



FINAL REPORT 2016

For Public Release

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CSE1306

**Project Title: Managing Bt resistance and the landscape ecology
of *Helicoverpa* spp.**

Project Commencement Date: 1 July 2012 Project Completion Date: 30 June 2016

CRDC Research Program: 1 Farmers

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Date Submitted:

5 Oct 2016

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

It is well-recognised that the pest status of *Helicoverpa* spp. moths within Australian cotton crops has been reduced by the use of Bt cotton varieties, and that much less insecticide is now directed against these pests. But how has the abundance per se of *Helicoverpa* spp. changed over time within cotton landscapes and how much of the observed change can be attributed to the use of Bt cotton? Have the two key species, *H. armigera* and *H. punctigera*, been influenced to the same extent? Does the large “spring” immigration of *H. punctigera* into eastern cotton production landscapes, from the inland where it is unlikely to be influenced by Bt cotton, still occur to the extent it was believed to do in past years? In part to help answer these questions, CSIRO has been monitoring populations of *Helicoverpa* at landscape scale in the vicinity of ACRI, Narrabri (Namoi Valley) using pheromone traps since 1992. This work continued in this project.

Irrespective of any numerical suppression of these pests, the possibility of Bt resistance emerging in *Helicoverpa* spp. remains a major threat for the Australian cotton industry. A Bt Resistance Management Plan (RMP) was therefore established with the introduction of Bt cotton and is regularly reviewed for its effectiveness. Central to the RMP is the use of mandatory refuge crops (currently pigeon pea and conventional cotton) to release large numbers of Bt susceptible moths within cotton production landscapes and thereby limit the development of Bt resistance. At the inception of this project, unexpectedly high frequencies of resistance alleles (especially those for Cry2Ab) had been reported and seemed to be trending upwards, which was of much concern. Our previous research had identified the most productive (although highly variable) refuges. We had also shown that mandatory refuge performance in servicing landscapes with susceptible moths is likely to be very patchy and suggested that such would be inevitably weakened further by the industry decision to halve refuge area requirements. Other work had shown that mating of *Helicoverpa* moths is random with respect to plant host origin (the latter determined using stable isotope signatures that moths acquire from plants when feeding on them as larvae). Random mating was hitherto a key but untested assumption in the RMP. However, our understanding of the relative contributions of the various plant host origins of moths, be they Bt cotton crops, mandatory refuge crops or other [“unstructured”] refuges, to the total populations of moths throughout landscapes and regions, and the degree of movement / spatial mixing of such moths within and between such areas, is still rudimentary. Such information is critical to an effective RMP. In particular, the scales at which the movements and mixing occur (and their spatial consistency) are directly relevant to the optimal development and use of modelling tools to adequately predict the development of resistance and its management. What is the most appropriate scale to use in such modelling? Possibly it’s landscape scale, or perhaps something larger is more befitting for very mobile insects?

In addition, the efficacies of other aspects of the RMP, most notably the mandatory requirement of planting windows for Bt cotton (designed to limit the number of generations of *Helicoverpa* exposed to Bt toxins) and post-harvest “pupae busting” of Bt cotton (designed to reduce the over-winter survival of resistant *Helicoverpa*, i.e. from one season to the next), have been questioned. Much has changed in cotton production since the original development of the RMP, or is likely to in the near future (e.g. increased dry-land cotton, greater emphasis on soil health, new tools provided to manipulate crop growth & maturity, the future release of Bollgard 3[®] cotton with three Bt toxins) which put pressure on the perceived benefits of these RMP-related practices.

The broad aims of this project were therefore to :

- i) Continue monitoring for *Helicoverpa* moths in the Namoi Valley to demonstrate long-term trends in abundance in relation to ongoing environmental change, in particular the advent of Bt cotton and prevailing weather.
- ii) Demonstrate the importance of regional landscape complexity in determining the mixing of *Helicoverpa* populations from different plant host origins, within contrasting production systems. Do moth populations reflect the landscapes they are found within? For landscape scale to be appropriate

for modelling resistance development, we argue they should. (Note : This reasoning includes mismatches explainable via the balance of both source and sink crops available to the pests).

iii) Build an overview of sowing and defoliation dates for cotton throughout the industry against which the efficacy of the planting window strategy for the Bt RMP can be gauged.

iv) Provide a review of the current knowledge of the efficacy of “pupae busting”, and, as an outcome from this, identify the need for further experimentation that would assist decision making within RMP development that is achievable within a single season. [Note : this work was added during 2015-16, thus providing a more limited 4th year extension to the project]

Note : Geoff Baker retired at the end of November 2014, but has continued from then on as a Post-Retirement Fellow with CSIRO, providing ongoing, but reduced, input to the project in that role.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

Objective 1. Monitor long-term trends and seasonal abundance of *Helicoverpa* spp. weekly through landscape-scale grid of pheromone traps operating all year round near Narrabri (with a secondary grid at St George, in-season only).

Milestone 1.1. Trapping data collated annually and related to previous year’s trends, prevailing weather etc.

Performance Indicator 1.1. Trapping data included in all progress reports and available for peers / industry on demand (within a week of collection for Narrabri data sets). [Start Date 1/7/2012 : End Date 30/6/2015]

Achieved, with the exception of the secondary grid of traps at St George. Our long-term collaborators there were unable to deliver the required input for us. Whilst unfortunate, this had negligible impact on the core aspects of the study.

Objective 2. Demonstrate importance of regional landscape complexity in determining the mixing of *Helicoverpa* spp. populations from different plant host origins, within contrasting cotton production systems.

Milestone 2.1. Grids of light traps established at strategic times (3 generation peaks) during cotton growing season in regions of different landscape complexity. Concurrent insect pressure (egg & larval densities) measured locally on crops, in particular in St George & Dirranbandi (D. Downs equivalent data available from companion project).

Performance Indicator 2.1. Traps run / insect pressure documented during early, mid & late season (corresponding with generational peaks of *Helicoverpa*) in 3 sub-regions of D. Downs, St George and Dirranbandi (+ Cunnamulla if logistics allow). [1/9/2012 : 31/3/2015]

Achieved.

Milestone 2.2. *Helicoverpa* moths sorted from bi-catch in light traps, prepared for and analysed in mass spectrometer for stable isotope signatures. Mated / reproductive status of trapped moths determined. Sub-sets of eggs / larvae reared & ID’d to further substantiate species balance.

Performance Indicator 2.2. Catch / reproductive status / chemical signature / insect rearing statistics documented in progress reports. Light trap catches supplemented by direct night & pheromone trap collections if needs be to provide adequate sample sizes for analyses. [1/11/2012 : 31/5/2015]

Achieved.

Milestone 2.3. Proportional incidence of different plant host origins of trapped moths related to measures of landscape heterogeneity and insect abundance on crops (includes linkages with data generated in Schellhorn project in D. Downs).

Performance Indicator 2.3. Statistical relationships (including spatial patterns) of linkages between landscape heterogeneity data and composition of moth populations presented in progress reports. [1/6/2013 : 30/6/2015]

Achieved.

Objective 3. Construct a representative overview of current year, cotton planting and defoliation dates, for the Australian cotton industry. Data for earlier years included, where available.

Milestone 3.1. Overview, and analysis relevant to seasonality of insect abundance & crop growth, submitted to TIMS & CRDC annually (June).

Performance Indicator 3.1. Overview and analysis accepted by TIMS & CRDC. [30/6/2013 : 30/6/2015]

Achieved.

Objective 4. Extend research results to industry & scientific colleagues.

Milestone 4.1. At least one scientific MS and one industry magazine article submitted and one seminar / grower talk presented per year. Findings also presented by project staff at relevant industry meetings (e.g. TIMS, REFCOM).

Performance Indicator 4.1. Publications & talks delivered. Details of observed importance of migrant *H. punctigera* to cropping population structure also provided to Bt Resistance Monitoring Project & related modellers. [1/1/2013 : 30/6/2015]

Achieved.

Objective 5. Provide a review of historic research outputs on the biology and management of *Helicoverpa* pupae, of relevance to the efficacy of “pupae busting”.

Milestone 5.1. Literature gathered and discussions with former scientists held. Review focuses especially on assessing our current knowledge of pupae busting, thus determining its practical application, consistency and utility within the current RMP for Bollgard. In particular, improved clarity on tillage depth and target area relative to crop row are required.

Performance Indicator 5.1. Review submitted to CRDC. Research gaps identified and field experiments conducted where relevant and achievable in one season. [1/7/2015 : 30/6/2016]

Achieved, review attached with this report. Whilst the review recognised the need for flexibility within a highly variable system, it did not identify any pressing need for further experimentation, except perhaps in the field of engineering tillage equipment specific to growers needs i.e. that could achieve adequate pupae busting under the specific on-farm conditions present.

Objective 6. Complete Final Report.

Milestone 6.1. Report submitted by end Sept 2016, & oral presentation of final report delivered as required.

Performance Indicator 6.1. Report & oral presentation accepted by CRDC. [30/6/ 2016 : 30/9/2016]

Achieved to the point of submission to CRDC.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Monitor long-term trends and seasonal abundance of *Helicoverpa* spp. through landscape-scale grid of pheromone traps.

Helicoverpa punctigera and *H. armigera* moths were caught using a grid of 14 pairs of Agrisense™ canister pheromone traps (1 for each species at each site) which was maintained continuously within about a 10km radius near the Australian Cotton Research Institute (ACRI), Narrabri, in northern New South Wales (Fig. 1) from July 2012 to June 2015. This followed continuous trapping at most of these same sites since 1992. The traps were usually emptied weekly, with occasional slight variations in timing due to inclement weather and resultant access difficulties. Lures, specific for *H. punctigera* and *H. punctigera*, were changed monthly, and pesticide strips (dichlorvos) were changed bi-monthly. The traps were mounted on metal poles, approximately 1.5m above the ground, and adjacent to agricultural fields. The traps only caught male moths.

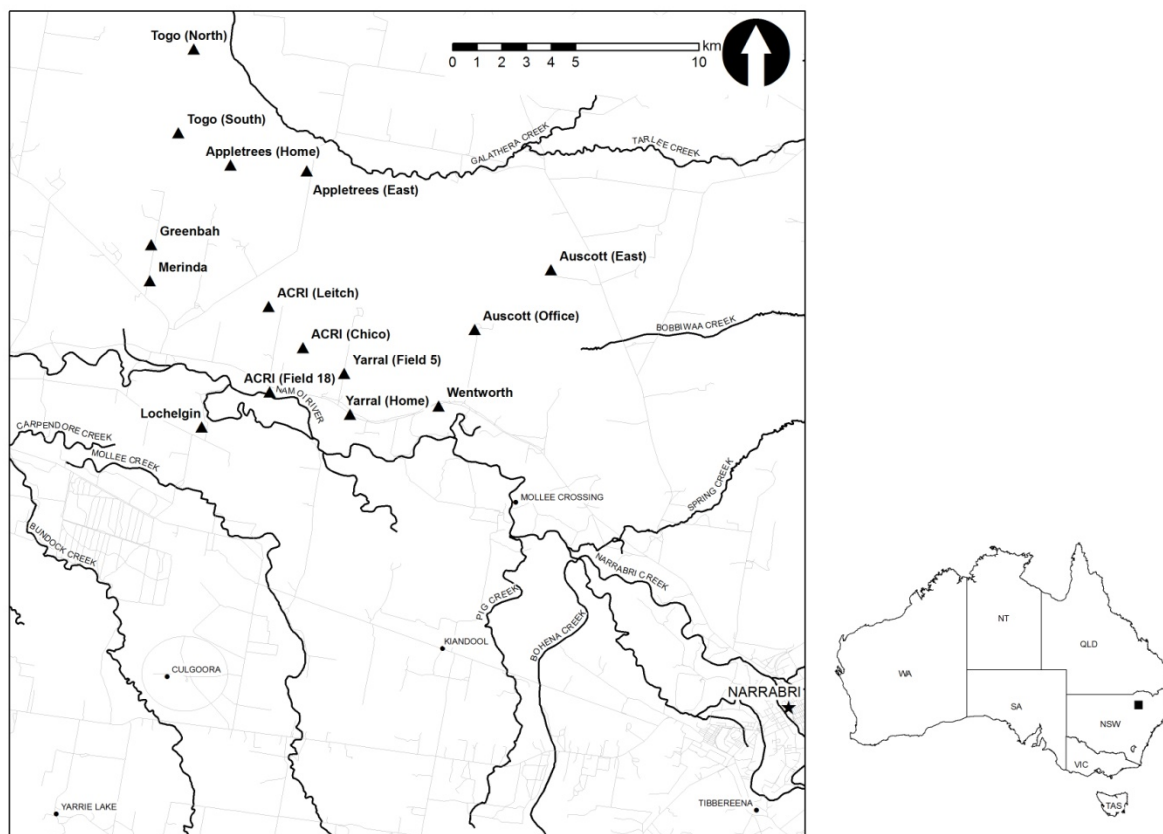


Fig. 1. Locations of the 15 pheromone trap sites near Narrabri, northern New South Wales. Main rivers (bold lines); roads (faint lines)

Previous work (Baker *et al.*, 2011) recognised a 1st generation of moths which was caught between weeks 8-20 inclusive (weeks being counted from July 1), a 2nd generation caught between weeks 21-30, and 3rd + generations caught between weeks 31-44. We continue to use the same cohort groupings. The 1st generation of moths must be mostly immigrants to the Namoi region; the majority are caught before local over-wintering pupae are due to emerge from the soil (Baker *et al.*, 2015).

The spring and summer crops, that were grown in the two fields nearest to each trap, were recorded each year, as had been the case in previous years. These crops mostly included cotton (*Gossypium hirsutum* L. – transgenic and conventional), soy bean (*Glycine max* Merr.), sorghum (*Sorghum bicolor* (L.)), wheat (*Triticum aestivum* L.) or wheat stubble, and chickpea (*Cicer arietinum* L.). Often, one or both of the fields was in fallow, which was also recorded. In many instances, the trapping sites were bordered, on one side, by land that was not used for cropping (referred to here as

verge). Verge was recorded instead of crop if greater in area than the second nearest crop. Verge was variable in nature and included, for example, patches of native and weedy roadside vegetation. Temporal trends in moth catches (1992 to 2015) were sought using data for 1) all the traps set throughout the study (thus local habitats varied between traps in space and time) and 2) where local habitat was the same for subsets of the traps being run. For the latter, we used data associated with fallow fields in spring and cotton fields in summer, because they were the most common land use at these times.

