



FINAL REPORT

Part 1 - Summary Details

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Project Title: The role of *Bt* cotton in pest suppressive landscapes

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Cotton CRC Program: Farm

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Background

Since its introduction in 1996, Australia has faced a rapid adoption of *Bacillus thuringiensis* (*Bt*) expressing cotton for the control of lepidopteran pests *Helicoverpa punctigera* and *Helicoverpa armigera*. In 2007, 95% of the cotton acreage was planted to *Bt* varieties. However, the proportion of *Bt* cotton in any agricultural landscape is quite variable, and is often a part of a cropping system which includes other crops that are susceptible to *Helicoverpa* spp. Most research on arthropod populations and pest control function in *Bt* cotton and other *Bt* crops is limited to the field level, e.g. comparison of *Bt* crops with non-*Bt* crops. However, the culmination of many farms at the regional scale planting *Bt* cotton may result in changes in landscape scale pest suppression for *Helicoverpa* - susceptible crops, e.g. non-*Bt* cotton and grains. Even though large scale planting of *Bt* cotton started a decade ago, consequences on a landscape-scale have hardly been studied. The exceptions being data collected in six Chinese provinces which provided the first evidence that highly efficient control of *H. armigera* in 3 million hectares of *Bt* cotton reduced the pest in 22 million hectares of other *H. armigera* susceptible crops grown in the same region (Wu et al., 2008). More recently, Lu et al., (2012) showed widespread adoption of *Bt* cotton and insecticide decrease promoted biological control in the surrounding landscape. In addition, work by Hutchison et al. (2010) showed that non-*Bt* grain crops on the landscape play a role in resistance management, and can be the beneficiary of lower pest pressure when the crops are grown concurrently or in conjunction with *Bt* crops.

At the field scale, *Bt* cotton has the potential to influence pest pressure via different mechanisms. First, *Bt* cotton is attractive to *Helicoverpa* spp. for oviposition, and the emerging larvae die quickly after ingestion of *Bt* proteins. Thus, *Bt* cotton functions as a sink for *Helicoverpa* spp. (i.e. a habitat where their population declines). Second, the reduced pesticide use in *Bt* cotton is likely to favour natural enemy populations that can provide essential pest control services in *Bt* cotton and other crops. However, not all natural enemies are likely to show the same response to *Bt* cotton. Due to the highly efficient elimination of lepidopteran larvae in *Bt* cotton, parasitoids that are specialized on larval and pupal stages of *Helicoverpa* may suffer from the lack of hosts in this crop, whereas generalist natural enemies may still be able to find sufficient prey or hosts. *Bt* cotton may therefore be a sink for parasitoids specialized on larval and pupal stages of *Helicoverpa*, and a potential source (i.e. a habitat where populations increase) for generalist natural enemies. Therefore, depending on the landscape configuration of *Bt* cotton and *Helicoverpa* -susceptible grain crops, *Bt* cotton may have the potential to change pest and natural enemy interactions at the landscape scale, and have implications for resistance management.

The aim of our research is to determine how the configuration (e.g. size, location, type and amount) of *Bt* cotton and grain crops influence *Helicoverpa* population dynamics and how this could contribute to reduced pest pressure and more effective resistance management.

Objectives

The objectives of the project were based on testing the following hypotheses:

1. In *Bt* cotton landscapes, large grain crops (1 km blocks) will have higher *Helicoverpa* spp. egg densities compared to small grain crops.
2. In *Bt* crops, moth density is expected to be higher and less variable when there are more neighbouring grain crops, i.e several small crops, compared to single large crops.
3. In *Bt* crops, egg parasitism is expected to be the same regardless of the surrounding sorghum configuration.

We have been able to conduct analysis beyond these original objectives that allow us to speak to the resistance management plan – this includes: analysing all 3 years of data (even though final reports were produced for each of the years separately) and to consider crop and non-crop habitat as predictors for *Helicoverpa* egg load into *Bt* cotton and sorghum.

Using a robust sampling design we collected data on the abundance of *Helicoverpa* spp (eggs and moths) and parasitism of *Helicoverpa* spp eggs in grain and *Bt* cotton at the spatial scale of fields, groups of fields for each of three landscapes across the Darling Downs, QLD for three seasons. In addition, we generated detailed land use metrics of each of the three landscapes for each of the seasons. Together this data was integrated and used in spatially explicit statistical analyses that indicate how landscape configuration of various crops (eg. size, location, type and amount) influences *Helicoverpa* spp.egg loads and moth densities in sorghum and *Bt* cotton, and was used to test our hypotheses to achieve the key objectives of the project.

In achieving the above objectives the results of our research show that:

- The number of *H. armigera* eggs in *Bt* cotton is explained primarily by the landscape –level aggregate of large blocks of *Bt* cotton;
- *H. armigera* eggs in *Bt* cotton decrease as the aggregate amount of *Bt* cotton at a landscape level increase;
- Sorghum fields surrounded by large areas of *Bt* cotton receive more *H. armigera* eggs and have more moths than sorghum field surrounded by sorghum;
- Egg parasitism is not influenced by the same landscape scale variables as *Helicoverpa* spp., although there is some indication that the degree of parasitism increases as the area of sorghum increases at a landscape scale of 1km radius.

Overall we have achieved the objectives of the project and have greatly increased our understanding of how landscape composition and configuration influences pest pressure in grain and *Bt* cotton crops, and the possible implications for resistance management.

Methods

Landscapes

Field work was conducted for three seasons, December -March 2009-10, 2010-11 and 2011-12 in the Darling Downs, QLD. The Darling Downs is situated 200 km west of Brisbane. This agricultural region is known for growing a diverse range of crops, the major summer crops being cotton, sorghum, mung beans, maize, and sunflowers all of which are host plants for *Helicoverpa* spp. The project was conducted in three replicate landscapes (Nandi, Cecil Plains and Pampas) within the Darling Downs region. Each of the landscapes is separated by 60 km and consists of a 10 km radius (20 km diameter) of mapped land-use (**Figure 1**).

Mapping and Land use Information

The research was conducted on growers' fields. At the end of September for each of the three seasons 2009-10, 2010-11 and 2011-12 letters were sent to approximately 80 growers. The letter introduced and explained the project as well invited growers to participate. This initial communication was then followed up by phone, email and or fax to confirm grower participation and request more specific information such as farm maps, crop varieties and planting dates for the season ahead. These land-use details were then confirmed by visiting and ground truthing the information which was then digitized and used to produce maps.

Maps for each landscape were created in ArcGIS 9.31. Aerial images of each landscape Google Earth Professional 2009, were rectified for the appropriate projected referencing system GDA_1994_MGA_Zone_56. Landscape features and land-use were then added as polygons. The polygons were used to calculate the land-use metrics such as proportion of land-use type for given areas in concentric rings with different radii (i.e. 250 m, 500 m, 1 km, 1.5 km and 2 km) around each field sampled (**Figure 2**). We characterized the landscape into 21 different land uses including roads, buildings, water storage and different crop categories at five spatial scales around all fields.

To compare broad land use across landscapes over the seasons we divided up the 21 land use categories for each of the three 20 km diameter landscapes into three categories based on host plants and the degree to which various crops and land use were suitable to support *Helicoverpa* spp. The three broad categories were: 1) matrix, land use that is unsuitable for *Helicoverpa* spp. including water storage, buildings, pasture and fallow or bare ground, 2) source crop, any suitable host crops for *Helicoverpa* spp. including conventional cotton, sorghum and pigeon pea, and 3) *Bt* cotton. Categorising land use this way enabled us to broadly compare the proportions of host plants, suitable and non-suitable "habitat" for *Helicoverpa* spp and change across seasons.

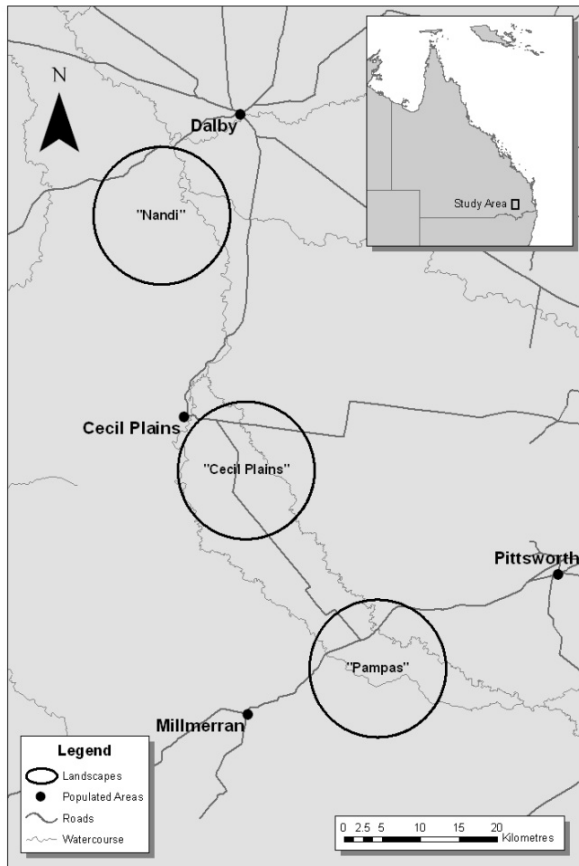


Fig.1. Location of the three landscapes across the Darling Downs.

