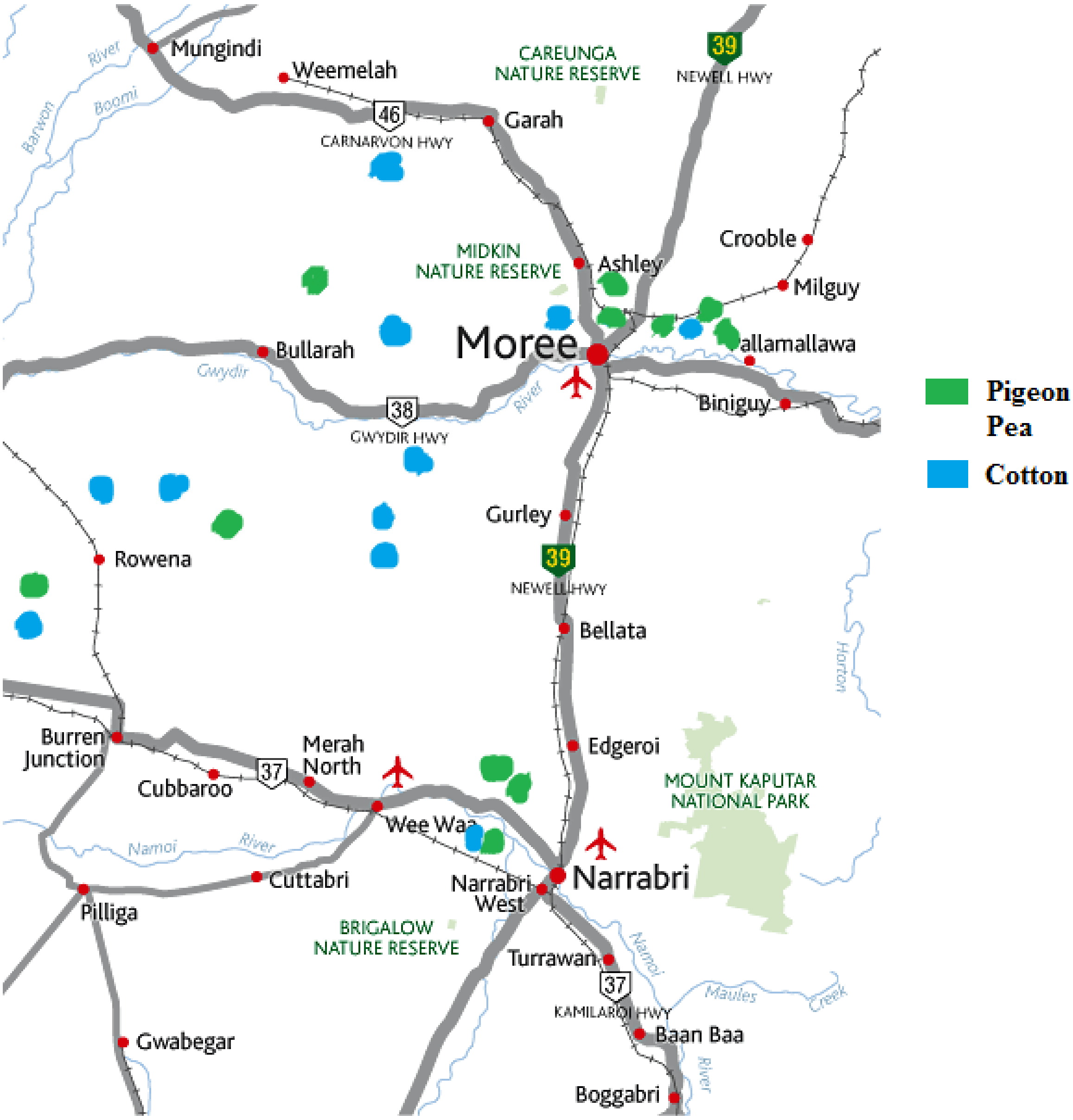

Figure 1. Map of North West NSW illustrating key field sites. Cotton fields are shown in blue, Pigeon Pea fields are shown in green.