Rainfall and temperature data for 1992-2015 were sourced from the Australian Government's Bureau of Meteorology website (<http://www.bom.gov.au>). Rainfall data were used for Narrabri (Mollee) (meteorological station number 53026; 30°26'S 149°68'E). Initially, temperature data were used for Narrabri at Narrabri West Post Office (station number 53030; 30°34'S 149°76'E), but with the closure of that station in 2002, data were subsequently used from the Narrabri Airport (station number 54038; 30°32'S 149°83'E). Rainfall data were also accessed for 36 additional recording stations in inland Australia (northern South Australia, southern Northern Territory, south-western Queensland and north-western New South Wales), as indicated in Baker *et al.* (2011).

Data for the hectares sown to different crops in the Namoi Valley region throughout 1992 to 2015 were provided by Neil Clark Business Intelligence (Bendigo, Vic.) and Cotton Australia (Sydney, N.S.W.) (see Fig. 2 for data from the Narrabri S.L.A.). Many crops are grown in this region, but continuous records are only available for a few that are dominant in the region and of particular relevance as host plants for *H. armigera* (here we use cotton, sorghum, chickpea and canola, *Brassica* spp.). Wheat was excluded here, because it is a poor host for *Helicoverpa*.

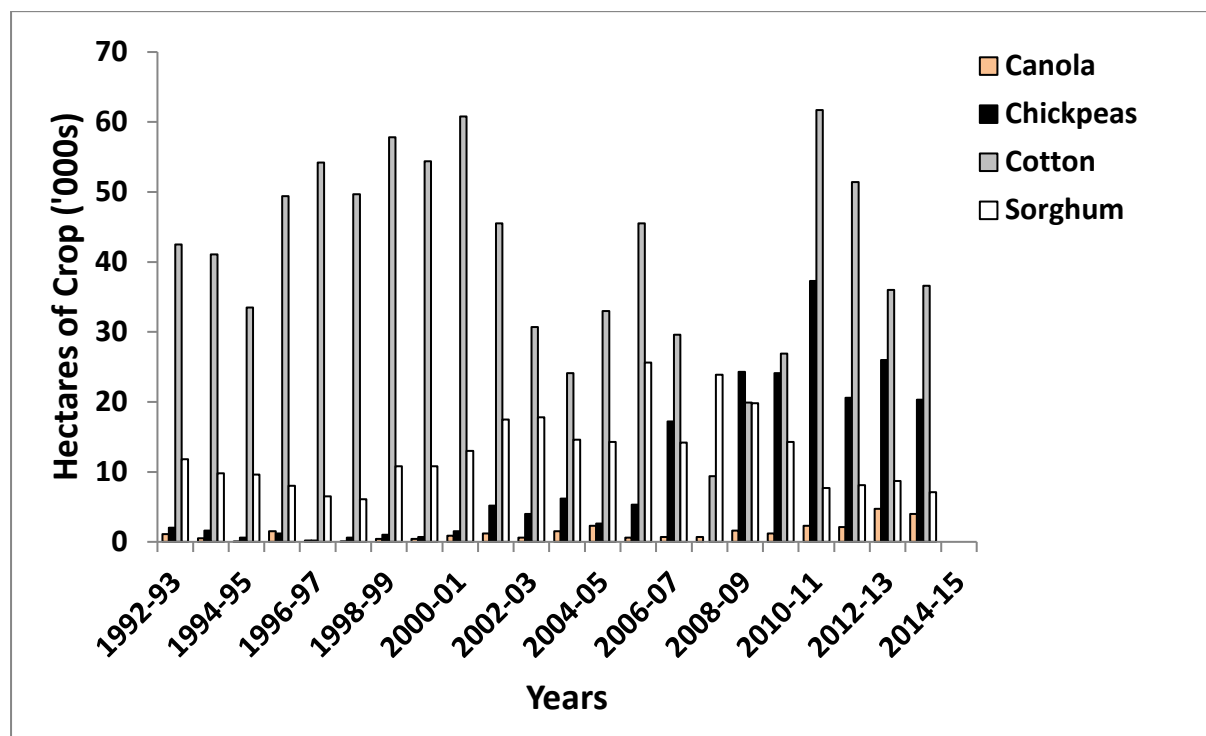


Fig. 2. Hectares of canola, chickpea, cotton and sorghum grown annually (1992 to 2014) in the Narrabri Statistical Local Areas (summed) of N.S.W. Data provided by Neil Clark Business Intelligence. No data were available for chickpeas in 2007-08.

Demonstrate importance of regional landscape complexity in determining the mixing of *Helicoverpa* spp. populations from different plant host origins.

Two contrasting agricultural regions, the Maranoa and Darling Downs, both in southern Queensland, (Fig. 3), were primarily used in this study, over a three year period (2012-15). During the last year of the study, moths were also collected from a third region, near Narrabri in the Lower Namoi, northern New South Wales. (The Narrabri study was in addition to what was agreed in the FRP for CSE1306). Within each of these three regions, three sub-regions were selected for *Helicoverpa* collections (St George, Dirranbandi &Thallon for the Maranoa; Pampas, Nandi & Cecil

Plains for the Darling Downs; Myall Vale, Merah North & Pilliga for the Lower Namoi). Within each sub-region, traps were set at three sites for *Helicoverpa* moths (see Fig. 3 for D. Downs & Maranoa). In most cases, these sites were reused each year throughout the study, but in Nandi, one site (Arrow Energy) had to be shifted about 10 km to The Meadows (between Mayfield & Dalby) in the 3rd year of the study. There were thus nine sites used on each sampling occasion within each region.

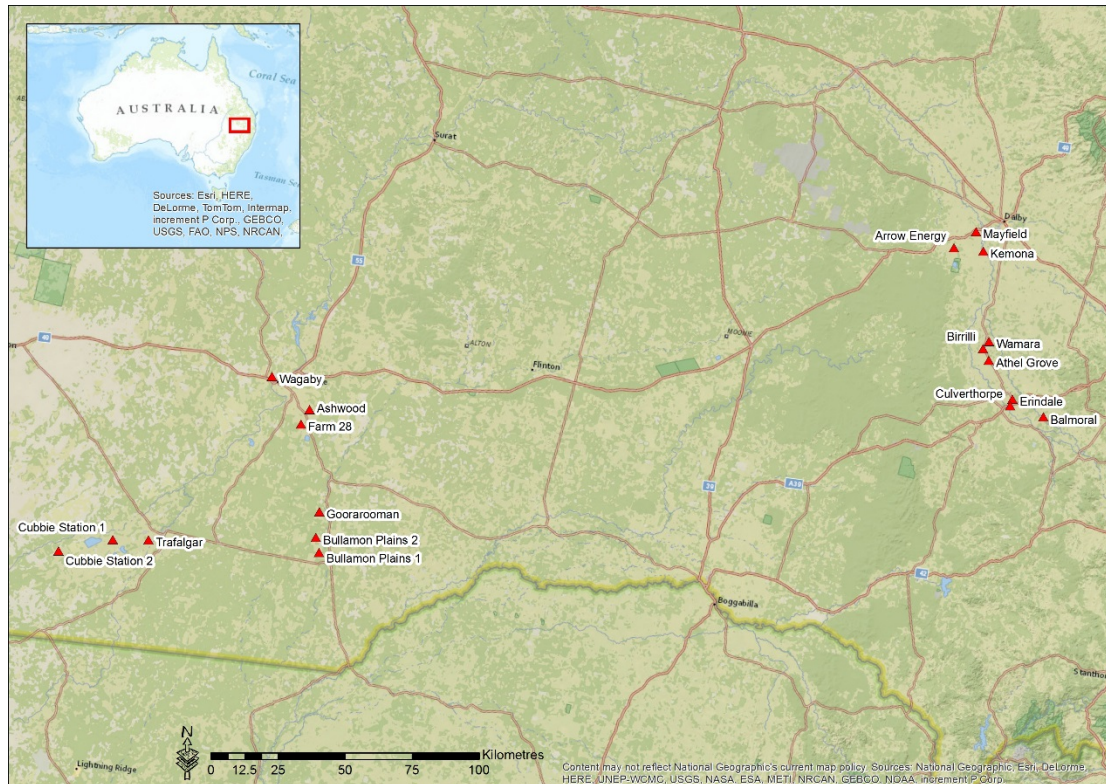


Fig. 3. Locations of the 19 trapping sites in the Darling Downs (cluster at the right of figure) and Maranoa regions of Queensland, Australia. The Arrow Energy site was used in 2012-13 and 2013-14. It was replaced by a nearby site at The Meadows in 2014-15.

We originally intended to only use light traps to catch moths. Light traps are preferable because they capture both male and female moths, unlike pheromone traps which catch only males. Light traps are, however, more cumbersome and require the sorting of desired species from bi-catch of other insects. Indeed, because of vast numbers of other insects fouling our light traps, especially in the Maranoa, on our first sampling occasion, we decided to set pheromone traps as well, to provide insurance against such trap fouling.

One light trap and two pheromone traps (one trap for each species) were established at each site, adjacent to the nearest Bollgard II cotton crop. Each trap was located approximately 100 m from its nearest neighbour. Traps were set for 2-3 nights on each sampling occasion. During 2012-13, 2013-14, and 2014-15, traps were set in the Maranoa and Darling Downs on four, five and four occasions respectively. During 2014-15, traps were also set on four occasions at the nine sites near Narrabri. A small number of moths were selected for stable isotope analyses from catches in the pheromone traps used in the monitoring grid in the Narrabri region (see above) during 2013-14.

We were unable to collect moths in the Darling Downs during mid-season (January) 2013. Fortunately, colleagues (N. Schellhorn team) working on a separate project in the Darling Downs during January 2013 did collect moths, with pheromone traps, within each of the same sub-regions that we used. We have incorporated data from these extra moth collections into our data set, with the caveat that they were not from the exact same sites per se within the three sub-regions.

On most moth sampling occasions, *Helicoverpa* spp. egg densities were also measured within the Bollgard II cotton crop to indicate insect pressure at the time of moth trapping. No egg checks were made in the Maranoa in February 2013 because local flooding prevented access to the cotton fields, nor in April 2014 (both Maranoa and Darling Downs) and March 2015 (all three regions) because the cotton plants had either senesced, been defoliated or were harvested at these times and

were thus deemed unattractive to moths. Eggs were reared in the laboratory to determine species prevalence (our eggs were provided to the Bt resistance monitoring team and reared through that project). No larvae were found on any sampling occasion.

Usually, all female moths caught in light traps were dissected to determine their reproductive maturity / mating status (gravid or not / numbers of spermatophores). However, when very large numbers of moths were collected, only a maximum of 20 moths / species / trap / night for each site were dissected. This applied especially to *H. punctigera* catches.

Sub-samples of the trapped moths were prepared for stable isotope analyses. Moths that were caught in light traps were selected first, then supplemented by moths caught in pheromone traps. Inevitably, some traps caught many moths, and others caught few. We chose all or very nearly all moths from traps with small catches and discarded more from traps with large catches. Overall, we analysed 7,609 *H. armigera* moths and 4,546 *H. punctigera* moths, collected during 2012-2015, for their stable isotope signatures.

A 1 mg (approximate) sample of head capsule was dissected from each air-dried moth selected for stable isotope analysis, placed in pressed tin cups (8 x 5mm), and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined at the University of New England, New South Wales using initially a Carlo Erba NA 1500 Solid Sample Analyser coupled to a TracerMass Stable Isotope Analyser and then this system was upgraded to a Sercon 20-22 continuous flow isotope ratio mass spectrometer connected to an ANCA-GSL solid sample preparation unit. Wheat flour (42.1% carbon, $\delta^{13}\text{C} = -22.9$) was used as the standard and this was referenced to Iso-Analytical Ltd (UK)'s wheat flour standard IA-R001.

We accepted that a $\delta^{13}\text{C}$ value of $< -20\text{‰}$ is indicative of a moth arising from a C3 host plant (e.g. cotton, pigeon pea, chickpea, wheat) and a $\delta^{13}\text{C} \geq -20\text{‰}$ is indicative of a moth resulting from a C4 host plant (e.g. sorghum, maize), as we have in the past. We anticipated that moths feeding on most legumes in the landscape would have a lower $\delta^{15}\text{N}$ than those feeding on non-legumes because of their fixing of atmospheric N and that such a difference would be passed on to larvae when feeding on the various plants.

Data for the hectares grazed by stock and sown to major crops in the Maranoa and the Darling Downs throughout 2012-2015 were provided by Neil Clark Business Intelligence (Bendigo, Vic.). This data is based on a geographical format (Australian Standard Geographical Structure) wherein the base unit for reporting data is the Statistical Area 2 (SA2). Data for the Darling Downs include inputs from the Wambo, Jondaryan, Pittsworth and Millmerran SA2s; data for the Maranoa include the Balonne SA2 only.

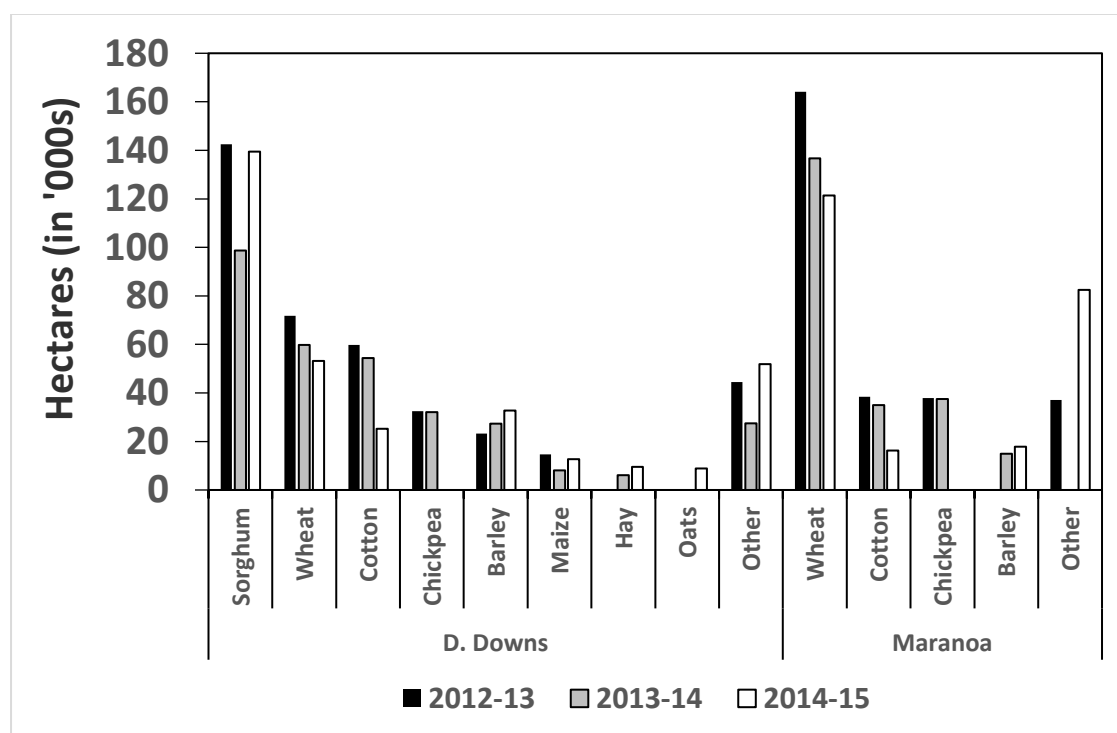


Fig. 4. Hectares of land used for crop production in the Darling Downs and Maranoa regions of Queensland during 2012-15. Data provided by Neil Clark Business Intelligence, Bendigo, Victoria.