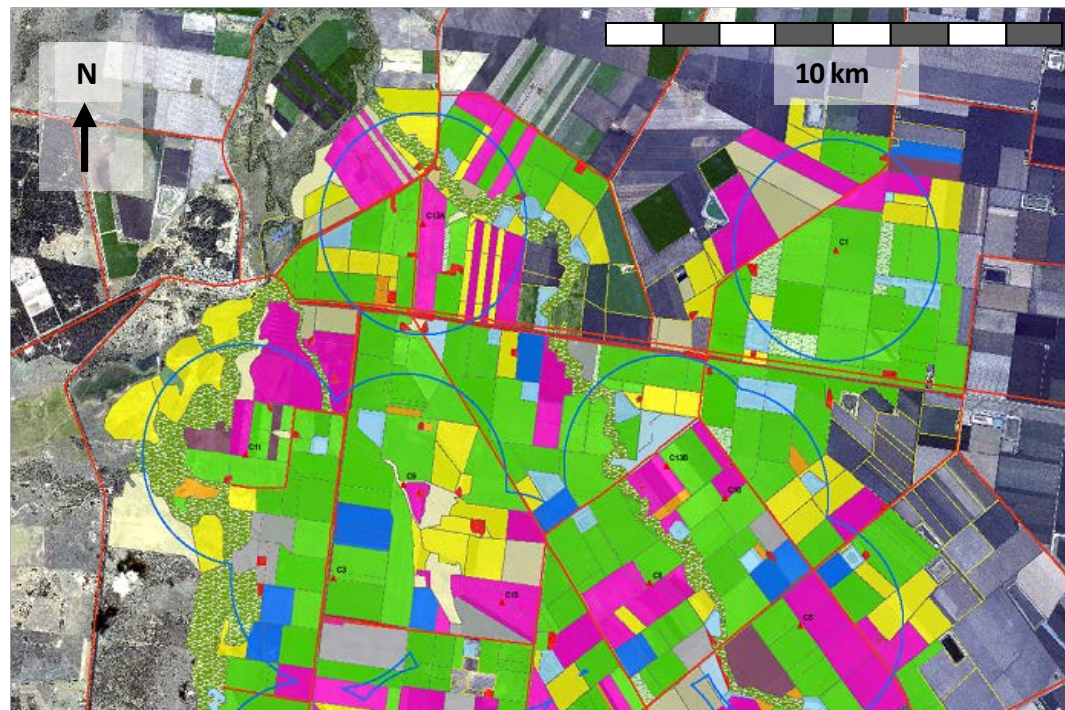


Fig.2. An example of a digitised map showing areas of land use for part of the Cecil Plains study landscape, 2011-12. The blue lined arcs represent the area at 2 km radius around each of the field sites. Coloured land use polygons = green –cotton, pink –sorghum, yellow -cereal stubble and blue –corn.

Sites and Sampling

Field site selection

In the first year of the project, 09-10, eight *Bt* cotton fields within each landscape were chosen based on the size of the crop. The details of this sampling design can be found in final report CRC #2.04.11. In subsequent years, using the land-use information, field sites were chosen ranging from large to small. The starting point was a standardized large-scale field measuring 1.5 km x 1.25 km, with the remaining seven fields decreasing in size, for a total of eight fields per landscape.

In 2010-11 and 11-12, in each of the three landscapes, eight fields of *Bt* cotton and eight fields of sorghum were selected for a total of 48 fields each year. Each field could be allocated to a treatment based on different proportions of *Bt* cotton and sorghum, ranging in size from big >75% to small < 25% respectively. The centre of the standardized large-scale was used to mark the point at which sampling for each field was conducted. No field site was used more than once in any of the three seasons. During the 2010-11 season one field trip was conducted before 19 December.

However, after this date, severe flooding along the Condamine River prevented access. Sampling resumed from February the 8th for 22 of the 24 cotton sites. The two-month gap in sampling meant that no data was collected from sorghum during the most critical period for *Helicoverpa* population development. The sorghum that was not too flood affected had senesced and harvesting had begun when field work resumed.

Egg and Moth Collection

Standardised timed counts were used to count and collect *Helicoverpa* eggs; the key response variable used to test our hypotheses. The advantages of using *Helicoverpa* spp. eggs was that they remain as eggs for approx. three days throughout summer and therefore provide a discrete developmental stage to quantify. With few exceptions, eggs are the only developmental stage of *Helicoverpa* spp. that survive in *Bt* cotton and are less likely to be affected by insecticides used for other cotton pests. A sub-set of eggs were collected, their development tracked (e.g. parasitized, hatched or unviable), and identified to species.

2009 – 2010

The sampling design for 2009-10 showed that *Helicoverpa* egg load was similar across a field and not influenced by cardinal direction. This allowed the egg sampling method for 2010-11 and 2011-12 to be modified to occur along two parallel transects in each field for each of the sampling dates (**Table 1**). For all three years there were 16 sampling points per field.

2010 -2012

Standardised timed searches of *Bt* cotton and sorghum plants for *Helicoverpa* eggs were undertaken by two people sampling along one of two transects at each field. Three minute timed searches of plants were conducted ever 30 meters, and repeated eight times along each of the 240 m transects (16 sampling points for each field) (**Figure 3**). At each of the eight points, plants were searched for three minutes and eggs and the total number of plants searched were counted and recorded. The principle growth stage and development of the crop was recorded for each field.

Table 1. Sampling dates for each season. NB * The eight week gap between the first and second sampling dates in 2010 -11 was due to the flood and access to the field sites was not possible.

Sample No Year / Date	1	2	3	4	5	6	7
2009-10	Dec 7	Jan 6	Jan 19	Feb 9	Feb 23	Mar 16	-
2010-11	Dec 6	* FLOOD	Feb 8	Feb 22	Mar 8	Mar 22	April 6
2011-12	Dec 6	Dec 20	Jan 3	Jan 17	Jan 31	Feb 14	Feb 28

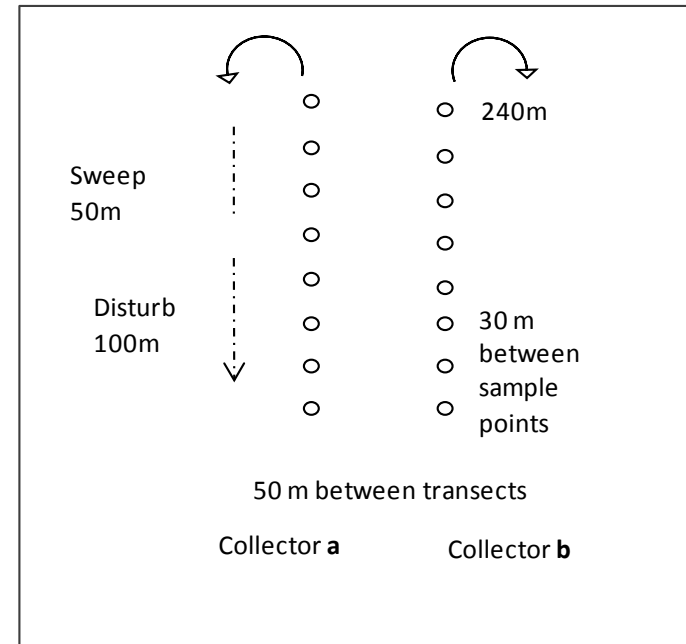


Fig.3. A schematic showing how timed egg searches were conducted in each field. Three minute searches for eggs were conducted at each point (30 m apart) along two transects (n=16) for each sampling date . Moth counts were conducted along the return sweep netting for 50 m and disturbing for 100 m.

After each timed search, a sub-set of eggs were collected from that point and were placed into gelatine capsules, details of the site, sample point and collector were recorded and capsules returned to the laboratory. The development of individual eggs was monitored and recorded daily. Any larvae that emerged were given an individual number and placed on diet and forwarded to Narrabri where larvae were identified as *H. punctigera* or *H. armigera*. The fate of the individual eggs that remained were recorded either as being parasitised or non-viable. In cases where a larva hatched from an egg but died, went missing or hatched too late to be sent for identification, it was recorded as an unidentified larva.