Visual surveys to measure attractiveness.

Each field was sampled twice by a two person team during the season; once in early January (5^{th} -13^{th}), and again three weeks later in late January/early February (24^{th} Jan – 7^{th} Feb). To sample each field, six reps, each consisting of a metre of row, were randomly chosen within the field (Fig. 2). The metre was carefully checked by eye, with each plant searched individually in order to record:

- Physical plant details: number of plants, plant height, number of nodes, number of nodes above white flower, and plant growth stage.

- Number of Helicoverpa eggs and their growth stage
- Number of Helicoverpa larvae and their growth stage
- Any other notable caterpillars/predators

Each metre was then marked with a flag so that it could be later checked for pupae.

Figure 2. Visual sample site showing a 1 meter length of one row of cotton refuge

Pupae samples to measure productivity

Three weeks after each visual sample, the same one-metre sample was dug for pupae. A two person team returned to the site of each visual sample to perform the pupae dig. A one metre length of soil on either side of the row was carefully removed, to a depth of approx 10cm and roughly a hand span away from the base of the plant, as shown in (Fig. 3). Any pupae found were collected in specimen jars and labelled.

Each pupae dig recorded:

- Plant growth stage
- Number of Helicoverpa pupae and their classification (prepupae, intact, damaged, parasitized)
- Number of emerged Helicoverpa pupae and their classification (moth emerged, parasitoid emerged)

Any collected pre-pupae were transferred to jars containing vermiculite and allowed to pupate, whilst being monitored daily. The date of emergence was recorded along with what species of moth or parasite was reared from the pupae.

Figure 3. Pupae dig site, showing 1 meter length dug for pupae.

Statistical analysis:

Because most of the data collected included many zeros and therefore was not normally distributed, most analyses were non-parametric, including the non-parametric Kruskal-Wallis ANOVA, Spearman's rank correlation coefficient, Mann-Whitney U test and Chi-square goodness of fit. All analyses were conducted in Genstat version 12.

Results

Visual surveys to measure attractiveness.

The January sample included some heavy egg lay events, although there was substantial variation between samples (range: 0 – 75 eggs/metre in cotton; 0-59 eggs/metre in pigeon pea; Fig.4). Fewer eggs were collected in February compared with the first sample (range: 0 -14 eggs/metre in cotton, 0 – 7 eggs/metre in pigeon pea; Kruskal Wallis one-way ANOVA: $H = 79.69$, $n = 264$, $df = 1$, $P < 0.001$). Nevertheless there was no difference in the number of eggs collected between cotton and pigeon pea crops for either January or February (Kruskal Wallis one-way ANOVA; January: H = 0.78, n=132, df = 1, NS; February: H = 1.63, n= 132, df = 1, NS).

In pigeon pea samples, the number of eggs collected in January was more strongly associated with early and mid flowering plants than plants that had not flowered or had finished flowering (Kruskal-Wallis one-way ANOVA: H=16.82,df=2, P<0.001; ; average rank for - 0% flowering (pre-flowering): 25, 5-50% flowering: 51, 80-100% flowering: 32).

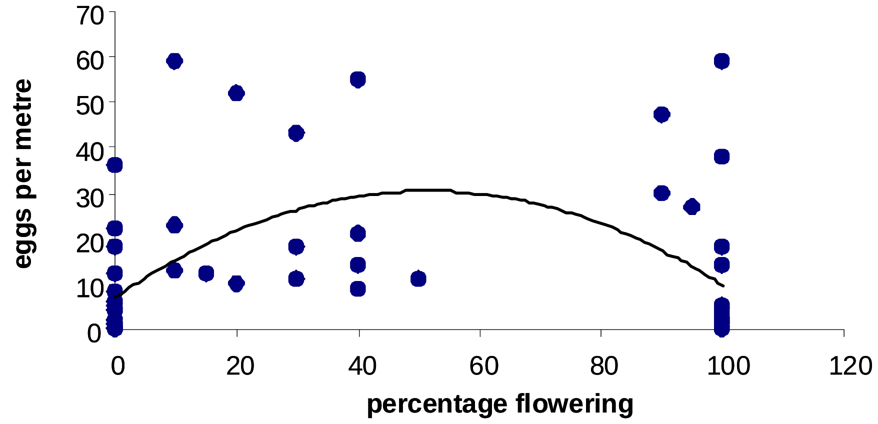

Figure 4. Relationship between percentage flowering and eggs found per metre in pigeon pea in January showing trend line. Eggs were more common in early flowering pigeon pea.