The agricultural land in the Maranoa is, as a whole, dominated by grazing on unimproved land. The main crops are wheat, cotton and chickpea (Fig. 4). Cropping is intensive within relatively small regions of the Maranoa where there is access to irrigation. Further afield, dryland wheat and, to a lesser extent, chickpea are interspersed amongst permanent grazing land, with crop stubbles also used for grazing. Cropping and grazing are more evenly represented in the Darling Downs, with a greater variety of crop types used there compared with the Maranoa (Fig. 4), most notably sorghum. Grazing on unimproved land is more common towards the west of the Darling Downs, with cropping most common towards the east, especially where there is irrigation.

The Lower Namoi is different again from the Darling Downs and the Maranoa, with wheat the most common crop. In recent years, cotton and chick pea have been more widely grown in the Lower Namoi than sorghum (see Fig. 2 referred to above in the section on long-term monitoring). The Lower Namoi is thus intermediate between The Darling Downs and the Maranoa in terms of cropping structure.

During each season that moths were trapped at the study sites, the crops sown in all fields within a 2 km radius of each pair of traps were recorded. These data were included in a geographical information system for us by Peter Verwey (NSW DPI) using ArcGIS, which enabled measures of the various most common land-uses in the near vicinity of each trapping site. The 2km radius was chosen to match other studies of landscape effects on the abundance of *Helicoverpa* eggs, in particular in the Darling Downs (N. Schellhorn team), but also similar work conducted overseas. The Schellhorn team also worked in the same sub-regions there as we did (i.e. Nandi, Cecil Plains and Pampas).

Construct a representative overview of current year, cotton planting and defoliation dates, for the Australian cotton industry.

The effectiveness of the planting windows strategy has not been previously evaluated using concurrent field data for both the incidence of the insects and the growth phases of the crops in the one analysis. Here we used long-term, industry-wide data-bases (2002-13) of sowing, harvesting and pupae destruction dates on-farm (see below) and related them to pheromone trapping records for *Helicoverpa* over the same time period (as described above). In addition, predicted seasonal timings of the onsets of winter (pupal) diapause and spring emergence as moths were calculated. Southern Qld and NSW were treated separately from central Qld, as these regions were also considered separately in the Bt RMP at the time of the study. The study period spanned growing seasons when Ingard® and later, Bollgard II®, cotton varieties were grown commercially in Australia.

Monsanto Australia Ltd (Monsanto) maintains a data-base of the dates (day/month/year) of Bt cotton cropping practices at the level of contractual agreements made with farmers (Technology User Agreements, or TUAs). The recorded data varies in content with growing region, according to differences in the RMP tasks required of farmers. Thus whilst both the dates when planting started and ended for each TUA are recorded in all cases, defoliation and harvest completions are only recorded in central Qld and pupae destruction is only recorded in regions further south. Overall 42,231 date records, across 15 cropping regions, were used in the Monsanto data-base (after occasional, clear mistakes were deleted – e.g. where harvest was marked as occurring before sowing). Slightly fewer records were available later in the season compared with planting start. Reasons for such are not known but may in part reflect abandonment of some crops due to lack of available water. The records include both irrigated and rain-fed crops (the latter being much less common in the Australian cotton industry). Available data varied in extent from one region to another, according to the degree of cropping conducted. Available data also varied from one year (season) to another, sometimes reflecting prevailing weather and farmer decisions to plant or not (e.g. Emerald & Macintyre), but also recent expansion of the cotton industry in “new” regions (e.g. Murrumbidgee). Some data were available in the Monsanto data-base for 5 other, small regions (Belyando, Burdekin, McKenzie River, Tandou and The Ord), but despite all growers there complying with the legal requirement to report their planting dates, these records were considered too limited to merit inclusion in the data analysis.

Because of the large amount of cropping data analysed for all 15 cotton growing regions, only selected examples for particular regions can necessarily be reported here. We focussed mostly on examples from 3 representative, and geographically distinct, regions (Emerald, central Qld; Darling Downs, southern Qld; Lower Namoi, NSW). Data for all individual regions are available from the authors. Monthly rainfall data for Emerald, Dalby (Darling Downs) and Narrabri (Lower Namoi)

were obtained from the Australian Bureau of Meteorology (Stations 35264, 41522 and 54038 respectively) for the period 2002-2013 inclusive to assist with some interpretations of planting and harvest events.

For ease of handling, dates were converted from individual days to being within particular weeks of the year, with the 1st week being the first week of July and the 52nd week being the last week of June the next year. This approach centred each growing season within the range of weeks considered for each year. On some occasions, defoliation, harvest or pupae busting did not occur before July in the year following sowing. In such cases, week counts simply continued (thus a data point for a harvest in late August could be week 60).

The CottASSIST website provides tools which predict when *H. armigera* moths will emerge in spring and when pupae will enter winter diapause, for locations throughout the cotton growing regions of Australia. Moth emergence for a particular location is represented as a cumulative percentage of moths that will have emerged on a daily basis. The diapause induction tool indicates the likelihood of a pupa entering diapause on any particular day, and is not a cumulative measure. Patterns of emergence and diapause induction throughout 2002-13 were accessed via the CottASSIST website for selected locations throughout the Australian cotton industry (Emerald, Theodore [Dawson-Callide], St George, Dalby [Darling Downs], Wee Waa [Lower Namoi] and Hillston [Lachlan]). Similar tools have not been developed for *H. punctigera*.

The CottASSIST website provides historic weather data, including soil temperatures (at 10 cm depth), for Myall Vale (i.e. ACRI) laboratories in the Lower Namoi, NSW). The optimum soil temperature for cotton establishment is 16-28°C. Daily minimum soil temperatures for August to October, 2002-13 (i.e. pre-sowing to post-sowing for cotton), were accessed via the CottASSIST website. No similar minimum soil temperature data are available for central Queensland or the Darling Downs. We therefore estimated minimum soil temperatures from minimum air temperatures recorded at Emerald (weather station no : 035264) for central Queensland and Dalby (weather station no : 041522) for the Darling Downs (data obtained from <http://www.bom.gov.au>), assuming the same relationship would hold there as it does between air and soil temperatures at Myall Vale (Soil Min °C = 8.77 + 0.65 Air Min °C).

Given the lack of harvest date information for the Lower Namoi and the Darling Downs in the Monsanto data-base, similar information was accessed from records listed in Cotton Seed Distributors (CSD) Annual Variety Trials Booklets for each growing season from 2002- 2013 (Cotton Seed Distributors 2002-13). Only the records for irrigated cotton were used in the CSD database. Such records (n = 93 and 51 across all seasons for the Namoi [both Upper and Lower Namoi collectively] and Darling Downs respectively) are fewer in number than those for Emerald in the Monsanto database (n = 466).

The grid of Agrisense™ canister pheromone traps operated near ACRI, NSW has been described above. A smaller number of the same traps (5-10 pairs) were monitored in the same way within the St George, Qld, cropping region from 1997 to 2009, except they were usually only run during the cotton growing season (weeks 16-44; mid October to end of April). Pheromone traps have their limitations. In particular, their catches are measures of both activity and abundance. We particularly used pheromone trapping records in this work on planting windows to identify separate generations of *Helicoverpa*. We placed little emphasis here on the numbers of moths trapped per se.

Extend research results to industry & scientific colleagues.

[See below in sections 4, 8 & 9 where details are provided of completed and planned extension & publication activities]

Provide a review of historic research outputs on the biology and management of *Helicoverpa* pupae, of relevance to the efficacy of “pupae busting”.

The methodology taken in preparing this review (done primarily by Colin Tann and Michael Braunack, CSIRO Narrabri) was a standard approach for such a deliverable : review of all existing literature, both in international journals, conference proceedings and industry articles, discussions with relevant colleagues and internal critique.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Monitor long-term trends and seasonal abundance of *Helicoverpa* spp. through landscape-scale grid of pheromone traps.

Two manuscripts (one for each *Helicoverpa* species) have been submitted to Bulletin of Entomological Research and accepted for publication (expected during 2017) :

Baker, G.H. & Tann, C.R. Long-term changes in the numbers of *Helicoverpa punctigera* (Lepidoptera : Noctuidae) in a cotton production landscape in northern New South Wales, Australia. *Bull. Ent. Res.* In Press.

Baker, G.H. & Tann, C.R. Broad-scale suppression of cotton bollworm, *Helicoverpa armigera* (Lepidoptera : Noctuidae), associated with cotton crops in northern New South Wales, Australia. *Bull. Ent. Res.* In Press.

These two manuscripts (as galley proofs) are attached to this report (Attachments 1 & 2). A condensed version of the results presented in the two manuscripts is provided here. Some selected Figures are provided. The reader is directed to the manuscripts for additional relevant figures, tables, statistical analyses and extra discussion.

Rainfall varied greatly between years at Narrabri and was particularly high during the summers of 1996-97 and 2011-12. Air temperatures showed little variation across the two decades of observation. The most notable outliers were the relatively cool season (spring-summer) in 2011-12 (mean maximum air temperature = 28.1°C) and the relatively warm season in 2013-14 (32.5°C). The most common habitats near the pheromone traps were fallow fields, wheat crops and verge during spring, and cotton crops, fallow fields and verge during summer. The incidence of these varied throughout the 23 years of the study and between sites, presumably reflecting, in the main, farmer decisions in response to weather patterns and market demands. Most notably, cotton was rare during 2007-10 and 2014-15, reflecting a general scarcity of cotton plantings across the Australian cotton industry in those years.

H. punctigera

Overall, the 1st generation of moths (caught between weeks 8-20) dominated the trap catches. At least one more generation (between weeks 21-30) was also vaguely discernible in the aggregated data. There was, however, much variability between individual years. In several years (e.g. 1993-94), only a 1st generation was discernible, in some other years (e.g. 1996-97) a 1st and 2nd generation were trapped, and very occasionally (e.g. 2009-10) three or more generations were apparent. 2013-14 was an unusual year, when three apparent generations were trapped, more or less in equal abundance, and the 3rd generation occurred earlier than expected, i.e. mostly before week 31.

The average numbers of *H. punctigera* moths caught in the pheromone traps set at the 15 separate sites varied markedly within each generation. However, some of these traps were set for more years than others. Including all such data in an analysis of variability between sites could create temporal bias. When we selected only those traps which were set for all, or very nearly all, years of the study (at least 20 of the 23 years; N = 8) for analysis, no significant differences in catch could be detected between trap sites for any of the 3 generations, using total catch for each generation at each site in each season as the primary data.

The total numbers of 1st generation moths caught each spring decreased throughout the 23 years of study (using the data available for all 15 sites for the analysis). More moths were caught when wheat or verge were present in at least one of the two fields adjacent to a trap. The presence of fallow had no effect. Large numbers of 1st generation moths were occasionally caught in the few traps in spring where chickpea was nearby (up to 3,365 in total in one trap in 2000, mean \pm S.E. = 753.3 \pm 319.2; this compares with 408.2 \pm 55.8 for traps near wheat).

The numbers of 2nd generation moths caught in summer also decreased in time, with the presence of cotton in at least one field near traps having a negative effect on catch. The presence of

neither fallow nor verge near traps influenced the catch of 2nd generation moths. Similarly, the catch of 3rd + generation moths decreased in time, but no local influence of cotton, fallow or verge on catch was discernible. In addition, the average numbers of 2nd and 3rd + generation moths that were caught in the few traps in summer where sorghum and soybean were nearby were relatively low for the former and similar in abundance for the latter compared with numbers caught near cotton.

When these same data for moth catches were separated into subsets of the three cotton production eras, i.e. 1) Pre-Ingard[®] (1992-96), 2) Ingard[®] (1996-2005) and 3) Bollgard II[®] (2005-15), a relationship (negative) between moth numbers (2nd and 3rd + generations) and the presence of cotton nearby was demonstrated during the Bollgard II[®] era.

The average numbers of moths (all generations) caught in the traps, irrespective of local habitat types, varied between years (Fig. 5). As well as erratic variability between consecutive years, there was a general decline in numbers in time, in particular for 1st generation moths. However, there was no evidence to suggest a similar decline in the numbers of 2nd generation moths. For the 3rd + generation, there was a significant decline in numbers over time, but given that 2013-14 was such an exceptional year, in which the 3rd generation occurred unusually early, it seems valid to recalculate the regressions omitting data for the 2nd and 3rd + generations in that year. If so, there was still no temporal decline in catch for the 2nd generation, but very nearly so. A significant temporal decline remained in the catch of the 3rd + generation.

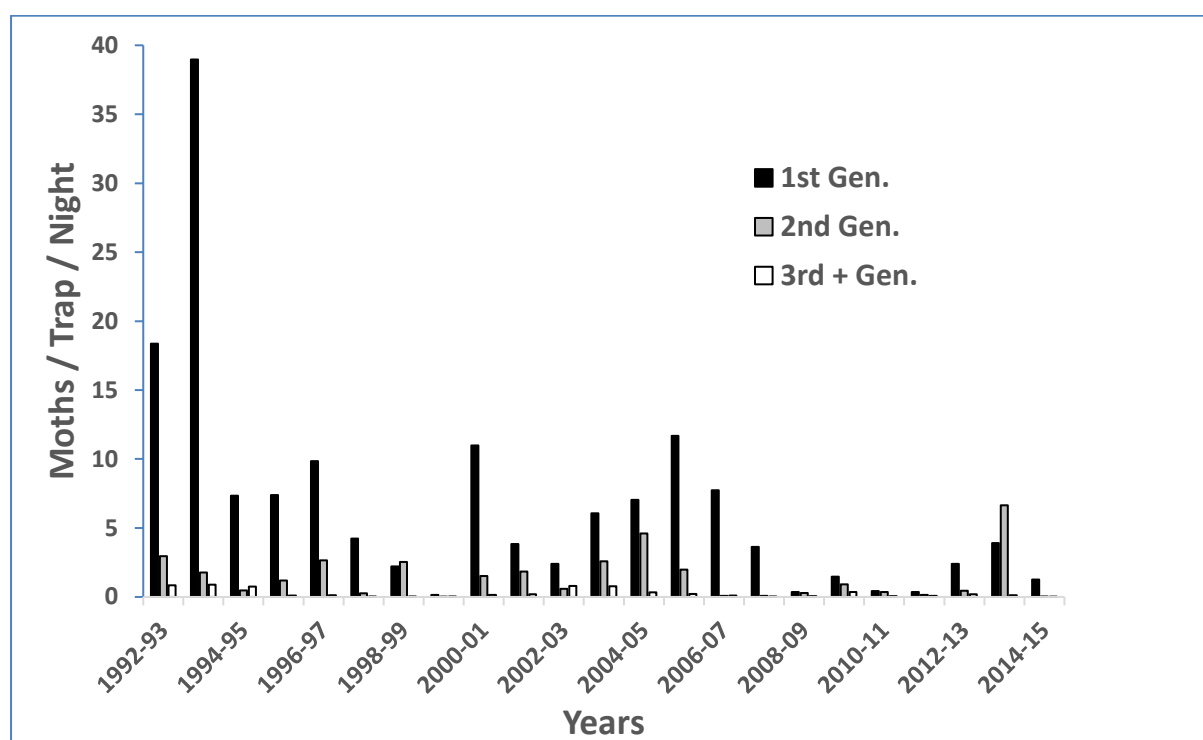


Fig. 5. Pheromone trap catches of male *H. punctigera* moths near ACRI throughout 1992 – 2015. Data are separated into moths in the 1st generation (caught during weeks 8-20), 2nd generation (weeks 21-30) and 3rd + generations (weeks 31-44), with weeks taken from 1 July.