Sorghum panicles

During the process of egg sampling in sorghum one panicle was collected randomly from every second sampling point (8 panicle per field per sample time or 32 panicles per field for the season). Individual sorghum panicles were placed in a paper bag which was labelled and taken back to the vehicle for processing. To remove the eggs individual panicles were struck three times against one meter square piece of velvet material which was spread out flat. The material trapped the eggs, which could then be easily collected using a fine paint brush. Eggs were processed and monitored for development as listed above. The growth and development stage of the plants and the individual panicles were recorded for each of the sampling times. As sorghum plants develop throughout the season most of the eggs are found in the panicles. Because of this the sampling protocol for sorghum was modified and timed searches of sorghum plants were concluded if, on the previous two sampling dates no eggs had been found, and instead an additional four panicles were collected from the field.

Moth Collecting

To estimate the moth density for each experimental field, moths were counted and collected. At the end of 240 m transect after egg sampling, each collector moved 10 m further away from each other to begin sampling moths while exiting the field. Moths were collected by sweep netting crop foliage for 50 meters, followed by moth counts, which were achieved by flushing moths from the crop using the handle of the sweep net for 100 meters (**Figure 3**).

Data Analysis

A range of summary statistics were generated to assess general trends in the abundance of *Helicoverpa* spp (eggs and moths) and parasitism of *Helicoverpa* eggs for each season, landscape and field. Egg load (mean total eggs present / time count), egg density (eggs/plant), moth load (mean total moths present / time count), and proportion parasitized were used as response variables, and land-use data at varying spatial scales (e.g. % *Bt* cotton at 0.5, 1.0, 1.5 km radii (r)) as predictors for all detailed analysis. Although early analyses were conducted using multiple linear regression, these limited our ability to speak directly to the issue of varying spatial scales.

Statistical Model

To determine how landscape composition and configuration (e.g. crop size, location, type and amount) influences the response variables of interest, we used hierarchical linear modelling (HLM), which included both fixed and random terms. This approach provides a powerful way to measure regression relationships and variance – covariance parameters in hierarchically structured data, e.g. data collected on plants, within fields, within landscapes, for factors that can be experimentally controlled (i.e. fixed), and for factors that the experimenter has little control (i.e. random effects). The ability to incorporate scale explicitly means such models are appropriate to analyse and test patterns from measured variables that are correlated across and or nested within different temporal and spatial scales (McMahon and Diez 2007). For example, by using HLM, we sequentially examined the effect of landscape (level-2) and field (level-1) predictors as explanation for the variation in egg load.

Modelling was guided by referring to the approach used in Singer (1998), and Raudenbush & Byrk (2002). Further, we rescaled the field level value to be centred around the landscape level mean for independent variables to allow for direct interpretation of parameter estimates.

Helicoverpa spp eggs and moths in *Bt* cotton & sorghum

To determine the spatial scale at which land-use predictors best account for the greatest amount of variation of *Helicoverpa* egg load in a *Bt* cotton field, we began with an unconditional means models. This model included the response variable mean eggs / plant or mean egg load / sampling period, no fixed effect, and a random effect grouping term - landscape nested within year. This grouping term was appropriate for two reasons, firstly there was no significant difference in explaining the variation in the mean *H. armigera* eggs among years, and more importantly landscapes (eg. Nandi, Cecil Plains, Pampas) are nested within years and there are many random – uncontrollable – variables that make up landscapes. This simple model established the baseline variation of the key response variable mean *Helicoverpa* spp egg loads as represented by the intercept term and by the group term, landscape nested within year, and is the model that all subsequent models are compared to. Subsequent models were constructed based on our hypotheses, and the result of the best-fit model is presented. To arrive at the best model, numerous models were fit that explored the effect of refuge (eg. conventional unsprayed cotton and pigeon pea), *Bt* cotton, grain (eg. sorghum and maize), native vegetation, pasture, fallow, and stubble.

During the 2012-11 season *Helicoverpa* egg data was collected from sorghum as well as *Bt* cotton. However, this data was not collected during 2010-11, due severe flooding from December to February; the suitable time of egg collection from sorghum. Egg collection from sorghum was not part of the 2009-10 data collection. Hypotheses testing and statistical modelling for eggs in sorghum were similar to that described above for *Bt* cotton, except that there was a single season of data collection. Therefore, the variable ‘landscape’ was used as a random grouping term, not ‘landscape’ nested within year.

Data on moths were collected and counted for two of the three seasons, 2010-11 and 2011-12. Moth species identification during flushing was not possible, and relatively few moths were captured during sweeping. Therefore, the response variable, mean moth load / sample period, likely represents both *H. armigera* and *H. punctigera*. Again, hypotheses testing and statistical modelling were similar to that described above. Prior to fitting models, correlations between predictors were identified and when variables were correlated only one was chosen for inclusion. Assumptions of the models were tested and met, and all final models converged.

Results

Although data was collected from three consecutive seasons across the Darling Downs, two of the three seasons were climatically extreme. The first seasons 2009-10 was the last year of a 10-year drought, 2010-11 experienced the worst flooding in 100 years, and 2011-12 conditions were regarded as ‘normal’.

Land use

Comparing the broad land-use categories shows that in 2009-10 over 65% of each of the landscapes was matrix, primarily in Pampas and Cecil Plains (**Figure. 4**). Most of the matrix consisted of fallow land. Essentially due to the drought and the lack of available water, there was reduced cropping. The subsequent seasons, 2010-11 and 2011-12, show similar proportions of source crops, which largely consist of sorghum and corn. The proportion of *Bt* cotton was similar between the last two seasons, however there was 3-4 times more *Bt* cotton grown for 2010-11 and 2011-12 compared to 2009-10. Interestingly in 2010-11 and 2011-12,

Cecil Plains had 15 and 20 percent more *Bt* cotton compared to the Nandi and the Pampas landscapes.

The abundance of *Helicoverpa* species

Timed-plant searches for all three seasons resulted in over 16,600 eggs being counted and 10,700 individual eggs being collected (**Table 2**).

There was a similar seasonal dynamic across landscapes for each of the seasons. The egg density gradually increased from early December, peaked mid-January to early February, before gradually declining as the season progressed (**Figure 5**). There was a difference in peak density among the years. For all three seasons the highest egg density, 7.8 ± 0.59 per plant was recorded in 2009-10 in Pampas. This was four times higher than the mean egg density for Cecil Plains and all but one of the fields in the Nandi landscape, for the same season. The Nandi landscape consistently had lower egg densities compared to other landscapes, especially for 2010-11 and 2011-12 where egg density only exceeded two eggs per plant for one field. There was a large amount of variation in mean egg density among fields within landscapes.

Composition of *Helicoverpa* spp.

The composition of *Helicoverpa* species (as indicated from egg collections) was similar among landscapes, but varied dramatically among years (**Figure 6**). In 2010-11, only 6% of the population was *H. punctigera*; the lowest recorded across all three years. This contrasts with 40% and 20% of *H. punctigera*, in 2009-10 and 2011-12 respectively.

***Helicoverpa* egg parasitism**

The degree of parasitism was calculated by dividing the total number of parasitoids by the total number of eggs collected for each field for each of the sampling periods (**Figure 7**). In general, parasitism gradually increased throughout the season and for most of the fields in each landscape, for all three years, the degree of parasitism fluctuated between 20 and 60 %. Interestingly, for all but one field in Cecil Plains, parasitism declined mid-season, January 17, 2011-12. This decline coincided with the peak *Helicoverpa* egg density (**Figure 5**), and so far there is little explanation.

Species composition of *Helicoverpa* egg parasitoids

Parasitoids of *Helicoverpa* eggs were dominated by species of Trichogrammatidae, a complex of species that belong to one of two genera, *Trichogramma* or *Trichogrammatoidea*, and a species of *Telenomus*. *Trichogramma* or *Trichogrammatoidea* are easy to distinguish from *Telenomus* spp. However the lack reliable morphological characters coupled with their small size makes *Trichogramma* or *Trichogrammatoidea* difficult to identify to genus and species. Throughout the project, for convenience all *Trichogramma* spp. and *Trichogrammatoidea* spp were recorded and referred to as *Trichogramma* spp. *Trichogramma* spp. were responsible for the greatest proportion of egg parasitism; 86%, 98% and 61% for 2009-10, 2010-11 and 2011-12 seasons respectively. The balance of parasitism for each of the seasons was confirmed as *Telenomus* sp. To begin understanding which *Trichogramma* species were present in the landscapes, DNA was extracted from a sub sample of parasitoids (n=38) that emerged from *Helicoverpa* spp eggs which had been collected from *Bt* cotton fields in December 2010. The samples were sequenced and analysed and the results confirmed two of the specimens as being *Telenomus* sp., nine were assigned to the genus *Trichogrammatoidea*, seven were identified as *Trichogramma funiculatum* and 20 were *Trichogramma pretiosum*.