In cotton there was no similar association, although fields which recorded high egg lays in January were correlated with high egg lays in February (Spearman's rank correlation coefficient: t approximation = 3.87, df = 64, P < 0.001, Fig. 5). In pigeon pea fields, there was no correlation between egg lays from the two samples (Spearman's rank correlation coefficient: t approximation = 0.86, df = 64, NS).

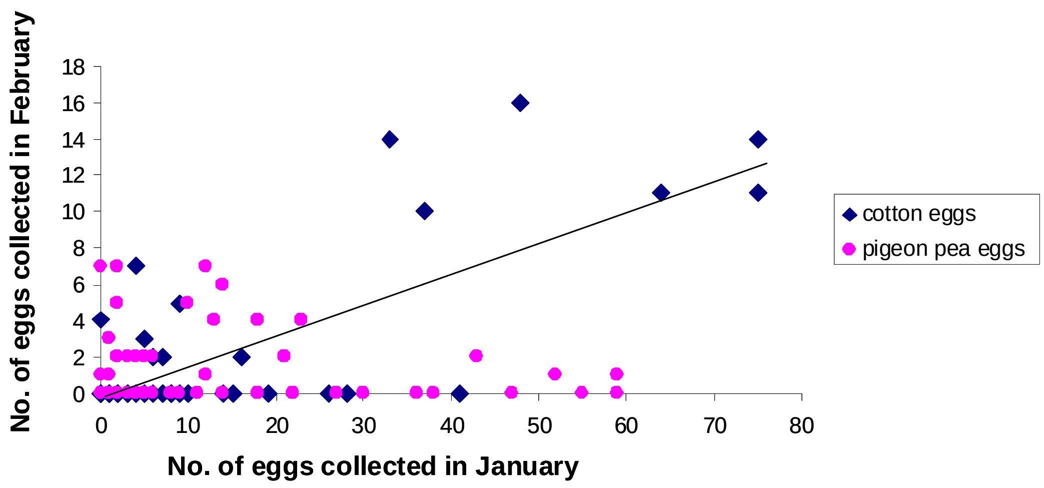

Figure 5. Number of eggs per metre found in the same cotton and pigeon pea fields in January and February. The line illustrates the correlation between January and February egg lays in cotton. There was no such correlation in egg lays in pigeon pea.

Pupae samples to measure productivity

The number of collected pupae and their emergences are shown in Fig. 6. The total number of pupae collected over the two sampling periods was quite low for both cotton (38 in January, 32 in February) and pigeon pea (50 in January, 12 in February) refuges. For example, out of the 22 fields dug for pupae during the first digs in late January, only 13 fields yielded pupae for collection. Although the low numbers of pupae made statistical analysis difficult, overall more moths emerged from pupae collected from cotton rather than pigeon pea fields (χ^2 = 9.78, df = 2, P = 0.008; Fig. 6).