There was also a significant decrease in the number of 1st generation moths caught in traps set where fallow fields predominated in spring (n = 36 cases where the two nearby fields were both fallow) throughout the 23 years of study. However, no similar decrease in catch (of 2nd & 3rd + generations) could be demonstrated for traps set near two cotton fields in summer (n = 24).

In addition, there was no correlation between the average numbers of 1st and 2nd generation moths caught in the traps, nor between 2nd and 3rd + generation moths, but there was between 1st and 3rd + generation moths (where data for all years and traps were included, but data for years were treated separately, thus N = 23). In contrast, there was no correlation between the average numbers of 1st and 2nd generation moths caught in the traps, but there was between 2nd and 3rd + generation moths and 1st and 3rd + generation moths (where data for all years and traps were again included, but data for sites were treated separately, thus N = 15). Thus analysing the data in a temporal context (across years) yielded a slightly different result than analysing it in a spatial context (across sites). There was

also a significant correlation between the catch of 3rd + generation moths in one year and the number of 1st generation moths in the next.

There were no significant associations, detected by multiple regression, between the catch of 1st generation moths and the hectares of canola and chickpea grown in the Narrabri SLA. There were also no significant associations detected between the catch of 2nd generation moths and the hectares of canola, chickpea or cotton. However, there was a negative relationship between the catch of 3rd + generation moths and the hectares of cotton grown.

The catches of 1st generation moths during both the Ingard[®] and Bollgard II[®] eras were lower than those during the preceding years when only conventional cotton was grown. Note, for this calculation, the catch for spring 1996 was included with the preceding conventional years and the catch for spring 2005 was included with the preceding Ingard[®] years, because 1st generation moths were caught prior to the sowing of cotton. There was no significant difference between the catches in the Ingard[®] and Bollgard II[®] eras. In contrast, there was no difference between catches of 2nd generation moths across the three cotton eras, but there was for the 3rd generation (differences between eras were as per 1st generation).

There were no significant associations between local rainfalls and average trap catches of 1st and 2nd generation moths each year, where rainfalls were calculated (as relevant for the particular moth generations) for the 1) preceding autumn (March to May inclusive), 2) preceding winter (June to August), 3) spring (September to November), 4) summer (December to February), and finally 5) autumn at the end of the cotton season (March & April)(1-3 deemed appropriate for 1st gen., 1-4 for 2nd gen. and 1-5 for 3rd + gen.). However, a significant, but weak, negative association was found between spring rainfall and the catch of 3rd + generation moths.

No associations were detected between temperature and the catches of 1st and 2nd generations of moths, using the same seasonal groupings as mentioned above in relation to rainfall. Some significant associations were detected for temperature and 3rd generation moths ; positive for the preceding winter and prevailing summer temperature , negative for spring and autumn (end of season).

The above is not to say that trap catches and weather at finer temporal scales (e.g. weekly) were not related (such data are not presented here).

Rainfall has varied greatly over the past 4 decades in inland Australia. However, there was no significant correlation between average rainfalls in autumn – winter (April to July inclusive) at the 36 meteorological stations in inland Australia and the numbers of 1st generation *H. punctigera* moths caught subsequently (weeks 8-20) in the trapping grid near Narrabri throughout 1992 – 2014, nor was there any correlation with those caught throughout the moth's active season, thus including 2nd to 3rd + generations (weeks 8-44). In addition, no significant correlations were obtained using rainfalls for individual months (April to July), nor the annual rainfalls (e.g. 1992 rainfall paired with the 1992-93 moth catch, and so on).

H. armigera

In most years, the 1st, 2nd and 3rd + generations of moths were reasonably discernible within the timings identified above. However, the 3rd and later generations were often difficult to separate, and there was large variability between years in the abundance of moths in each generation. In a minority of years, the 2nd and 3rd + generations were discrete, but occurred slightly earlier or later than expected (e.g. in 2005-06, 3rd generation earlier than weeks 31-44; in 2010-11, 2nd generation later than weeks 21-30).

The average numbers of *H. armigera* moths caught in the pheromone traps set at the 15 separate sites varied markedly within each generation. However, as explained above, some of these traps were set for more years than others. Including all such data in an analysis of variability between sites could create temporal bias. When we selected only those traps which were set for all, or very nearly all, years of the study (at least 20 of the 23 years; N = 8), the numbers of 1st generation moths varied significantly between the trap sites, but no significant differences in catch could be detected between sites for the 2nd or 3rd + generation moths (using total catch for each generation at each site in each season as the primary data). Amongst the 8 traps which were set for at least 20 years, most 1st generation moths were caught at Auscott (East) and least were caught at ACRI (Leitch). There was, however, no correlation between 1st generation moth catch at these 8 sites and the overall proportions of local habitat nearby that were either fallow, wheat or verge (proportions based on aggregated data for field use near each site across all years of study).

When the data were examined in finer detail (taking the catches of each moth generation and habitat details for each of the 15 traps separately in each year as the primary data), no effect of year could be demonstrated for either 1st or 2nd generation moth catch, but the numbers of 3rd + generation moths decreased through time. There was no significant effect of wheat or verge on the catch of 1st generation moths when they were present in at least one of the two fields adjacent to a trap. Cotton was negatively associated with the catch of 2nd and 3rd + generation moths. Verge was positively associated with the 3rd + generation catch. No influence of nearby fallow on the catch of *H. armigera* was observed throughout the study. Very large numbers of 1st generation moths were occasionally caught in the few traps in spring where chickpea was nearby (up to 2,340 in total in one trap in 2010, mean \pm S.E. = 470.8 ± 180.1 ; this compares with 210.6 ± 16.1 for traps near wheat). In contrast, the average numbers of 2nd and 3rd generation moths that were caught in the few traps in summer where sorghum and soybean were nearby were reasonably similar to those caught near cotton at the same time.

When the data for moth catches at each of the 15 sites were separated in time, into the three cotton production eras, i.e. 1) Pre-Ingard[®] (1992-1996), 2) Ingard[®] (1996-2005) and 3) Bollgard II[®] (2005-2015), negative relationships between moth numbers and the presence of cotton nearby were demonstrated for both 2nd and 3rd + generations during the Bollgard II[®] era, but not for either generation during the Ingard[®] era, and for only the 3rd + generation during the Pre-Ingard[®] era.

The numbers of moths (all generations) caught in the traps varied between years (Fig. 6). There was, however, no discernible trend through time in the catches of 1st generation moths (e.g. no indication of a decline in numbers with the advent of Bt cotton). The catches of 2nd generation moths were more erratic, but there was also no temporal trend in the abundance of these moths throughout the study. In contrast, there was an obvious peak in the catches of 3rd + generation moths between 1996 and 2006, i.e. during the Ingard[®] cotton era.

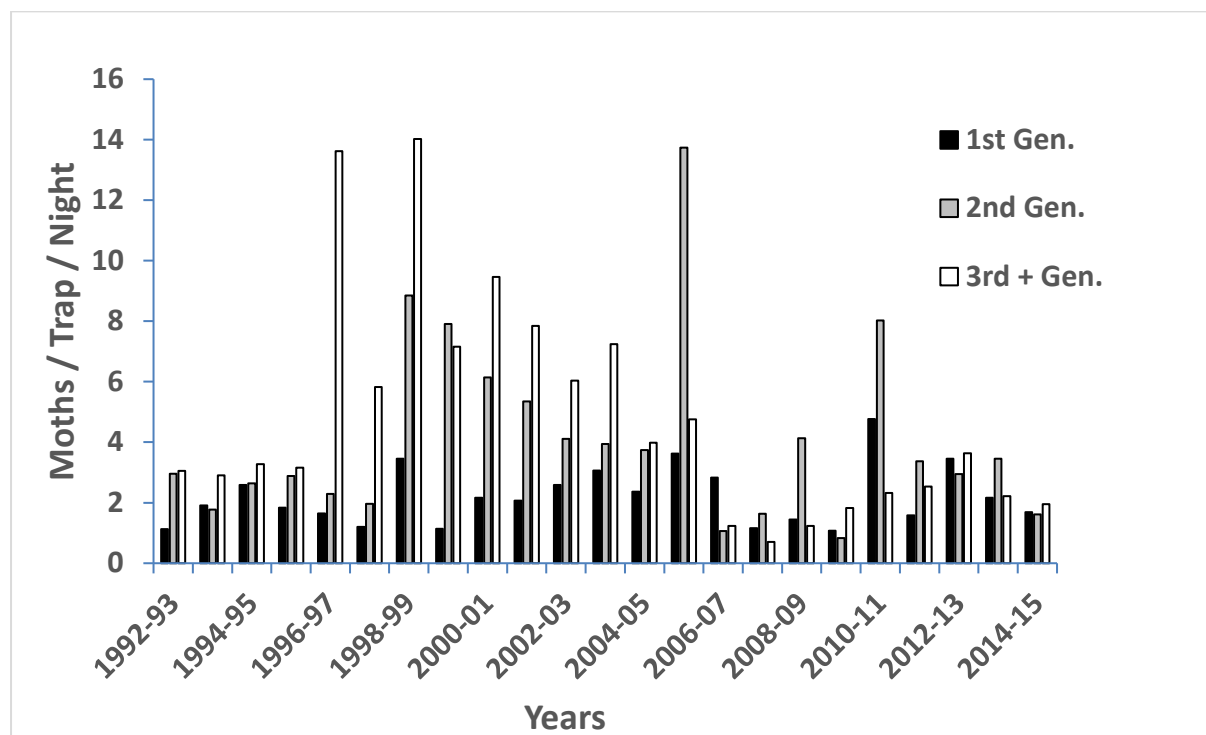


Fig. 6. Pheromone trap catches of male *H. armigera* moths near ACRI throughout 1992 – 2015. Data are separated into moths in the 1st generation (caught during weeks 8-20), 2nd generation (weeks 21-30) and 3rd + generations (weeks 31-44), with weeks taken from 1 July.

Evidence for temporal trends in moth catches was also sought where local habitat was the same for all traps. We used data associated with fallow fields in spring and cotton fields in summer, because they were the most common land usages at these times. There were 36 cases throughout the 23 years of study when the two fields near to individual traps were both fallow during spring, and 24 cases where both fields were used for cotton during summer. No temporal change was found in the abundance of 1st generation moths in the traps near fallow fields in spring, nor was there for the 2nd generation moths near cotton in summer. However, there was significant pattern amongst the catches

of 3rd + generation moths near cotton in summer (Fig. 7). There was no statistical difference between the catches during the Pre-Ingard[®] and Ingard[®] eras, but both of these were higher than the catch during the Bollgard II[®] era.

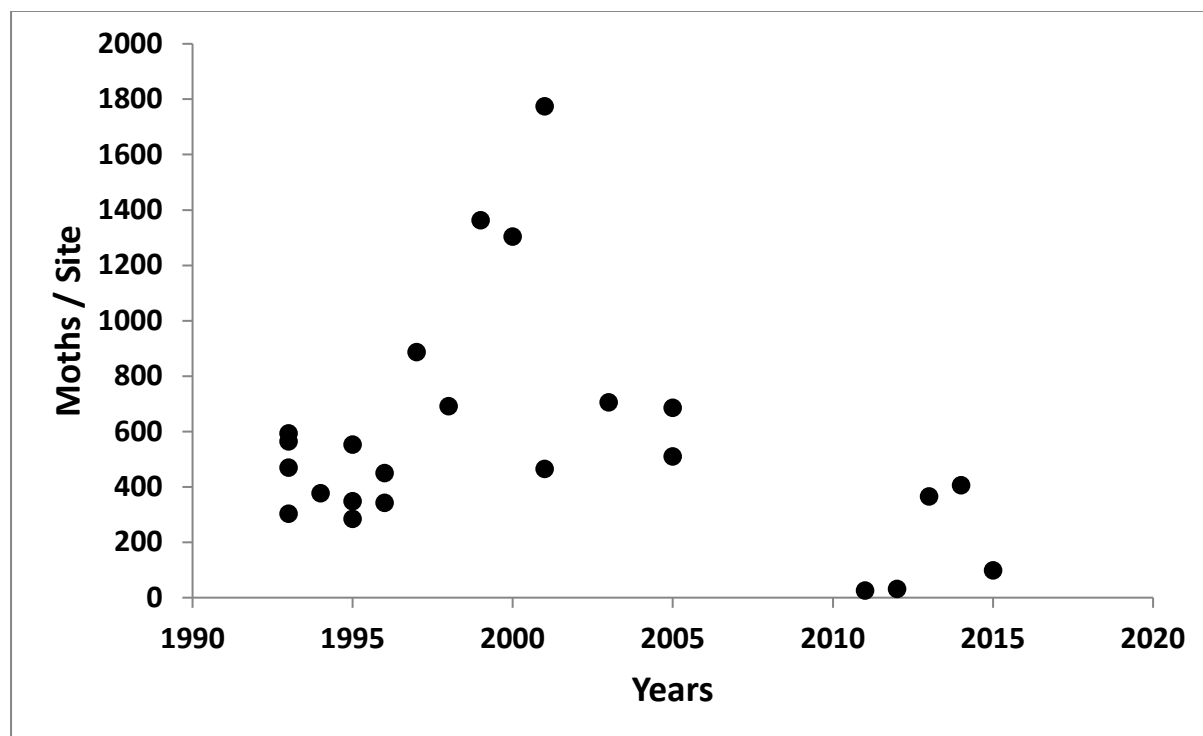


Fig. 7. Total numbers of 3rd + generation *H. armigera* moths trapped at individual sites near ACRI between 1993 and 2015, where cotton was being grown in the two nearby fields.