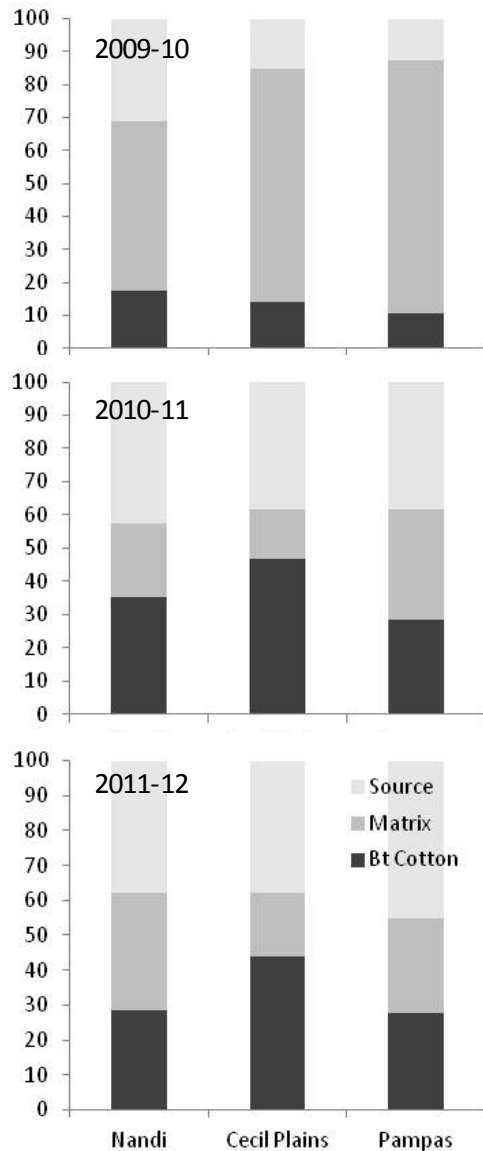


Table 2. A summary of the results from total sampling effort from each of the three seasons 2009-10, 2010-11 and 2011-12.

Season	Eggs collected	Eggs Counted	Plants searched	H. a	H. p	Helicoverpa sp. unknown	Telenomus spp	Trichogramma spp.	Non viable eggs	Parasitoids sp. unknown
Bt cotton										
2009-10	2889	3389	10620	822	538	98	16	1180	234	0
2010-11	3234	6251	10631	1569	103	308	6	986	264	0
2011-12	4042	6181	15822	1287	314	345	223	1114	695	64
Bt cotton Totals	10165	15821	37073	3678	955	742	245	3280	1193	64
Sorghum 2011-12										
plants	125	152	6953	75	0	11	1	13	25	0
panicles	476	699	974	121	0	19	3	248	78	6
Sorghum totals	601	851	7927	196	0	30	4	261	103	6
Total	10766	16672	45000	3874	955	781	249	3541	1296	70

H.a = *Helicoverpa armigera*
H.p = *Helicoverpa punctigera*

Fig.4. Percent land use for three broad categories, source = host crops for *Helicoverpa* spp. minus *Bt* cotton, matrix = land use in which *Helicoverpa* can not survive and *Bt* cotton = *Bt* cotton, calculated for each of the landscapes for each season .

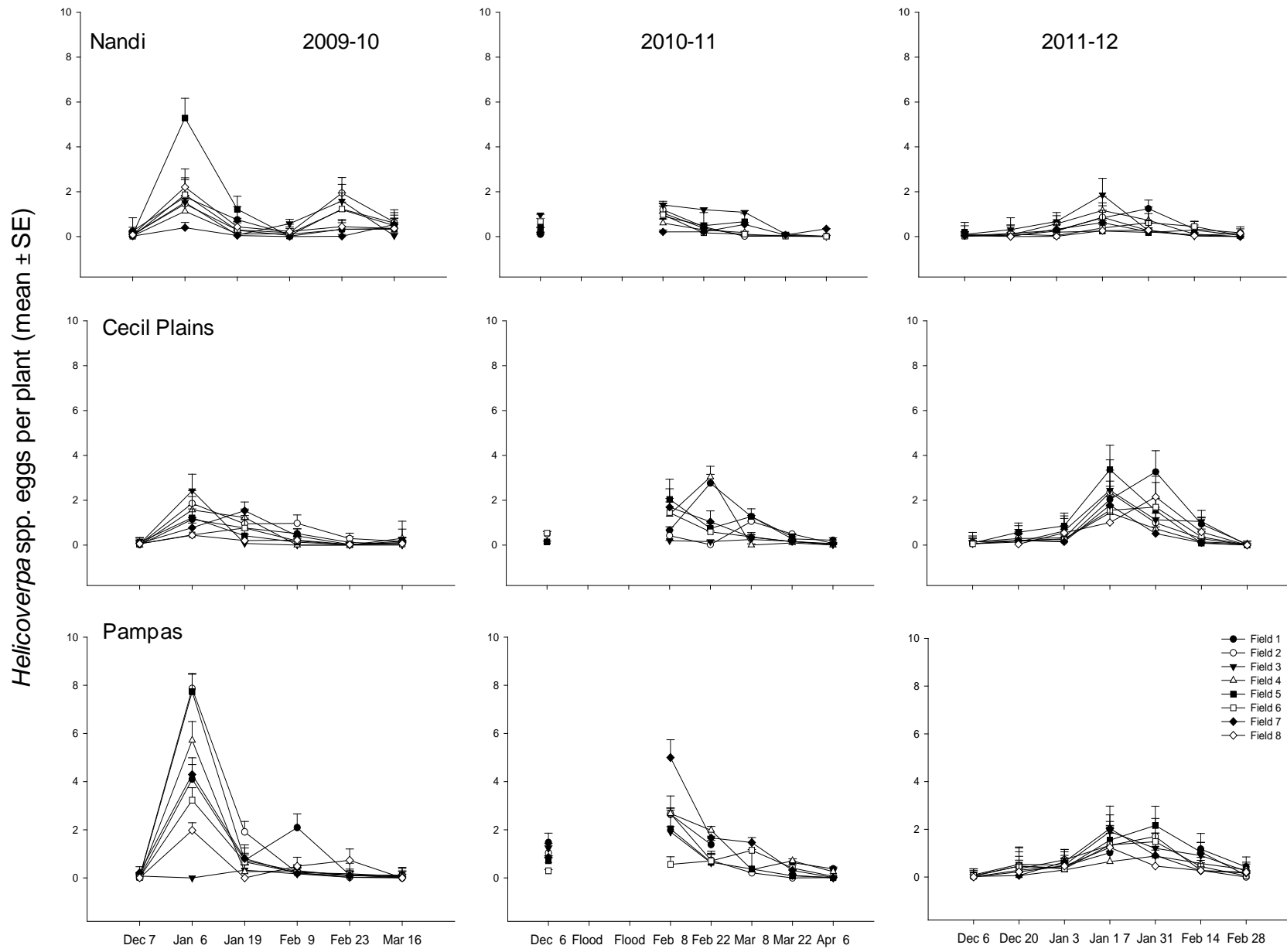


Fig. 5. Mean *Helicoverpa* spp. eggs per *Bt* cotton plant for each field in each of the landscapes