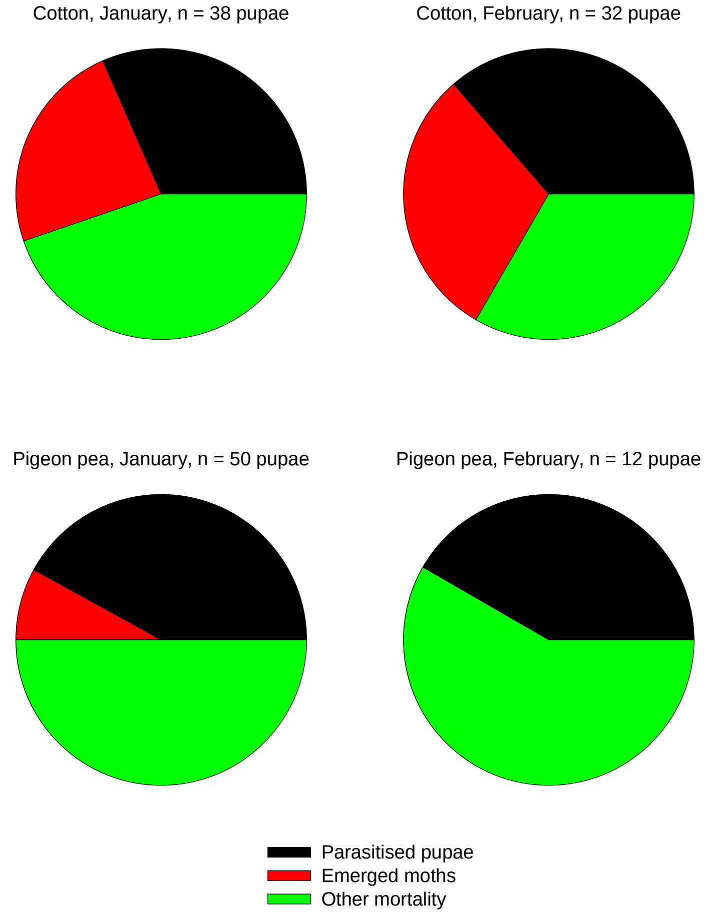

Figure 6. Emergence (%) of moths and parasitoids from collected pupae. More moths were collected from cotton refuges.

Parasitism rates were high in both cotton and pigeon pea for both January and February (Cotton: 32% and 38% respectively; and Pigeon pea: 43% and 42% respectively). Also of interest was the type of parasitoids which were reared from the collected *Helicoverpa* spp. pupae. In the past there has been a tendency for a good number of parasites to be Tachinidae, *Heteropelma* or *Ichneumon* (Baker *et al.* 2008). This pattern was again repeated with the Tachnids *Gonioptha lmus*, *Carcelia* and the wasp *Heteropelma* dominating the parasitoids (Fig. 7).

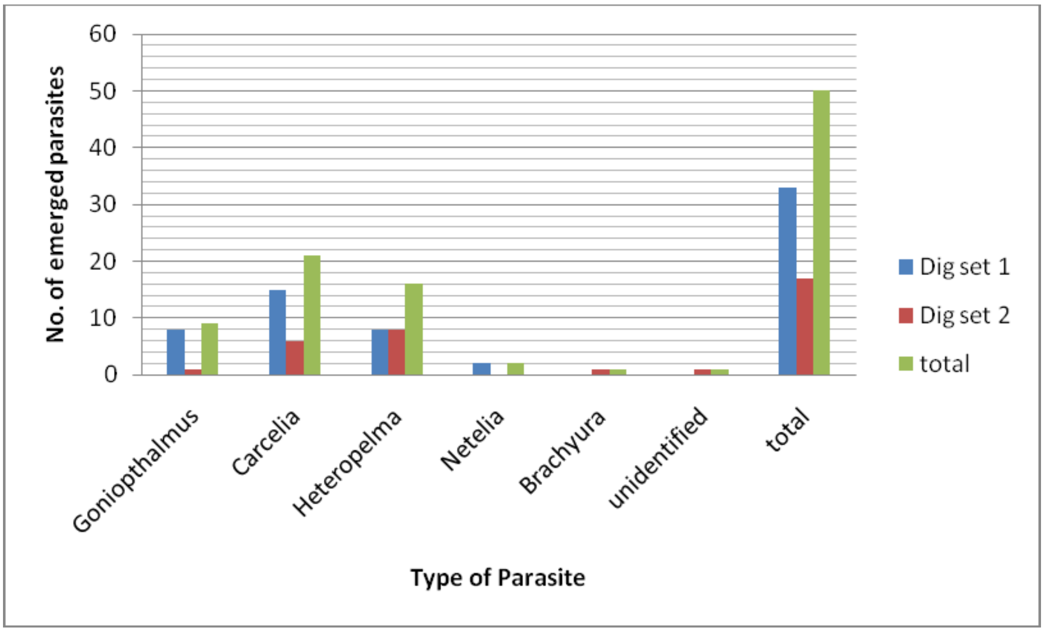

Figure 7. Numbers of parasites emerged according to each dig. Set 1 = January sample, and Set 2 = February

Is attractiveness correlated with productivity?