In addition, the average numbers of 1st and 2nd generation moths caught in the traps were positively correlated, but such was not the case between 2nd and 3rd + generation moths, nor between 1st and 3rd + generation moths (where data for all years and traps were included, but data for years were treated separately, thus N = 23). In contrast, there was no correlation between the average numbers of 1st and 2nd generation moths caught in the traps, 2nd and 3rd + generation moths, or 1st and 3rd + generation moths (where data for all years and traps were again included, but data for sites were treated separately, thus N = 15). Thus, as for *H. punctigera*, analysing the data in a temporal context (across years, N = 23) yielded a slightly different result than analysing it in a spatial context (across sites, N = 15). There was no correlation between the catch of 3rd + generation moths in one year and the number of 1st generation moths in the next.

There were no significant associations between local rainfalls and average trap catches of 1st and 2nd generation moths each year, where rainfalls were calculated (as relevant for the particular moth generations) for the various preceding & prevailing seasons as indicated above for *H. punctigera*. However, a significant, but weak, positive association was found between summer rainfall and the catch of 3rd + generation moths.

No significant associations were detected between temperatures and any generation of moths, using the same seasonal groupings as mentioned above. As indicated for *H. punctigera*, we stress that it is not to say that trap catches and weather at finer temporal scales (e.g. weekly) were not related.

Peak collections of individual generations were frequently too vague to enable analysis of the timings of generations in association with prevailing weather. It is worth noting however that in some seasons an apparent earliness was associated with warmer than average summer temperatures (e.g. 3rd generation in 2005-06) and an apparent lateness with colder than average winter temperatures (e.g. 1st and 2nd generations in 2010-11).

The areas (ha) sown to chickpea and canola (spring crops) and cotton and sorghum (summer crops) in the Narrabri, Gunnedah, Liverpool Plains and Walgett Statistical Local Areas (SLAs) within northern N.S.W. (which surround the trapping grid near ACRI) varied markedly throughout 1992 to 2014. Cotton hectares peaked between 1999 and 2002 and again in 2011 to 2013. Sorghum hectares were greater than cotton during the intervening years (2003 to 2010), when water for irrigation was less assured for farmers. Chickpea and canola hectares steadily increased throughout the study

period. Cropping patterns were quite different between SLAs. e.g. there was a greater predominance of cotton in the Narrabri SLA and sorghum in the Gunnedah SLA, and there was likewise a differential in the use of chickpea and canola as spring crops in the two regions.

There were no significant associations between the catch of 1st generation moths and the hectares of chickpea and canola grown in the Narrabri SLA, which immediately surrounded the ACRI trapping grid. However, there were positive associations detected between the numbers of 2nd generation moths and the hectares of cotton and sorghum, and a negative association between the numbers of 3rd + generation moths and hectares of chickpea. At a broader scale (the combined Narrabri and adjacent Gunnedah, Liverpool Plains and Walgett SLAs), there was no association between the catch of 1st generation moths and the hectares used for chickpea crops (canola could not be tested for here, because data were unavailable for the Walgett SLA). There were, however, positive associations between the catch of 2nd generation moths and hectares of sorghum, and between the catch of 3rd + generation moths and hectares of cotton.

Cotton Australia (C.A.) maintains an alternative database for cotton production, in particular for the Namoi Valley. This region (which combines two sub-regions, the Upper and Lower Namoi Valleys) is slightly different from the four SLAs (in total) listed above. In the C.A. database (which contained data for one more year, 2014-15), the area (ha) sown to cotton in the Namoi Valley peaked in 1998-99 and 2011-12, with a trough in 2008 which corresponded with the end of the drought in the region (Fig. 8). The relationship (+ve) between the numbers of 3rd + generation moths and the hectares of cotton grown was significant during the Pre-Ingard[®] and Ingard[®] eras (i.e. up to 2005), but not during the Bollgard II[®] era (post 2005).

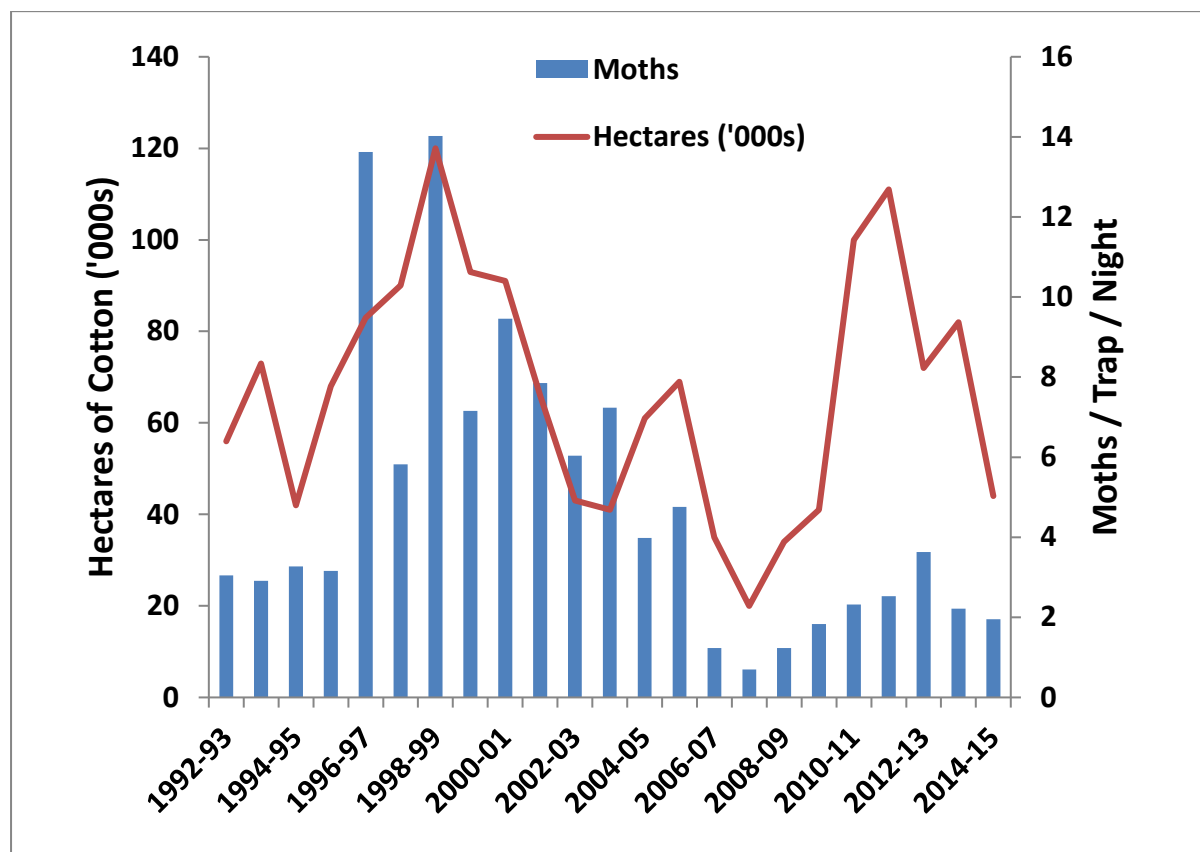


Fig. 8. Numbers of 3rd + generation *H. armigera* moths trapped each year near ACRI and hectares of cotton sown in the Namoi Valley from 1992 to 2015. Cotton data provided by Cotton Australia.

Brief Overview

This long-term trapping of *H. punctigera* and *H. armigera* has especially demonstrated temporal declines in the abundance of both species in a major cropping region (Lower Namoi), which are most likely explained by different factors. In the case of *H. punctigera*, the long-term decline in moth abundance is attributable to a reduction in the numbers of 1st generation moths being recruited into the cropping region from elsewhere (traditionally viewed as inland Australia). In the case of *H. armigera*,

there is good evidence that the advent of Bollgard II[®] cotton (but not Ingard[®]) has suppressed abundance at a broad-scale. The underlying mechanism(s) for this are not clear, but could be related to the greater toxicity of the two toxin product, no area cap on its use compared with the 30% applied to Ingard[®], an associated increase in natural enemies due to the ongoing reduction in insecticide use, or a combination of some or all of these. Both decreases in abundance are of strong relevance to the future pest management of *Helicoverpa* spp. and Bt resistance in particular.

The trapping grid was also run throughout 2015-16, but the data from this season were not available for the write up of the papers indicated above. Nevertheless this 2015-16 work simply reinforced the earlier findings ; the abundance of both moth species remained low.

We suggest there is little point in maintaining per se the trapping grid in it's current form into the future, unless a novel use for it is found. The grid has served its original purpose, and is time consuming to operate. However, the GPS coordinates are listed in the published papers. The traps could be easily re-established if needs be. e.g. if a check of shift in moth abundance seems sensible.

Demonstrate importance of regional landscape complexity in determining the mixing of *Helicoverpa* spp. populations from different plant host origin

A paper is in an advanced stage of preparation on this topic, and likely to be submitted to a journal in the next month or two :

Baker, G.H., Tann, C.R., Verwey, P. & Lisle, L. Do the plant host origins of *Helicoverpa* (Lepidoptera : Noctuidae) moth populations reflect the agricultural landscapes within which they are caught ?

Some key figures pertaining to this work are provided in the following pages. Other figures, tables and statistical analysis will appear in the paper when published.

The abundance of *Helicoverpa* eggs was reasonably similar across the three sub-regions within each of the Maranoa and the D. Downs during 2012-13. However, eggs were particularly common in general across the D. Downs in late January. During 2013-14, the abundance of eggs in the Maranoa was much higher in general than the previous year, but comparable between both years in the D. Downs, with again a peak during January. Egg lays were reasonably consistent between local sub-regions. During 2014-15, the abundance of eggs was different from previous years in the Maranoa, with most eggs recorded during February, but consistent in the D. Downs with again a peak in January. Egg densities were relatively low near Narrabri during 2014-15, with most found during January. Yet again, there was reasonable consistency in the magnitude of egg-lays across sub-regions within each of the three major regions surveyed.

Overall, 61.7 % of female moths (both species pooled) were gravid during 2012-13 and there was a mean of 2.2 spermatophores present / female moth (i.e. female moths had mated on average approximately twice). During 2013-14, 79.6% of females were gravid and there was a mean of 2.1 spermatophores / female moth, whilst in 2014-15, 64.9 % of females were gravid and there was a mean of 2.3 spermatophores / female moth. No consistent species, month of sampling or location (site, sub-region or region) differences were found; hence all data within individual seasons were combined here.

There were thus no marked spatial patterns evident in the reproduction of *Helicoverpa* within the three regions. There were, however, minor differences between regions in the timings of peak egg lays.

The stable isotope signatures for groups of moths caught concurrently in pheromone and light traps were very similar. Therefore, moths from these two different types of traps were pooled in the analyses that follow (but keeping the two species separate). (Note : given that pheromone traps catch only male moths, but light traps catch both sexes, this implies that the stable isotope signatures of the sexes were similar. This was indeed the case).

Early in the 2012-13 season (November and December), most *H. armigera* moths, in both the Maranoa and the D. Downs were of C₃ plant host origin (Fig. 9a). During January, most moths were of C₄ origin. This remained the case in the D. Downs in February, but there was a suggestion in the data that the proportion of C₃ origin moths had increased towards parity with C₄ origin moths in the Maranoa. The proportion of C₃ moths increased similarly in the D. Downs during March. Most *H.*

punctigera moths were of C₃ plant host origins throughout November to January in both regions, but in February there were similar proportions of C₃ and C₄ moths in the D. Downs (Fig. 9b).

During the 2013-14 season, patterns in C₃ : C₄ preponderance in *H. armigera* moths started much the same as in the 2012-13 season, with a bias towards C₃ moths in December and a switch to C₄ moths in January, in both the Maranoa and the D. Downs (Fig. 10a). This C₄ bias remained in February, with Maranoa moths reverting to a C₃ bias in March, but those in the D. Downs probably not doing so (at least to parity) until April (note the April collection of *H. armigera* in April was very small). Limited data for moths from Narrabri suggested a somewhat similar pattern of early season C₃ bias, mid-season C₄ bias, then a reversion to parity by end of season. *H. punctigera* moths were predominantly of C₃ origin throughout the 2013-14 season, but the proportion of C₄ moths was noticeably higher during January and February in the D. Downs compared with earlier (Fig. 10b).

Again in 2014-15, C₃ origin *H. armigera* moths dominated in December (Fig. 11a). During January, the switch to C₄ predominance was strongest in the D. Downs, compared with the Maranoa and near Narrabri, and from then on the proportions of C₃ : C₄ moths were generally close to parity in all three origins. *H. punctigera* moths were predominantly C₃ in origin during December, and in January in the Maranoa only (Fig. 11b). From then on most moths in all regions were C₃, but substantial proportions were nevertheless C₄ (approximately 20 – 50%).