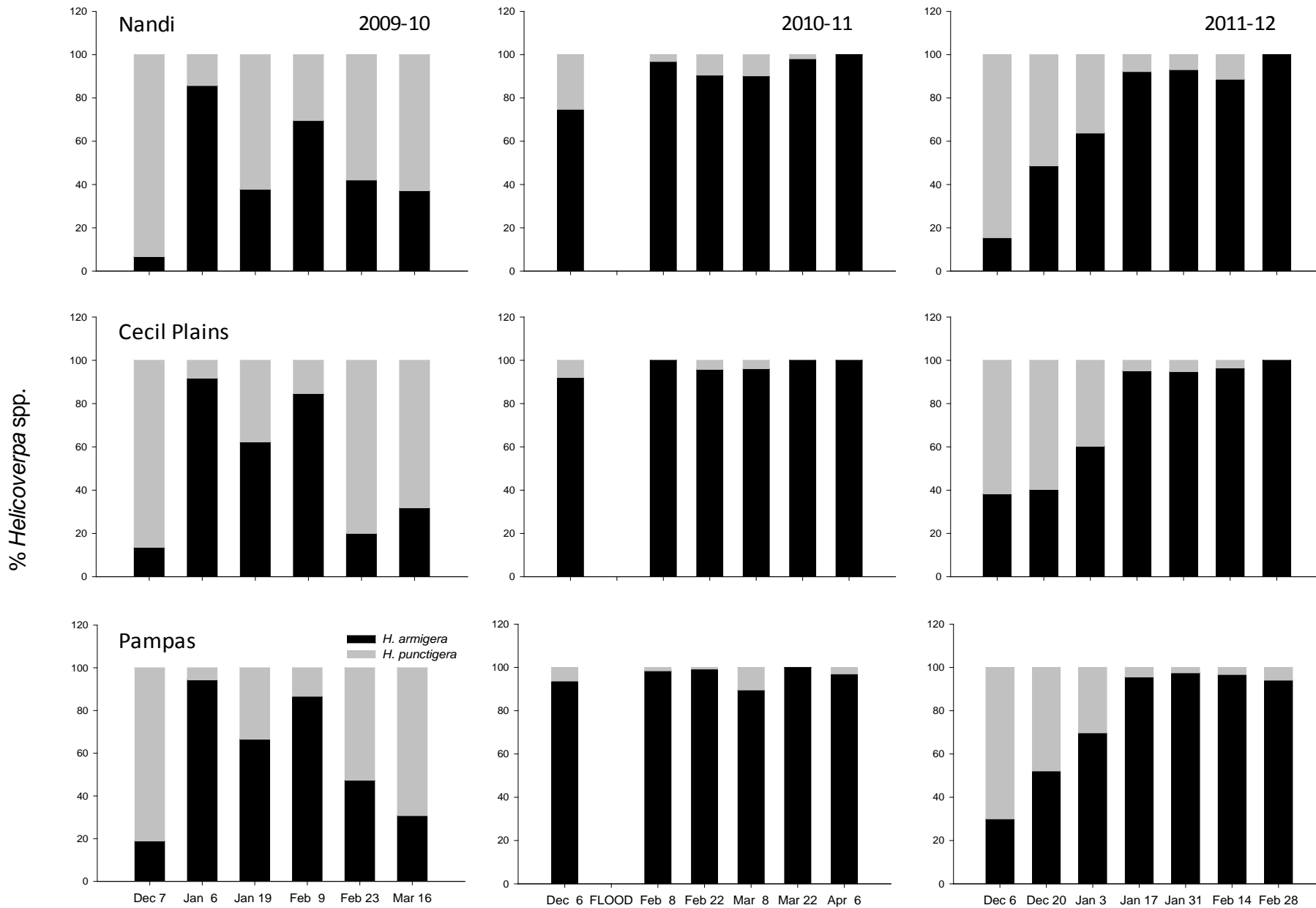


Fig. 6. Percent species composition of *Helicoverpa* spp. from eggs collected each sampling time for each landscape.

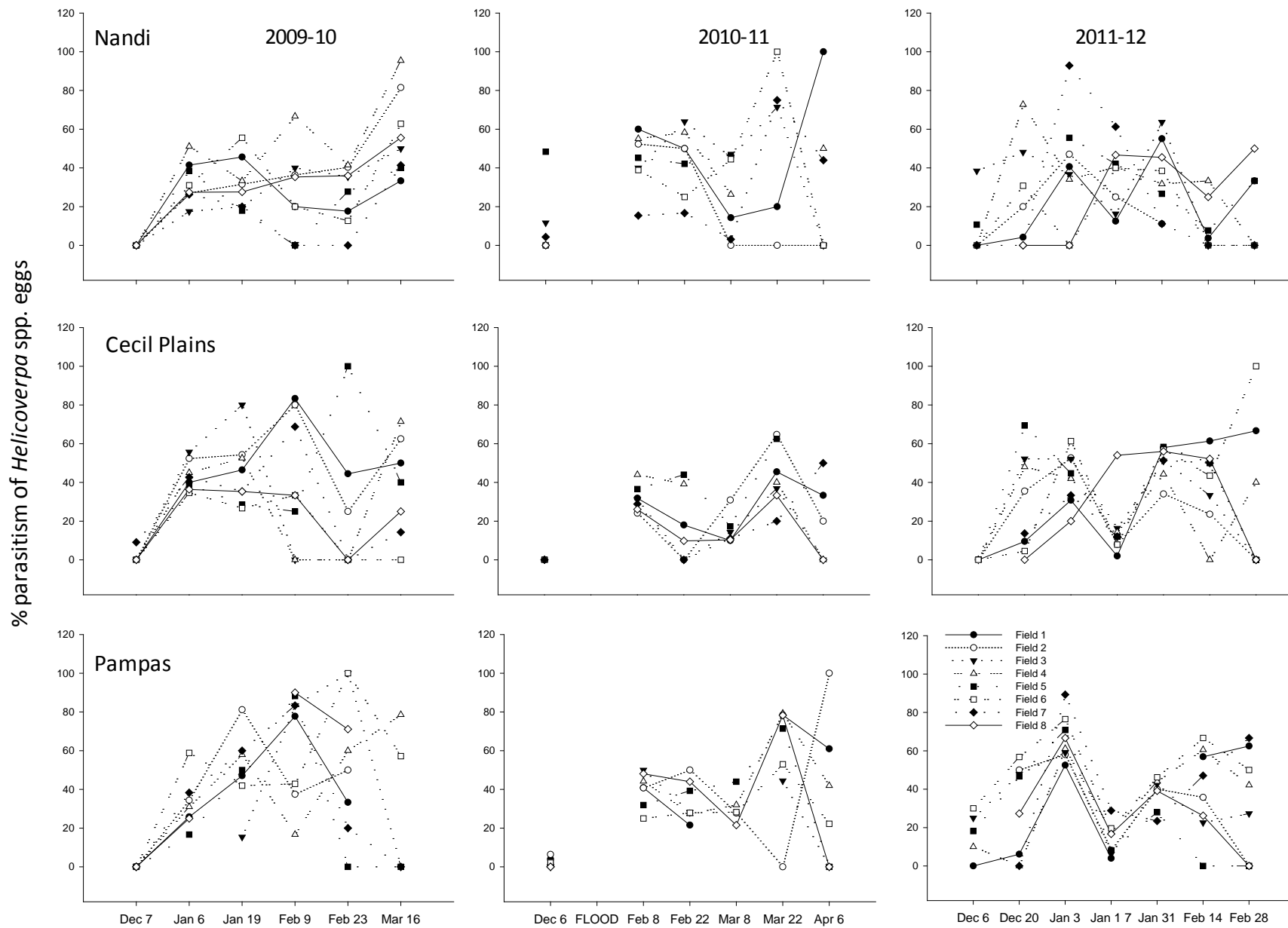


Fig. 7. Percent parasitism of *Helicoverpa* spp. egg collected from *Bt* cotton for each field in each landscape.

***Helicoverpa armigera* eggs from sorghum**

Of the total number of eggs collected from sorghum fields, 24% were from the plants and 76% were from the panicles. Similar to eggs collected from *Bt* cotton, the amount of *H. armigera* eggs collected from sorghum varied across fields and sampling times (**Figure 8**). No *H. punctigera* eggs were collected from sorghum.

The proportion of eggs that were collected from sorghum, and that were parasitised, increased in each of the landscapes throughout the season. This was especially evident in both Nandi and Cecil Plains (**Figure 9**). The proportion of parasitised eggs collected from panicles was over five times greater 54%, compared to the parasitism of eggs that were collected from plants, which was 9%. The proportions of eggs parasitised for each of the fields was regressed over the proportions of *Bt* cotton and sorghum at 0.5, 1 and 2km r. The results showed a trend for increased parasitism with an increase in the amount of sorghum at spatial scale of 1km r $F_{(1, 21)} = 4.19, p = 0.05$. However there was no relationship evident between parasitism and proportion of *Bt* cotton at any of the spatial scales tested.

Spatially explicit modelling

The statistical hierarchical modelling undertaken describes the relationship in variation of the response variable as it changes with the predictors across different levels or scales. The response variable was the mean *H. armigera* and *H. punctigera* eggs per field per sampling event, referred to here as mean egg load. Predictors included the amount (as a percentage) of different land use (e.g. crops and non-crop habitat) at increasing scales (0.5, 1.0, 1, 5, and 2.0 km r) surrounding the focal sample fields.