Comparing the relationship between egg lays and pupae collected was difficult because of the large number of samples where either no eggs or pupae or both were collected (Fig. 8). Because of this it was not possible to test for non-linear relationships between egg lays and pupae found. There was no linear correlation between egg lays and pupae found in cotton in either January or February, although there was a linear correlation between egg lays and pupae digs in pigeon pea in January only (Spearman's rank correlation coefficient, correlation =0.4, n=66, t approx.=3.48, P<0.001).

Nevertheless there was a difference in the relationship between egg lays and pupae found between the January and February samples for both cotton and pigeon pea. To conduct this analysis, the ratio between egg and pupae was calculated by dividing the number of pupae by the number of eggs found in each field (after first removing the zeros by adding 1 to all data). When these ratios were compared, more pupae were associated per egg lay in February than in January for both cotton and pigeon pea, suggesting better survival of Helicoverpa in February in comparison to January (Mann Whitney U test for pigeon pea ratios: U=630, February has the highest rank sum, P<0.001, n=66,57; cotton ratios: U=921, February has the highest rank sum, P<0.001, n=66,65).

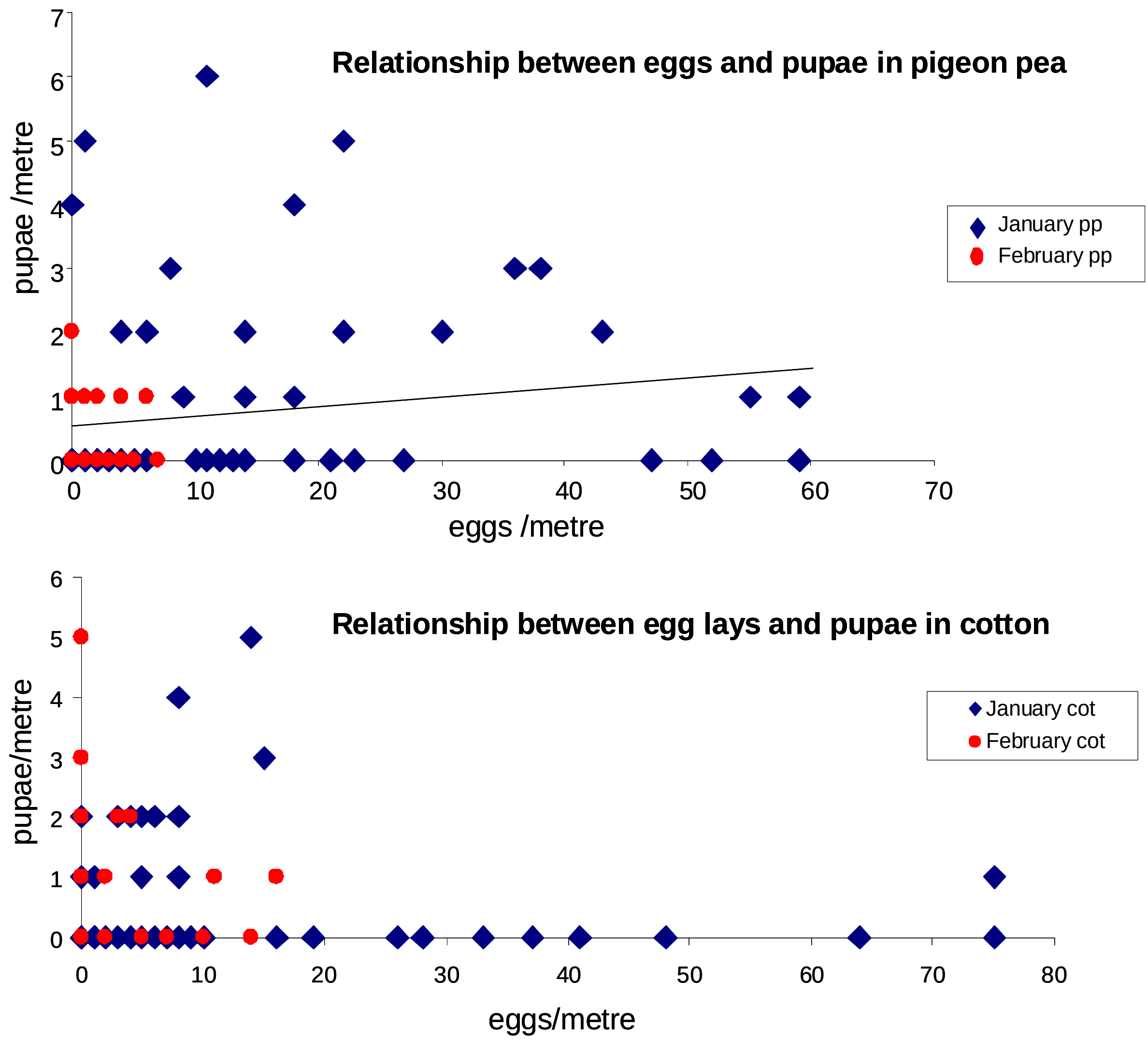

Figure 8. The relationship between egg lays and pupae collected in pigeon pea and cotton. Only in pigeon pea collected in January is there a linear relationship (as indicated by the line)

Discussion

Current Bt resistance management strategies in the Australian cotton industry are based on estimates of the relative production of *Helicoverpa* moths from different refuges compared with unsprayed (non-Bt) cotton (Baker et al 2008). These estimates determine the required ratio of different types of refuge crops. Under the strategy, unsprayed conventional cotton refuge must be planted at a rate of 10ha to every 100ha of Bt cotton, while unsprayed pigeon pea must be planted at a rate of 5ha to every 100ha of Bt cotton. This implies that unsprayed pigeon pea refuge is 2× more productive than unsprayed conventional cotton. Our field surveys taken over the period of Jan-Feb 2011 indicate that conventional cotton refuge was in fact more productive than pigeon pea refuge, with