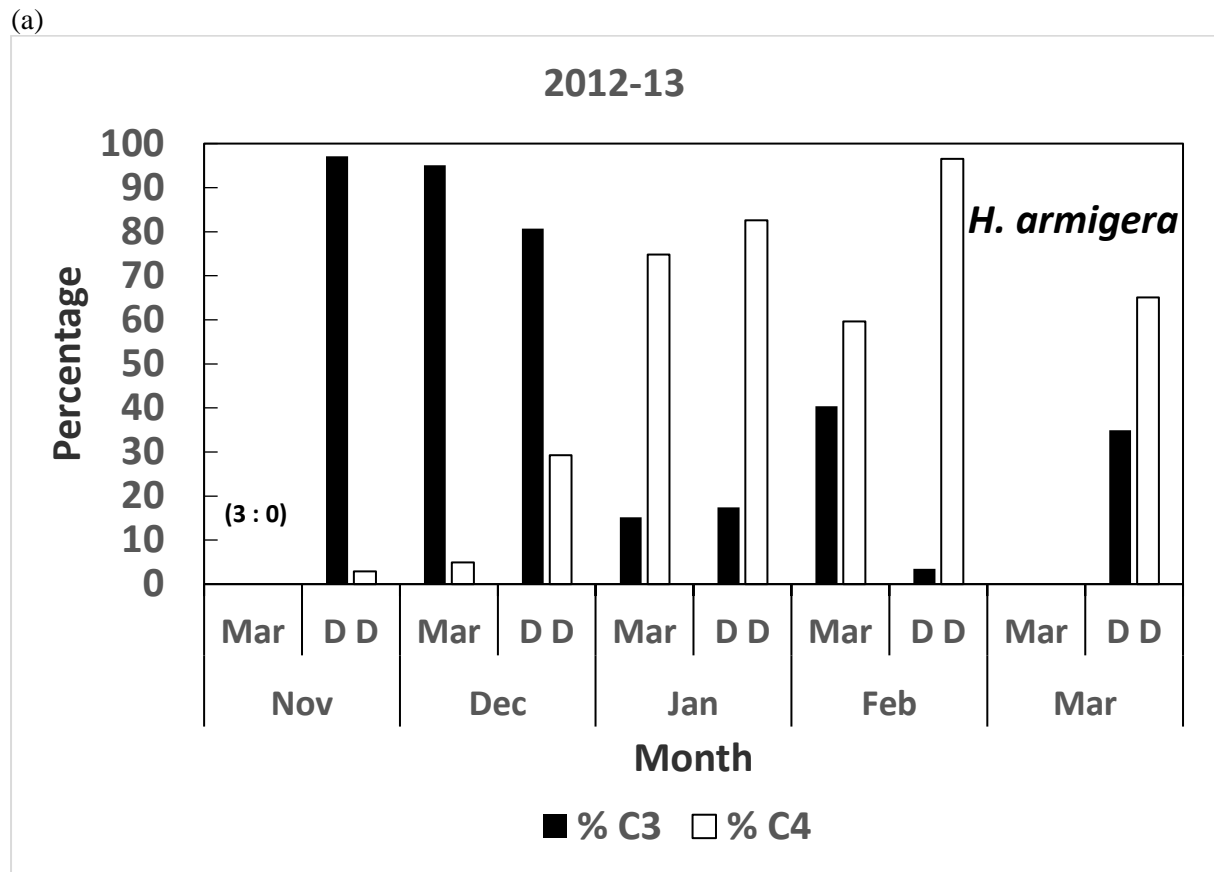


Fig. 9. (see legend next page under Fig. 9b)

(b)

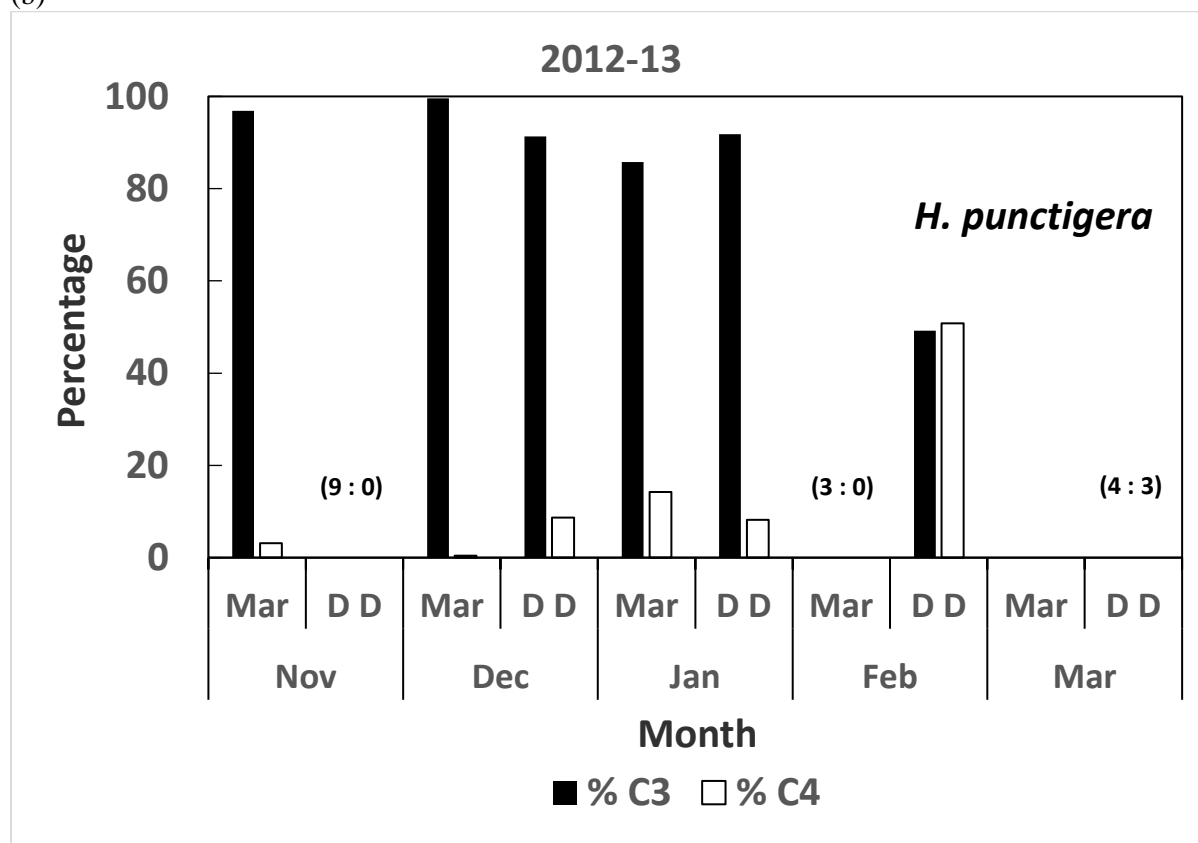


Fig. 9. Percentages of a) *H. armigera* and b) *H. punctigera* moths trapped in the Maranoa (Mar) and the Darling Downs (DD) during the 2012-13 season that were of either C₃ or C₄ plant host origins. Numbers in parentheses are absolute numbers of C₃ : C₄ moths (where too few moths were available for stable isotope analyses to justify percentage calculations).

(a)

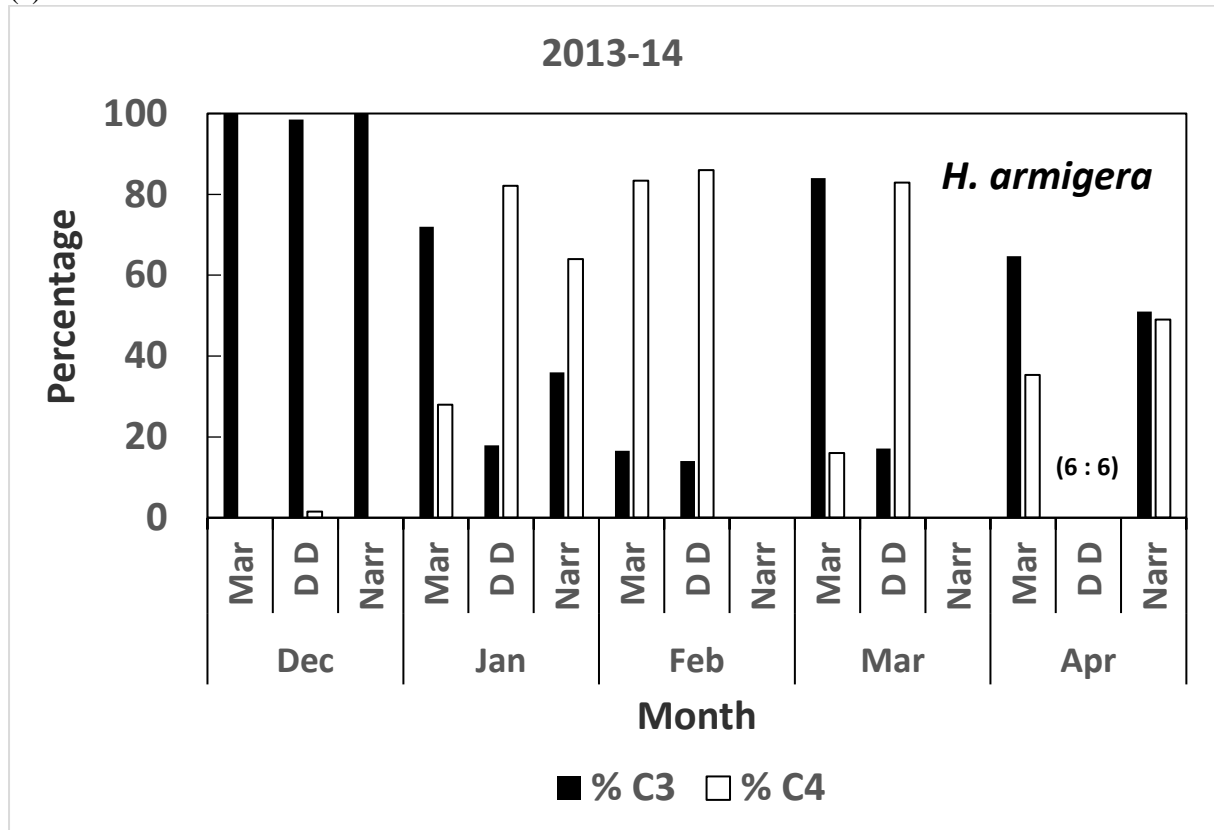


Fig. 10. (see legend next page under Fig. 10b)

(b)

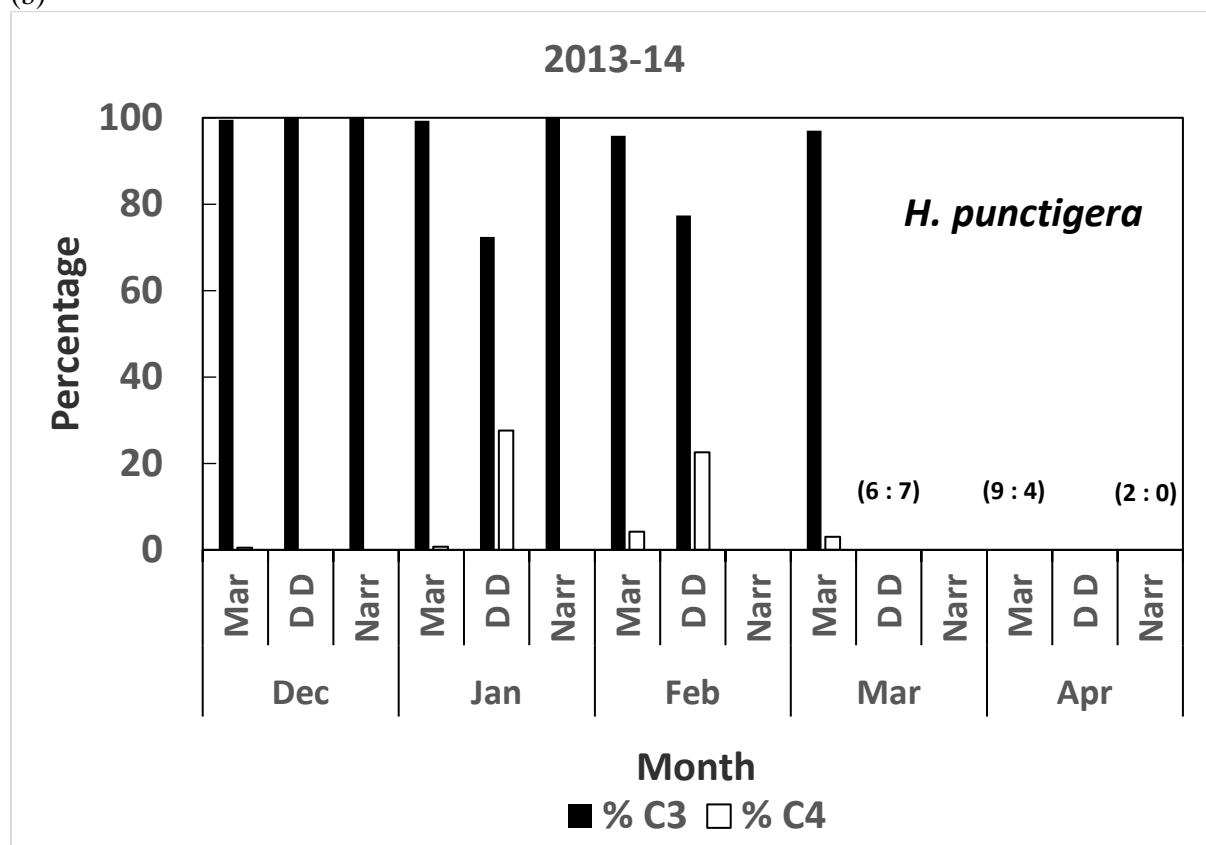


Fig. 10. Percentages of a) *H. armigera* and b) *H. punctigera* moths trapped in the Maranoa (Mar), Darling Downs (DD) and near Narrabri (Narr) during the 2013-14 season that were of either C₃ or C₄ plant host origins. Numbers in parentheses are absolute numbers of C₃ : C₄ moths (where too few moths were available for stable isotope analyses to justify percentage calculations).

(a)

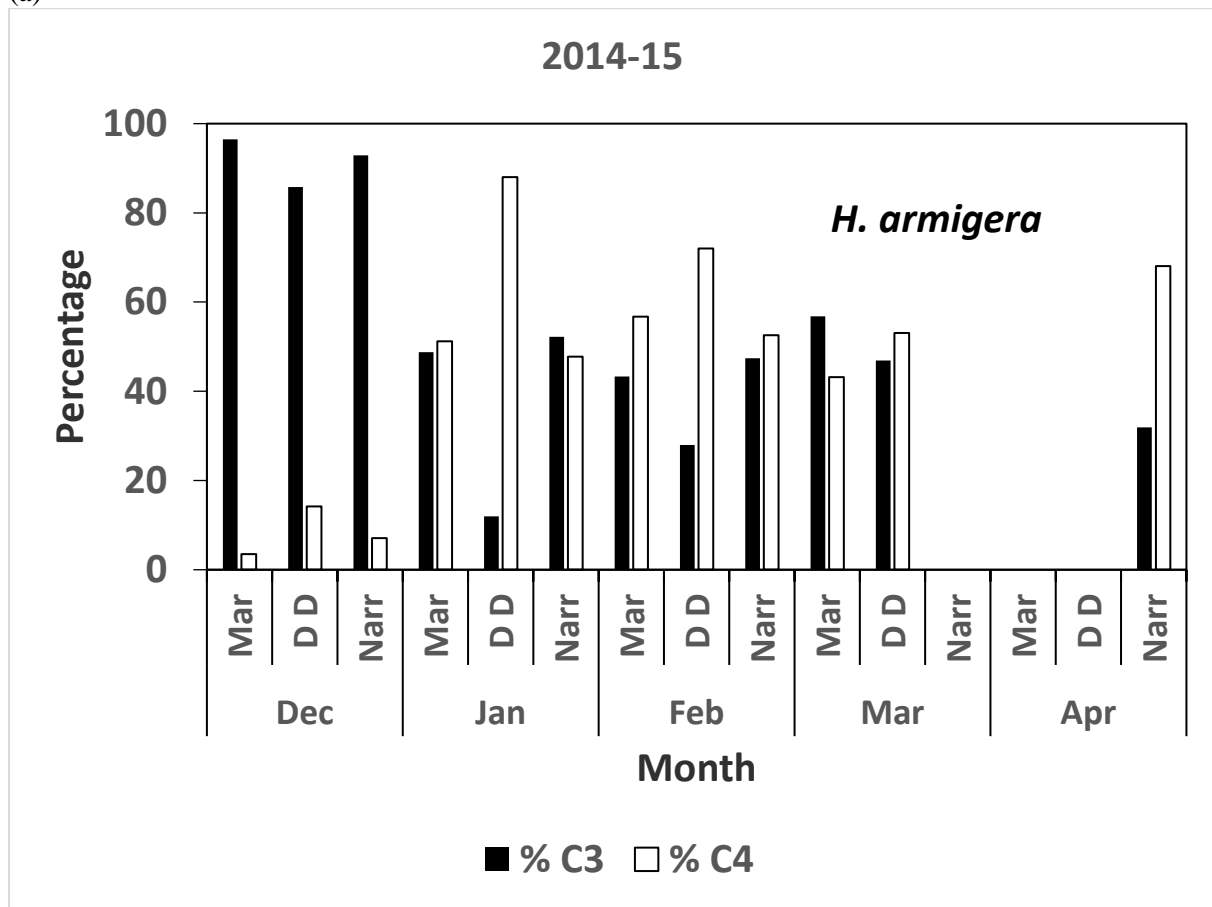


Fig. 11. (see legend next page under Fig. 11b)