***H. armigera* eggs in *Bt* cotton**

To understand the factors contributing to the variation in *H. armigera* eggs in *Bt* cotton crops we first fit an unconditional means model (**Table 3.1**). Some 65% of the variation in *Helicoverpa* spp mean egg load occurs among landscapes, suggesting similarity within a landscape, and differences between landscapes. On average there were 37.5 eggs / sampling event (or 1 egg located every 1.2 minutes of searching), and the range of plausible values estimated ranged from 7.14 – 82.06 mean egg load / sample. This range is to be expected as it includes egg lay across nine landscapes and over 60 fields. The response variable mean egg load translates into 0.37 *H. armigera* eggs / plant, and a plausible range from 0- 0.75 eggs/ plant.

Our next model incorporated the effects of landscape-level predictors. In other words, we wanted to understand why there is a landscape difference in *H. armigera* egg load in *Bt* cotton crops. Many models were tried, and the final, best-fit model, as indicated by the Akaike information criterion (AIC) included the average landscape-level amount of *Bt* cotton up to 0.5km r. This explained 50% of the variation between landscapes in mean egg load. The similarity among fields within each landscape was 0.48, calculated as the intraclass correlation coefficient (ICC) (**Table 3.1**).

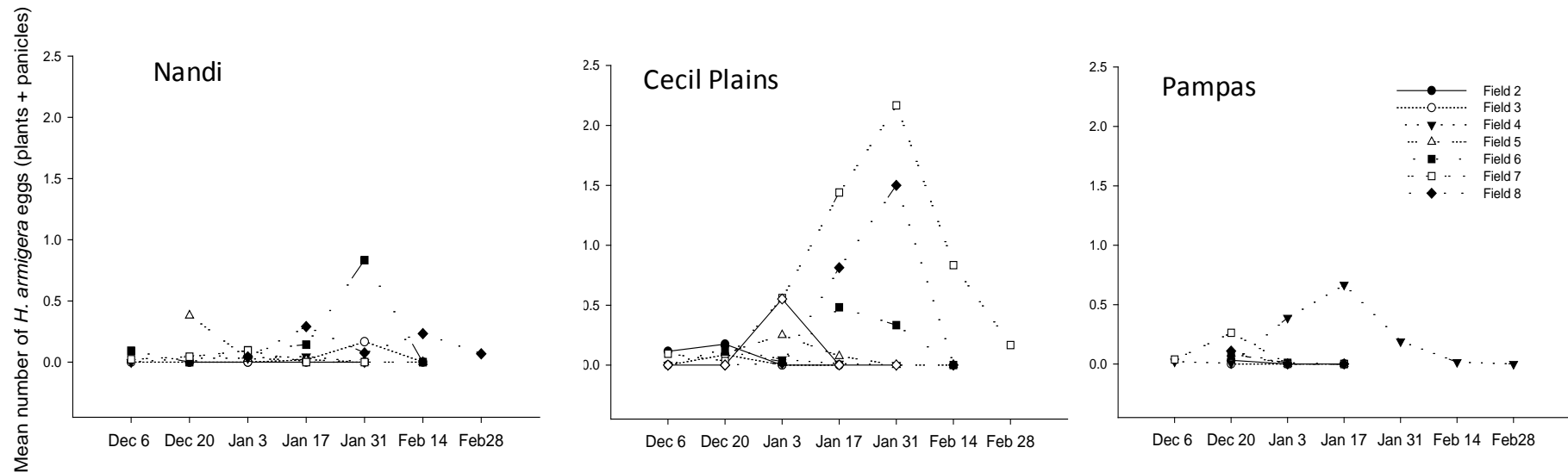


Fig.8. Mean number of *Helicoverpa* spp. eggs from sorghum (plants plus panicles) for each field 2011-12.

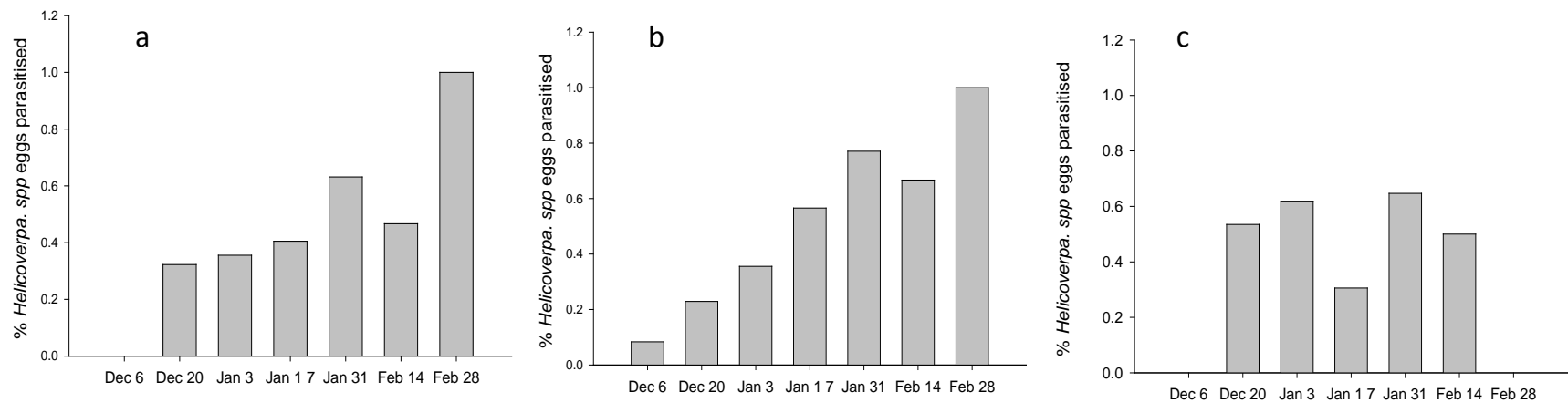


Fig.9. Percent egg parasitism for the total number of eggs collected from sorghum (plants plus panicles) for individual sample dates for each landscape a) Nandi, b) Cecil Plains and c) Pampas 2011-12.

The relationship between average landscape-level amount of *Bt* cotton up to 0.5km r and eggs being laid in *Bt* cotton is negative. For every one percent increase in the average landscape-level amount of *Bt* cotton at a 0.5km radius the mean *H. armigera* egg load decreases by 5.3 (Table 3.2).

Table 3.1. Summary of model variance - mean number of *H. armigera* eggs per sample in *Bt* cotton.

Variance Component Comparisons						
Model			Between fields within landscapes		Between Landscapes	
	Level 1 Field-level covariates	Level 2 Landscape-level covariates	Residual Variance	Percent of variance accounted for	Residual Variance	Percentage of variance accounted for
a	None	None	280.21	‡	517.76	‡
b		Average landscape-level <i>Bt</i> cotton @0.5 km r	280.12	3.3	256.12	50

a = unconditional means model, b = best fit model, c = best fit minus the single field outlier, ‡Not applicable.

Table 3.2. Comparison of model estimates - mean number of *H. armigera* eggs per sample in *Bt* cotton.

Fixed Effects Estimates							
Model			Model Intercept			Predictors	
	Level 1 Field-level covariates	Level 2 Landscape-level covariates	Estimate *	p value	AIC†	Estimate *	p value
a	None	None	37.45 (7.85)	0.001	606	‡	‡
b		Average landscape-level <i>Bt</i> cotton @0.5 km r	37.04 (5.32)	0.003	597	-5.32 (1.90)	0.006

a = unconditional means model, b = best fit model, c = best fit minus outlier, † Akaike information criterion.

*Estimate of mean egg load per sample, ‡Not applicable, NB standard errors of the estimates appear in parentheses.

$H. armigera$ egg load in *Bt* cotton = $37.04 - 5.32(\text{landscape-level Btcot}@0.5\text{km r})$

$H. armigera$ eggs / plant in *Bt* cotton = $0.36 - 0.30(\text{landscape-level Btcot}@0.5\text{km r})$

There is still significant amount of within landscape variation not yet explained by our models. This variation might be explained by factors that are not included in these models, such as crop development degree days and on farm management activities, such as pesticide application.

Numerous other models were tried using a range of predictors including field- and landscape-level *Bt* cotton at 1 and 1.5km r, crops (sorghum and corn), pasture, refuges and native vegetation. However, none of these models explained significant amount of variation or achieved a better fit than that presented above.

***H. punctigera* eggs in *Bt* cotton**

The model which best explained the variation of *H. punctigera* eggs in *Bt* cotton was the unconditional means model showed us that 42% of the variation in *H. punctigera* mean egg load occurred among landscapes. This suggests that for *H. punctigera* there is more similarity within a landscape than among, the difference wasn't as big as for *H. armigera*. On average there were 6.0 eggs/ sampling event (or 1 egg every 7.5 minutes searching), and the plausible values ranged from 0.067 to 12.05 eggs / sampling event.