conventional cotton producing more moths from reared pupae (Fig.6). However the surveys taken are relatively focused in a small area of space and time. Previous work by Baker, Tann & Fitt (2008) with surveys taken between the years 1996-2003, and with a broader area of sample locations, demonstrated that pigeon pea refuge (based on total pupae numbers) was reasonably well justified as being 2× more productive than conventional cotton. The data supports Baker, Tann & Fitt's (2008) suggestion that there is considerable variability in the abundance of *Helicoverpa* pupae in space and time. For example the collection of 50 total pupae in pigeon pea refuge during January, whilst only a total of 12 pupae were collected in February. Also the total number of collected pupae from our surveys was significantly lower than that of previous years.

The visual surveys to measure attractiveness supported this notion of variability between and within the cotton seasons. The data shows no consistency in the attractiveness of conventional cotton and pigeon pea refuge, with many fields attracting no eggs at all, and others attracting upwards of 40 per metre. As well as this variability in both refuge types, there was no difference between the attractiveness of conventional cotton and pigeon pea refuges. However, what the data does show is a correlation between the attractiveness of pigeon pea refuges and the plant's growth stage (Fig. 4). In pigeon pea samples, the number of eggs collected was more strongly associated with early and mid flowering plants than plants that had not begun to flower or had finished flowering. This means that depending on the sample times and the sowing times of each field, the number of recorded eggs from visuals was affected by growth of the refuge crops. For instance if all visual samples were conducted whilst the refuge was flowering or not flowering, the average number of egg/m would be considerably higher or lower respectively.

Although plant development stage in cotton did not significantly affect egg numbers, there was a correlation between the attractiveness of conventional cotton refuges between samples. Thus a cotton field that was attractive early in the season was likely to remain attractive throughout the season. Maintaining consistent attractiveness throughout the season would assist resistance management.