(b)

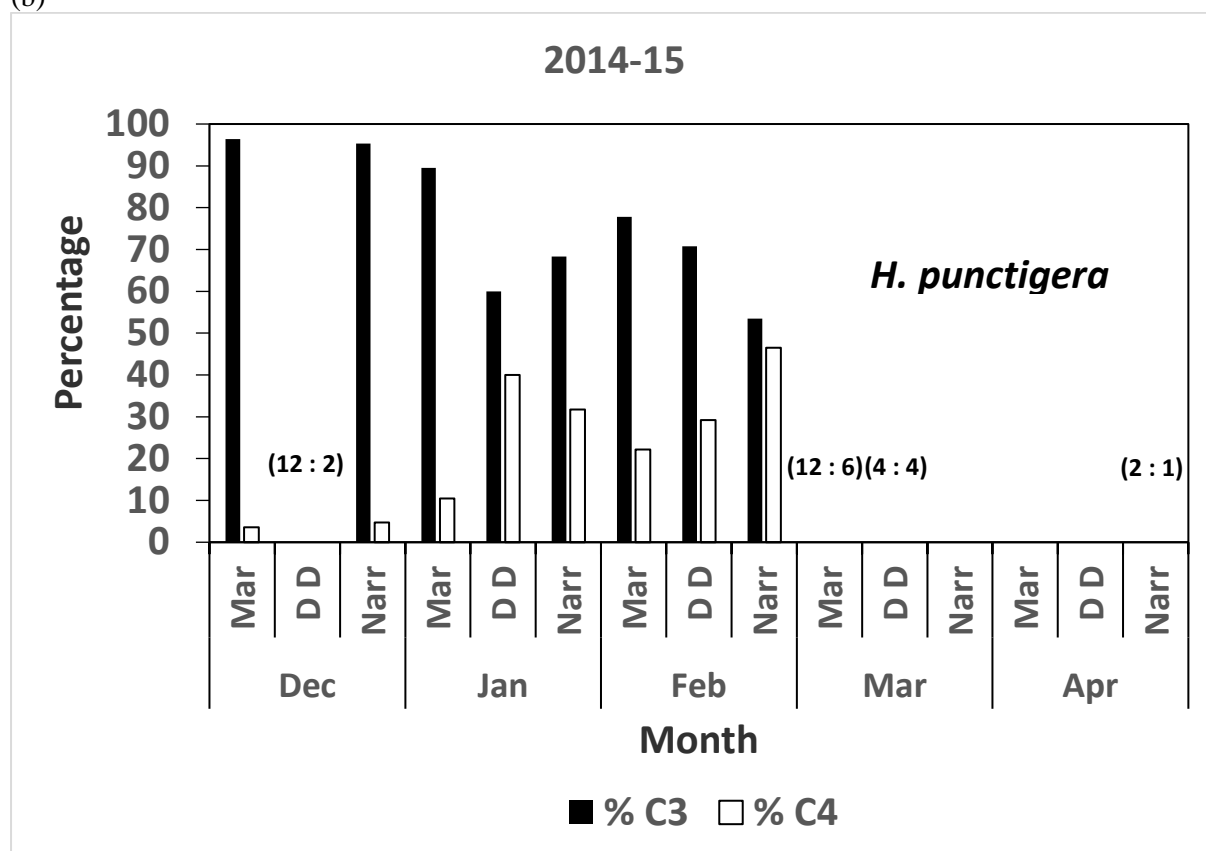


Fig. 11. Percentages of a) *H. armigera* and b) *H. punctigera* moths trapped in the Maranoa (Mar), Darling Downs (DD) and near Narrabri (Narr) during the 2014-15 season that were of either C₃ or C₄ plant host origins. Numbers in parentheses are absolute numbers of C₃ : C₄ moths (where too few moths were available for stable isotope analyses to justify percentage calculations).

The numbers of *H. punctigera* moths trapped and analysed for stable isotope signatures were occasionally too few at individual sites in the Maranoa and D. Downs (the latter especially), within individual seasons, to calculate meaningful proportions of C₃ : C₄ individuals at site level, and thereby test for significant variability between species, sites, sub-regions, regions and years within a single analysis. Data were therefore bulked, for initial analyses, at sub-region level (Figs 12 -14). At that level, there was a marked inter-specific difference in the proportion of moths that were of C₃ plant host origin. There were also significant effects of region and year on % C₃. Much higher % C₃ was found in the Maranoa than the D. Downs, and % C₃ was lower in 2012-13, in particular in *H. armigera*. There was however no effect of sub-region on % C₃, in either the Maranoa or the D. Downs. Alternatively, if the data were kept at site level, but bulked across the years (to provide larger numbers of moths at site level), there was no significant effect of site, nor sub-region, on % C₃ within either species in both regions, but the strong inter-specific and regional differences were retained.

In addition, during 2014-15 when all three regions (Maranoa, D. Downs and Narrabri) could be compared (but with data pooled at the sub-region level; catches, in particular of *H. punctigera*, were too small at some individual sites to enable analysis at site level), there was a significant difference in the proportions of moths that had C₃ origins between the regions, as well as a significant difference between species. C₃ proportion did not vary between the Maranoa and Narrabri, but both these regions had higher C₃ proportions than were found in the D. Downs; *H. punctigera* had higher C₃ than *H. armigera*. There was no effect of sub-region.

However, catches of *H. armigera* (i.e. excluding *H. punctigera*) were large enough to meaningfully analyse fully at site level across the whole study. In the case of the Maranoa and D. Downs, across all three years, there were significant differences in % C₃ of moths at regional and year

level, but not at sub-region or site level. % C₃ was higher in the Maranoa compared with the D. Downs, in particular in 2013-14. In the case of 2014-15 only, for all three regions, the only significant difference detected in % C₃ was between regions. % C₃ was lower in the D. Downs than in both the Maranoa and near Narrabri.

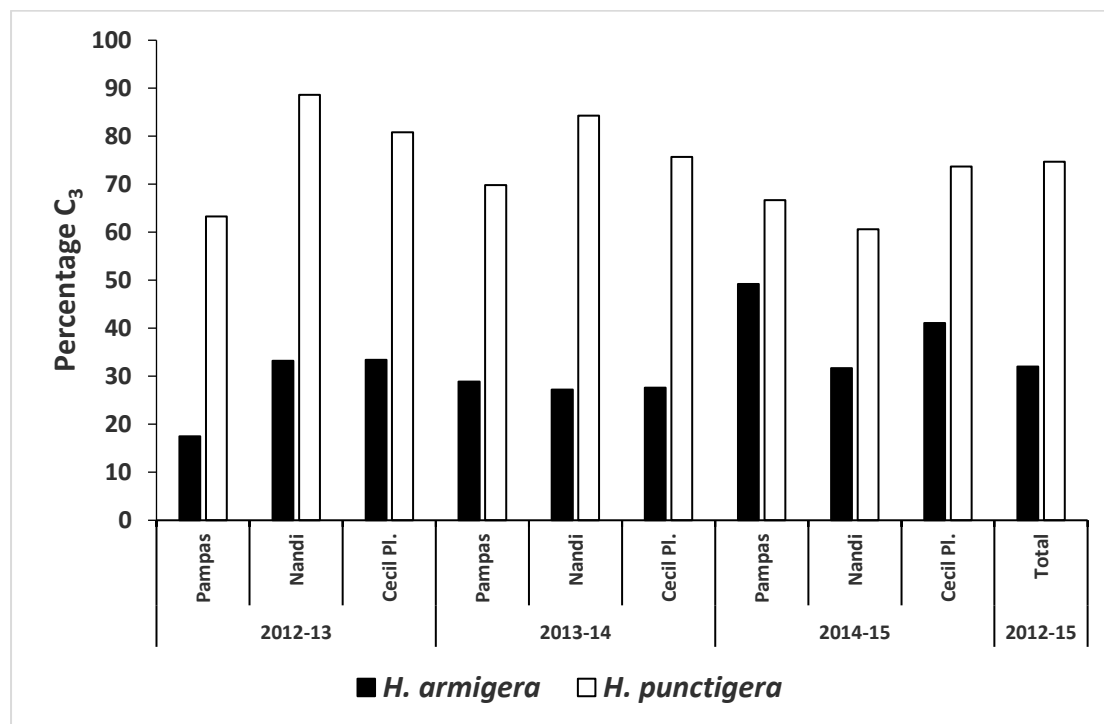


Fig. 12. Percentage of moths trapped in the three sub-regions of the Darling Downs each season that were C₃. Overall totals of 3,570 *H. armigera* and 1,102 *H. punctigera* were analysed for $\delta^{13}\text{C}$. Numbers per season in the sub-regions varied between 15 (*H. punctigera*, Pampas, 2014-15) to 658 (*H. armigera*, Cecil Plains, 2012-13).

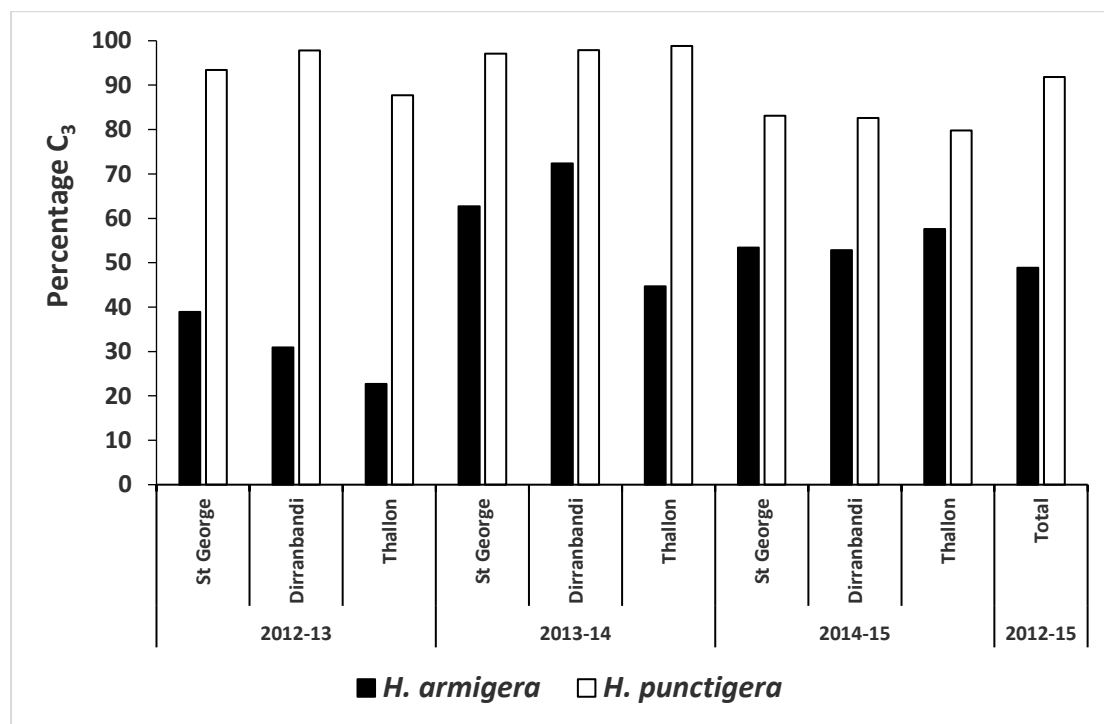


Fig. 13. Percentage of moths trapped in the three sub-regions of the Maranoa each season that were C₃. Overall totals of 2,821 *H. armigera* and 2,866 *H. punctigera* were analysed for $\delta^{13}\text{C}$. Numbers per season in the sub-regions varied between 91 (*H. punctigera*, Dirranbandi, 2012-13) to 694 (*H. punctigera*, St George, 2013-14).

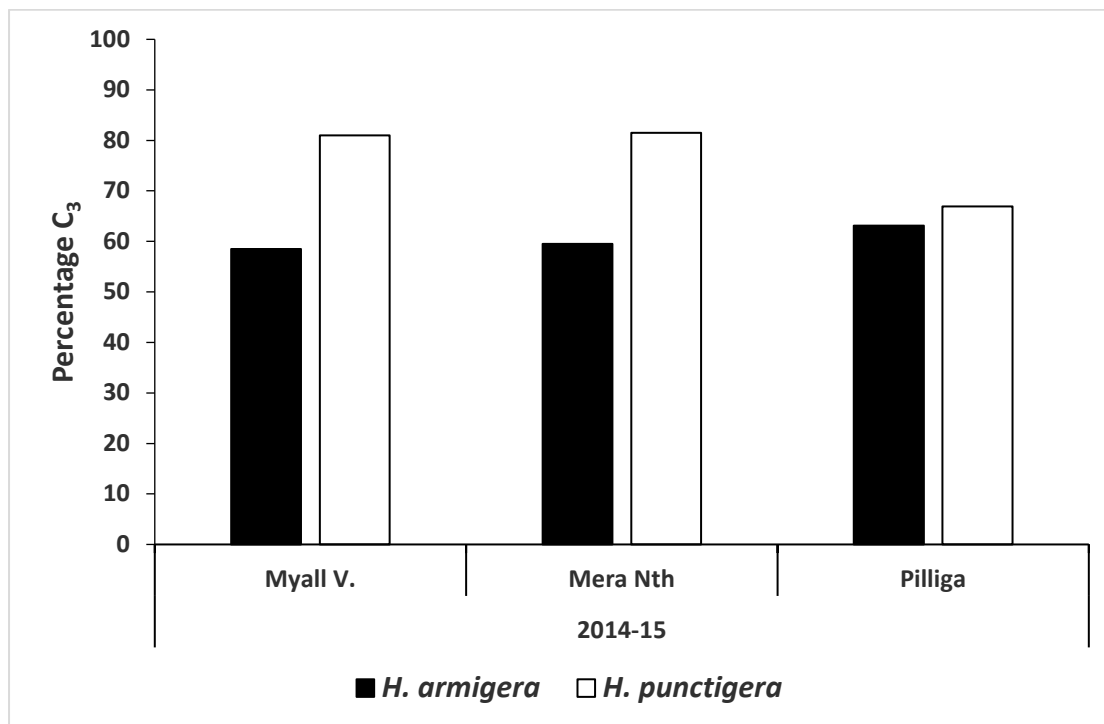


Fig. 14. Percentage of moths trapped in the three sub-regions near Narrabri in the 2014-15 season that were C₃. Overall totals of 1,095 *H. armigera* and 505 *H. punctigera* were analysed for $\delta^{13}\text{C}$. Numbers in the sub-regions varied between 121 (*H. punctigera*, Myall Vale) to 419 (*H. armigera*, Myall Vale).