The response variable, mean egg load, translates into an egg / plant mean value of 0.07, and a plausible range from 0.09- 0.17.

***H. armigera* eggs in sorghum**

Firstly, no *H. punctigera* eggs were found in sorghum; all eggs were *H. armigera*. The unconditional means model tells us that there is no difference among landscapes for *H. armigera*, mean egg load during 2011-12 (**Table 4.1**). On average there were 38.68 eggs / sampling event (or 1 egg every 1.16 minute). The response variable mean egg load translates into an egg / plant mean value of 0.089 *H. armigera* eggs / sorghum plant. Because there was no landscape difference, there was no reason to fit a landscape-level variable as was done above for *Bt* cotton.

Our final model incorporated the effects of field-level predictors, which allowed us to consider the average of the 3 regression equations (3 landscapes across 1 year), and ask how much do the regressions vary from landscape-to-landscape. Two field-level best-fit models resulted, each with nearly identical fit; the amount of field-level sorghum at 1km r and the amount of field-level *Bt* cotton at 1km r. These two predictors could not be fit in a single model because they were highly negatively correlated. Although the effect of each of these predictors was similar in strength, the direction was opposite. The mean egg load in sorghum surrounded by *Bt* cotton is 72.75 compared to 8.35 in sorghum surrounded by sorghum. This translates into 1.6 eggs for every 1 minute of searching, and 1 egg for every 5.4 minutes searching, respectively. If the amount of *Bt* cotton at 1.5km r increases by 1%, then the model predicts that the number of eggs in sorghum will decrease by 1.04/ sampling event (1 less egg per 333 plants or 2000 fewer eggs / ha at a planting density of 700,000 ha).

The opposite is true for sorghum surrounded by sorghum. If the amount of sorghum at 1.5km r increases by 1% then the model predicts that the number of eggs in sorghum will increase by 0.82 / sample (or 0.002/ sample or 1 additional egg per 500 sorghum plants or 1400 more eggs / ha at a planting density of 700,000 ha) (**Table 4.2**). However, there is also a significant interaction between the average landscape-level amount of sorghum and field-level amount of sorghum indicating that the intercepts and the slopes for field-level amounts of sorghum are different for different levels of average landscape-level amount of sorghum.

Table 4.1. Summary of model variance - mean number of *H. armigera* eggs per sample from sorghum.

Variance Component Comparisons						
Model			Between fields within landscapes		Between Landscapes	
	Level 1 Field-level covariates	Level 2 Landscape-level covariates	Residual Variance	Percent of variance accounted for	Residual Variance	Percentage of variance accounted for
a	None	None	2679.66	‡	0	‡
b	Field-level sorghum @1 km r	Average landscape-level sorghum @1 km r. Field-level sorghum @1 km r * Average landscape-level sorghum @1 km r	1948.81	27		
c	Field-level Bt cotton @1 km r		2194.21	18		

a = unconditional means model, b= sorghum surrounded by sorghum model, c = sorghum surrounded by Bt cotton model, ‡=Not applicable.

Table 4.2. Comparison of model estimates - mean number of *H. armigera* eggs per sample from sorghum.

Fixed Effects Estimates							
Model			Model Intercept			Predictors	
	Level 1 Field-level covariates	Level 2 Landscape-level covariates	Estimate *	p value	AIC †	Estimate *	p value
a	None	None	38.68 (11.04)	0.000	230.4	‡	‡
b	Field-level sorghum @1 km r	Average landscape-level sorghum @1 km r Field-level sorghum @1 km r * Average landscape-level sorghum @1 km r	8.35 (18.67)	0.7323	221.1	8.66 (5.65) 0.82 (0.09) 0.32 (0.14)	0.3681 0.025 0.032
c	Field-level Bt cotton @1 km r		72.75(17.35)	0.1491	220.1	-1.04 (0.43)	0.0278

a = unconditional means model, b = sorghum surrounded by sorghum model, c = sorghum surrounded by Bt cotton model.
*Estimate of mean egg load per sample, ‡Not applicable, NB standard error of the estimates appear in parentheses, † Akaike information criterion.

H. armigera egg load in Sorghum= 72.75 -1.04 (field-level Btcot @ 1.5km r).

H. armigera egg load in Sorghum= 8.35+0.83 (field-level Sorghum @ 1.5km r) + -0.32 (landscape-level * field-level sorghum @ 1.5km r).

OR

H. armigera eggs/ plant in Sorghum = 0.18 -0.003(field-level Btcot @ 1.53km r).

H. armigera eggs / plant in Sorghum = 0.003 - 0.002 (field-level Sorghum @ 31.5km r) - 0.0007 (landscape-level * field-level sorghum @ 31.5km r).

Numerous other models were fit using a range of predictors, however, none of these models explained significant amount of variation or achieved a better fit.

***Helicoverpa* moths in *Bt* cotton**

A similar modelling approach was taken to account for the variation in moth density. The base line model used landscape nested within year for 2010-11 and 2011-12 data. The variance component within landscapes is half of the variance component among landscapes, but the among landscape difference was marginal (p=0.07). The similarity among fields within each landscape was correlated at 0.64. Although numerous models were tried, we were unable to account for additional variation. Only when we analysed the last season's data, 2011-12, could we explain any additional within-landscape variation. The amount of field-level *Bt* cotton at 0.5 km r explains 34% of the within landscape variation.

2011-12 Moth numbers in *Bt* cotton = 14 + 0.19 (field-level Btcot @ 0.5km r)

***Helicoverpa* moths in Sorghum**

There was no difference in moths in sorghum among landscapes in 2011-12. However, there is a significant positive relationship between moths in sorghum and the average landscape-level amount of *Bt* cotton at 1.5 km r (**Table 5.1**). For every 1% increase in the average landscape-level *Bt* cotton at 1.5 km r, there is a 0.4 increase in moths in sorghum crops per sample. This result for moths follows that of *H. armigera* eggs; more eggs in sorghum surrounded by *Bt* cotton than sorghum surrounded by sorghum:

Moth numbers in Sorghum = 2.18 + 0.39 (landscape-level Btcot @ 1.5km r).

We cannot compare the moth densities between sorghum and cotton because sweep net samples can vary between the two crops due to the difference in crop morphology. For example, an estimate of 2.1 moths in sorghum compared to 14 in cotton is not necessarily an accurate estimate of the relative populations between crop types (**Table 5.2**).

Table 5.1. Summary of model variance - number of *Helicoverpa* spp. moths per sample from sorghum.

Variance Component Comparisons						
Model			Between fields within landscapes		Between Landscapes	
	Level 1 Field-level covariates	Level 2 Field-level covariates	Residual Variance	Percent of variance accounted for	Residual Variance	Percentage of variance accounted for
a	None	None	5.38	‡	122.47	‡
b		Average landscape-level <i>Bt</i> cotton @0.5 km r	5.34	<0.1	0	100

a = unconditional means model, b= best fit model.

Table 5.2. Comparison of model estimates - number of *Helicoverpa spp.* moths per sample from sorghum.

Fixed Effects Estimates							
Model			Model Intercept			Predictors	
	Level 1 Field-level covariates	Level 2 Landscape-level covariates	Estimate *	p value	AIC †	Estimate *	p value
<i>a</i>	None	None	2.10 (1.10)	0.1973	331	‡	‡
<i>b</i>		Average landscape-level <i>Bt</i> cotton @0.5 km r	2.18 (0.49)	0.1416	101	0.39 (0.13)	0.0063

a = unconditional means model, *b* = best fit model *Estimate of mean number of moths per sample, ‡Not applicable. NB standard error of the estimates appear in parentheses, † Akaike information criterion.

2011-12 Season

The three field seasons were extreme, drought, flood, and ‘normal’. Therefore, we also fit models for the ‘normal’ year 2011-12. The results for *H. armigera* egg load, mirrors that of the full three year analysis with a minor difference. The mean *H. armigera* egg load/ sample in *Bt* cotton was 26, compared to the full three year analysis season result of 37.5. There is no significant average landscape-level affect, but there is a significant field-level affect of *Bt* cotton, but at a large scale of 1.5 km r, instead of 0.5 km r. Similarly, if the amount of *Bt* cotton at 1.5 km increases by 1%, then the model predicts that the number of eggs in *Bt* cotton will increase by 0.29/ sample (0.0007 eggs / plant or 1 in 1430 plants or 489 additional eggs/ ha).

H. armigera egg load in *Bt* cotton= 72.75 +1.04 (field-level Btcot @ 1.5km r).

The mean *H. punctigera* egg load/ sample in *Bt* cotton was 6.7 compared to the full season result of 6.0. There is very little variation among landscapes, only 7%.

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Outcomes

How project outputs contribute to planned outcomes.