Even though the pupae samples conducted to measure productivity were affected by low numbers of pupae collected and highly variable moth productivity, they did yield some interesting results. The rate of parasitism was very high (Cotton: 32% and 38% respectively; and Pigeon pea: 43% and 42% respectively) with the types and ratios of parasites emerging from reared pupae consistent with previous work by Baker, Tann & Fitt (2008) indicating no change over time in parasitoid composition and a stable parasitoid community over many years. Conventional cotton refuges produced more moths than pigeon pea refuges, especially in February (Fig. 5). This indicates that the conventional cotton refuges in this study were more reliable than the pigeon pea refuges.

The relationship between attractiveness and productivity of conventional cotton and pigeon pea refuge is not evident from our data. High egg lays in January did not correlate with high pupae numbers (Fig. 8) and from the pupae that were collected from these lays, there was a low production of moths. Last year's study (Whitehouse unpublished data) showed very few pupae were produced from egg lays in early January (as was the case here) but that there could be a correlation between eggs lays and pupae production in late January/early February. In the current study no strong linear relationship between egg lays and pupae was observed. These results suggest that egg lays cannot be indicative of moth production from a field, as the relationship is weak at best.

Nevertheless, Helicoverpa survival to pupae was higher in February than January. This is consistent with last year's results (Whitehouse unpublished data) indicating that egg and larval survival increased from January to February. Thus we may expect greater survival of Helicoverpa from eggs laid in early February compared to early January. This has ramifications for resistance management.

The large variability in attractiveness and moth productivity shown by our data has potential to impact on the ability of refuge crops to serve their purpose. For a refuge to be successful it must produce susceptible moths consistently to dilute the resistant moths emerging from the Bt cotton. If the attractiveness and productivity of refuges are so variable, then it is possible that within and between seasons some farms may produce more resistant moths than non-resistant, and tolerance to the Bt toxin may accumulate. Down at the field level our data shows that a conventional cotton field that is attractive in January will be also attractive in February. The other side of that result is that initially unattractive fields remained unattractive for the entire sampling period, which could compromise moth production, even though the link between egg and pupae production was weak in our study. As such it is possible that those fields which were unproductive produced an insufficient number of susceptible moths to dilute the resistant moths emerging from Bt cotton in their area. Maintaining the productivity of refuges is key to their success at managing resistance to Bt in Helicoverpa.

Conclusion

Overall the attractiveness and productivity of both conventional cotton and pigeon pea was highly variable. Pigeon pea was more attractive for oviposition when flowering but this did not necessarily translate into more moths, and Pigeon pea fields did not maintain their attractiveness. Cotton fields which were attractive maintained their attractiveness and so were more reliable, but there were many fields which were consistently unattractive. However, attractiveness, as indicated by egg lay, was a very poor indicator of pupae production in this study. Pigeon pea fields were not twice as productive as cotton fields at producing pupae, and indeed cotton fields produced more moths.

Helicoverpa survived to pupae better in February compared to January. This change in survivorship will affect refuge productivity during the season.

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Presentations and Public Relations

This work will be presented as part of the IPM forum discussions in winter, and will be sent to the participating growers. It will also be discussed at other industry meetings by MW. DH will submit a report on this project as part of the professional experience program for his BScAgr degree.

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Executive Summary

Refuge crops are used in the cotton industry to dampen the expression of resistance in *Helicoverpa* moths, and are an integral part in the use of transgenic cotton varieties; as such it is important to confirm refuge assumptions. This experiment sampled 11 Conventional Cotton and 11 Pigeon Pea fields in January and February to compare the survivorship of *Helicoverpa* spp. It was found that both the attractiveness and productivity of Pigeon Pea and Conventional Cotton refuges were highly variable. Pigeon pea was more attractive for egg lays when flowering, but this attractiveness did not translate into more moths. Conventional Cotton Fields which were attractive in January, remained attractive throughout the sampling period, and were more reliable at producing moths than other fields; however there were many fields that were consistently unattractive. Overall there was no difference in *Helicoverpa* productivity between Conventional Cotton and Pigeon Pea refuges, contrary to the established notion that Pigeon Pea is twice as effective as unsprayed conventional cotton.