Predicted Outputs:

- *Data on the abundance of *Helicoverpa* spp (eggs and moths) and parasitism of *Helicoverpa* spp eggs in grain and Bt cotton at the spatial scale of fields, groups of fields and landscapes across the Darling Downs, QLD.*

The comprehensive nature of our research spanning three consecutive but very different climatic seasons has allowed us to identify and predict the effect of landscape composition and configuration on the population dynamics of *Helicoverpa* spp. and their egg parasitoids.

- *Results from spatially explicit analyses that indicate how landscape configuration, namely; the size of fields, location, type and amount of grain and Bt cotton crops influence *Helicoverpa* egg loads and moth densities.*

These results have a number of clear implications and outcomes for industry. Specifically especially at the landscape scale aggregate at 1.5 km radius is having a strong influence on *H. armigera*, but no such influence is evident on *H. punctigera*. In addition sorghum surrounded by *Bt* cotton attracts more *H. armigera* eggs compared to sorghum surrounded by sorghum, and that this may act to reduce the selection pressure of *H. armigera* in *Bt* cotton. Such findings will be important for resistance modelling, and need to be considered for any future development of resistance management strategies.

- *Dependent on the results and in consultation with industry and growers we will produce and distribute guidelines for best management practice when considering on-farm crop configuration to enhance pest management.*

Given the results and potential implications of these findings, full consultation and discussion with CRDC, specialists in resistance management and TIMS committee is recommended prior to making any recommendations to the RMP or developing guidelines.

Technical advances and Intellectual Property

No commercially significant technical advances were achieved, and there are no changes to the IP register required. However, application of hierarchical linear modelling has allowed us to elucidate the spatial resolution of egg load going into fields.

Conclusion

The economic and environmental benefits of *Bt* cotton to the Australian cotton industry are without question. However the development of resistance in *H. armigera* and *H. punctigera* remains a threat, and therefore protecting *Bt* technology will remain a priority. *Helicoverpa armigera* and *H. punctigera* are highly mobile organisms existing across broad acre landscapes that are constantly changing, yet little is known about how they respond to these landscapes dynamics. Our research involved modelling data generated from large scale field sampling of *Helicoverpa* spp, combined with landscape scale information for three seasons. Modelling this data allowed our research to move beyond the limits of traditional small scale experiments to multiple spatial scales, which more appropriately reflects the relationship between landscape scale patterns and the population dynamics and ecology of *Helicoverpa* spp. These results have contributed significantly to understanding how *H. armigera* and *H. punctigera* populations respond to the proportion of crop type such as *Bt* cotton at a landscape scale and have important implications for industry.

Using a range of landscape predictors we showed that crop type, amount and arrangement can explain a significant amount of variation in *Helicoverpa* egg loads across landscapes. These results have generated predictions about how changes in the type, amount and location of crops, especially *Bt* cotton, and sorghum are likely to change *H. armigera* egg load in *Bt* cotton and sorghum. While we have begun to identify how proportions of different crop effect *H. armigera*, no clear relationship was established for *H. punctigera*. For example *H. armigera* is strongly affected by landscape composition and configuration at the scale of tens of kilometres. The landscape-scale average of many large areas of *Bt* cotton has the biggest effect – more so than the immediate surrounding field effect – and results in fewer eggs laid in *Bt* cotton. In comparison *H. punctigera* appears to interact with crops at a much greater spatial scale, beyond that of our 20 km diameter landscapes. In the case of sorghum and *H. armigera*, where sorghum is surrounded by *Bt* cotton, our results indicate that crop type and configuration is influencing their behaviour resulting in more eggs and moths within sorghum.

Together with these important results our research has generated a comprehensive data set, which is available for further interrogation and useful to answer specific questions about resistance. Together these data and the models will provide valuable base-line information and reference models against which to measure any changes in the future.

Although these findings have the potential for positive outcomes in relation to the control of resistance and area wide pest control, the ways in which they might be applied has many challenges. For example, from a *Helicoverpa* species perspective when we consider combining crops it may mean that “one size may not fit all” and any development of strategies may require careful consideration.

These findings will be valuable for initiating discussion with all industry stakeholders and be useful for informing the development of any management activities that directly influence refuge strategies.

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Extension Opportunities

We plan to communicate the results to industry leaders working in resistance management, RefCom (Aug 2012) and TIMS, and discuss the implications for resistance management. In addition, our plan for future presentation and dissemination of project outcomes is to present results at the Entomological Society of America meetings in Knoxville, TN November 2012. Furthermore we will identify field days where we can communicate findings more broadly to industry.

We will apply the results to the newly funded CRDC project titled, 'Area-wide pest suppression in transgenic landscapes: Implications for resistance management' (see below). The aim of our project is to increase the flexibility and value of the Resistance Management Plan (RMP), one that extends refuge options for *H. punctigera* and *H. armigera*. We will achieve this aim by establishing how the configuration of refuges and crops in cotton-grain landscapes influence the mechanisms responsible for delaying the evolution of resistance, and area-wide pest suppression. Specifically we will investigate how the size, proportion and location of crops (*Bt* and non-*Bt*) in the landscape alters: (i) the proportion of the *Helicoverpa* population under selection, (ii) their reproductive potential (per capita reproductive rate), and (iii) their ability to find mates.

Publications

Two final reports from 2009-10, and 2010-11 field season have been submitted, but no peer reviewed reports at this time.

Preliminary results of the project were presented by Dr Nancy Schellhorn at the CRC Science Forum Narrabri Oct 2010 and March 2012.

Part 4 – Final Report Executive Summary

Even though large scale planting of *Bt* cotton started a decade ago, consequences on a landscape-scale have hardly been studied. *Helicoverpa armigera* and *H. punctigera* are highly mobile organisms existing across broad-acre landscapes that are constantly changing, yet little is known about how they respond to crop and non-crop composition and configuration. Using a robust sampling design we collected data on the abundance of *Helicoverpa* spp (eggs and moths) and egg parasitoids in *Bt* cotton and sorghum at the spatial scale of fields, groups of fields and landscapes (20 km diameter) across the Darling Downs, QLD over three years. Detailed land-use metrics were generated at various distances from each sampled field in each landscape and season. These data were combined and used in spatially-explicit statistical analyses to identify and predict how crop composition and configuration at the landscape-level and field-level influence *Helicoverpa* egg and moth density.

Helicoverpa armigera egg and moth density is strongly affected by landscape composition and configuration at the scale of landscapes, or tens of kilometres. The landscape explains most of the variation in *H. armigera* egg density in *Bt* cotton, not what immediately surrounds the *Bt* cotton field. The best-fit model accounts for 50% of the variance among landscapes with large areas of *Bt* cotton (at a scale of 1.5 km radius) having the biggest affect, resulting in fewer *H. armigera* eggs laid in *Bt* cotton. The explanation for this result may be that *Bt* cotton functions as a population sink and overtime fewer moths are available to lay eggs

Combinations of crop at various scales could not explain *Helicoverpa punctigera* egg and moth density. This maybe because *H. punctigera* interacts with crops at a spatial scale larger than landscapes of 20 km diameter.

No *H. punctigera* were found in sorghum. For *H. armigera* as sorghum fields increase in size so does the *H. armigera* egg density. However, sorghum surrounded by sorghum never has as many eggs as sorghum surrounded by *Bt* cotton. This could be due to either more immigrants to sorghum over time or moths less willing to leave sorghum when surrounded by large areas of *Bt* cotton.

In *Bt* cotton, there was a positive relationship between the number of moths (species not separated) and the field-level amount of *Bt* cotton at 0.5 km r. However this was only found for 2011-12 season. In sorghum, there was a positive relationship between the number of moths and the average landscape-level amount of *Bt* cotton at 1.5km r. The moth and *H. armigera* egg result are similar. There are more moths and eggs in sorghum surrounded by *Bt* cotton than sorghum surrounded by sorghum. Sorghum in a sea of *Bt* cotton either ‘attracts’ and / or ‘holds’ *H. armigera* moths.

Interpretation and Implications

These results provide clear demonstration of the importance of landscape composition and configuration. Further, variation was often best explained by landscape-level predictors, and less often by field-level predictors. This demonstrates the importance of area-wide approach to pest and resistance management. Further, the strength of selection for resistance is largely determined by the proportion of the population under selection. Our results allow us to identify and predict some of the factors contributing to eggs deposited by female moths in *Bt* cotton.

These findings have the potential for positive outcomes in relation to resistance management and area-wide pest control, however the ways in which they might be developed for application will be challenging. Regardless, our approach and results can directly contribute to selection of crop type, amount and location for delaying resistance in *Helicoverpa* spp.