The land-use data recorded within 2km radii of the individual trap sites (Table 1 provides aggregated data for 2012-13 as an example) were in broad agreement with the major cropping differences that were evident between the D. Downs and Maranoa in Fig. 4 (i.e. regional statistics). Sorghum was common near the traps in the D. Downs, but was not recorded in the Maranoa. Cotton was recorded more often in the D. Downs than sorghum, which may seem at first glance to be at odds with the data in Fig. 4 – but we need to be mindful that by always placing our traps next to a cotton crop, such action automatically inflated the relative abundance of cotton in our data.

There was much variability within the land-use data recorded within the 2km radii of the traps. Using cotton and sorghum grown in the D. Downs and Maranoa as examples (given they were the dominant summer crops), significant variation was recorded in the land-use proportions of sorghum between regions and years (obviously higher in the Maranoa, but also low in 2014-15), but not between sub-regions and sites. For cotton, significant variation was only demonstrated between regions (higher in the Maranoa). Using the data for all three regions (i.e. D. Downs, Maranoa and Narrabri) in 2014-15, there was significant variation between regions for sorghum (but not between sub-regions and sites), and no significant variations at all were demonstrable for cotton.

No significant correlations were detected between the proportions of *H. armigera* that were of C₄ origin in the D. Downs collections (calculated at site level, within individual years, data transformed to arcsine) and the corresponding proportions of sorghum, maize or sorghum + maize in the local vicinity (i.e. within 2 km radius). In addition, no correlations of this nature were detected at sub-region scale either.

“Other” categories of land-use were often scored, especially in the Maranoa. These were made up of various non-agricultural habitats such as flood-ways, water storages, woodlands, roads, buildings etc which occasionally made up large proportions of the landscape.

Whilst $\delta^{13}\text{C}$ signatures enabled the separation of C₃ and C₄ plant host origins, $\delta^{15}\text{N}$ signatures did not readily assist this separation further (e.g. Figs 15 & 16 provide bi-plots of $\delta^{13}\text{C}$ x $\delta^{15}\text{N}$ for *H. armigera* and *H. punctigera* moths trapped in January 2013 in the Maranoa as examples). However, Baker & Tann (2013) recorded the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for individual *H. armigera*, collected (as pupae) from beneath particular crops (cotton, pigeon pea, sorghum and maize), then reared to moths without further feeding. These data represent stable isotope measures of moths from known host plant sources. The means of these values are plotted in Fig. 15. Most notably, the mean coordinates

for *H. armigera* that fed on sorghum match very well with the C₄ moths of unknown origin collected in the Maranoa. It is also interesting that such coordinates are quite central to the distribution of C₄ *H. punctigera* in Fig. 16.

Table 1. Mean proportions (%) of various (summer) land-uses in the Darling Downs and Maranoa during 2012-13 (N = 9 sites for each region). Data recorded for land-uses within 2km radii of moth traps.

Land-Use	Darling Downs	Maranoa
Sorghum	19.3	0
Cotton	30.2	39.5
Legumes	2.2	0
Maize	4.6	0
Pigeon Pea	0.8	1.4
Cereal Stubble	12.2	0
Fallow	11.2	0
Grazing	0.8	10.1
Other	18.7	49.0

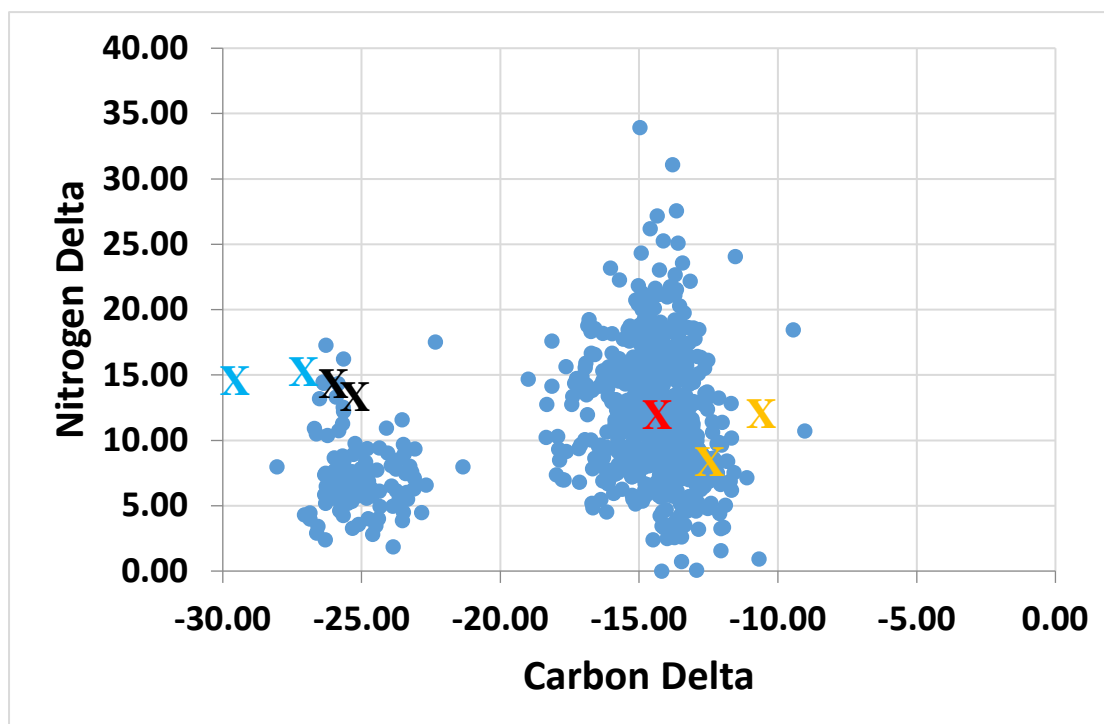


Fig. 15. Bi-plots of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for all individual *H. armigera* moths caught in the Maranoa during January 2013 (●). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *H. armigera* moths reared from pupae collected beneath cotton (blue X), pigeon pea (black), maize (orange) and sorghum (red) crops (thus of known plant host origin) are also marked.

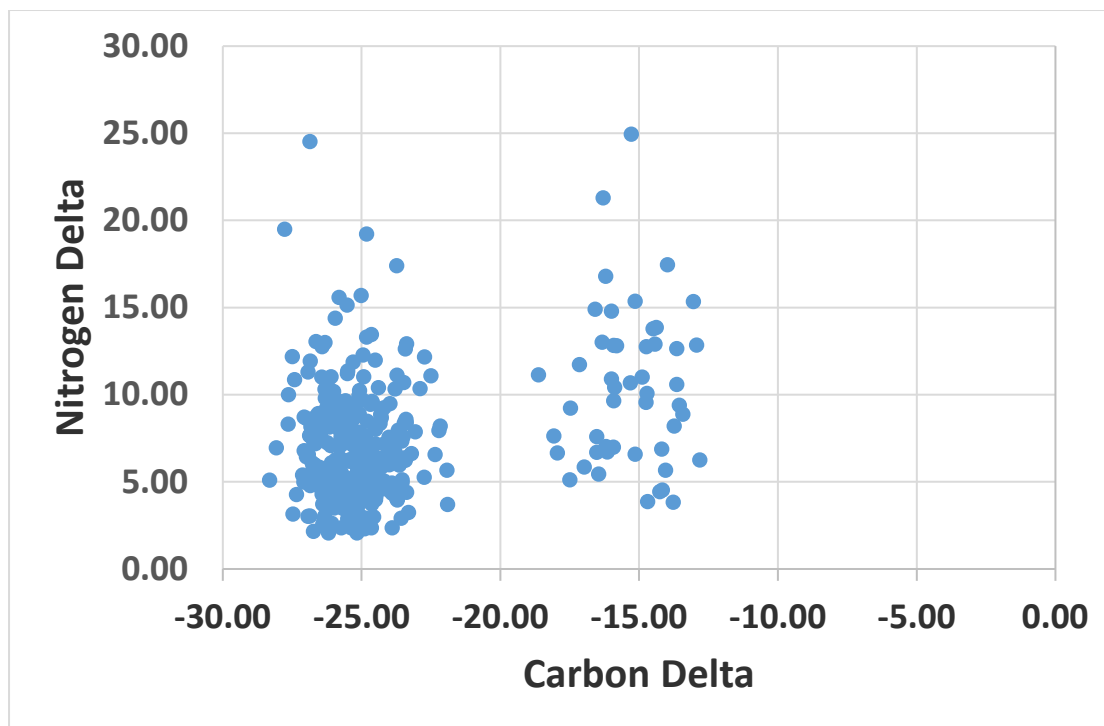


Fig. 16. Bi-plots of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for all individual *H. punctigera* moths caught in the Maranoa during January 2013 (●).

Brief overview

An understanding of the relative contributions of the various plant host origins of *Helicoverpa* moths, be they Bt or conventional cotton crops, mandatory refuge crops or other (“unstructured”) refuges (e.g. crops such as sorghum, maize, soybean, chickpea and sunflowers, weeds and native plants) to the total populations of moths throughout cotton production regions, and the scales of movement / spatial mixing of such moths within and between such areas is fundamental to developing an effective Bt resistance management strategy. But, our knowledge on these fronts is very limited. In this part of the project, we used stable isotope signatures (especially $\delta^{13}\text{C}$) to help identify the larval feeding origins of moths, and hence to determine the degree to which moth communities reflect the cropping structures they were found within.

We worked within two primary cotton production regions, the Maranoa and Darling Downs, which have very different cropping structures, relatively simple in the former and more complex in the latter. Most notably, sorghum (a C_4 plant and favoured host of *H. armigera*) is a prominent crop in the D. Downs but rare in the Maranoa. We supplemented the work in the 3rd year by including additional data from the Namoi Valley, which is intermediate in cropping complexity. The results high-lighted the numerical importance of “unstructured” refuges in contributing to the populations of moths in all three regions. We also found no significant differentiation in moth population structure (based on feeding origins) at trap and sub-region scale. Such differentiation was only observed at regional scale. This could suggest that moth communities do not reflect the local landscape scale land-use they are found within. Perhaps larger scale movements and mixing of moths from different habitats override any differences generated locally in moth community structure. An understanding, e.g. modelling, of the development of Bt resistance needs to recognise such scales of interaction amongst moths.

The proportions of moths with C_4 origins that were caught in the D. Downs were higher compared with those in the Maranoa, as was to be expected, given the preponderance there of C_4 host plants such as sorghum. However, the proportions of C_4 moths collected in the Maranoa were nevertheless surprisingly high, given the lack of C_4 crops grown there. This begs the question, where did these moths come from? We provided some (admittedly limited) evidence that the C_4 *H. armigera* moths found in the Maranoa might have sorghum origins (which would presumably be far afield from the Maranoa). In addition, C_4 *H. punctigera* moths were often caught in all three regions, yet this species has not traditionally been thought to commonly use C_4 plant hosts. Further research needs to provide a better understanding of the plant host origins, both taxonomically and spatially, of *Helicoverpa* spp.

Construct a representative overview of current year, cotton planting and defoliation dates, for the Australian cotton industry.

One paper has already been published from this work in *Austral Entomology* :

Baker, G.H., Leven, T., May, A. & Tann, C.R. 2016. Planting window requirements for Bt cotton in Australia : do they limit the exposure of *Helicoverpa* spp. (Lepidoptera : Noctuidae) to Bt toxins ? *Aust. Entomol.* **55**, 32-42.

This paper, along with a supplementary file that accompanied it on-line, is attached to this report (Attachments 3 & 4). A condensed version of the results presented in the paper is provided here. Some selected figures are included. Again, the reader is directed to the paper per se for other relevant figures, tables, statistical analyses and extra discussion.

It is important to stress here that the above mentioned paper was greatly influenced by discussions held at various TIMS Bt Tech Panel meetings. The authorship of the paper, which includes staff from CSIRO, CRDC & Monsanto, is indicative of this.

Rainfall varied markedly between years throughout the observational period (2002-2013) providing a broad range of cropping conditions in each region, from very dry cropping seasons such as 2002-03 to very wet seasons such as 2010-11 at Emerald.

Annual variability in planting and harvest-related dates

Planting dates (start & end), accessed via the Monsanto database, varied little between years, but significant differences were nevertheless detected in the data, probably enabled by the large numbers of records available. In addition, there was significant annual variability in the timing of harvest within the Emerald region, with a particularly late harvest in 2010-11. Whilst the Monsanto database does not provide harvest times for the more southern regions, there were significant differences between years in other related data (e.g. crop residue destruction) indicative of end of season annual variability. Harvest dates obtained from the CSD Variety Trials also varied between years, e.g. for both the Darling Downs and the Lower Namoi, with the earliest harvests in 2003 and 2005 and the latest in 2011 and 2012.

Seasonal patterns in planting and harvest-related dates

In NSW and southern and central Qld, most planting occurred between weeks 13-19 (mid September – early November), 14-22 (late September – late November) and 12-19 (early September – early November) as exemplified by the Lower Namoi, Darling Downs and Emerald respectively (Figs 17-19). Harvest occurred throughout late March to end of June (weeks 38-52) in the Namoi, with a mean date of week 44 (late April). A similar pattern was apparent in the Darling Downs (weeks 40-56, mean week 45). Crop residue destruction was most common in weeks 44-50 (late April – early June) in the Lower Namoi, but more prolonged in the Darling Downs, where it was still quite common up to week 56 (late July). Likewise, pupae busting peaked during weeks 45-52 in the Lower Namoi and much later (weeks 55 – 59) in the Darling Downs. Although defoliation, harvest and crop destruction were mostly completed by week 47 (mid May) in Emerald, several crops remained unharvested well into winter (\geq week 49). Mean harvest time in Emerald was at week 42.3.

The average minimum soil temperatures (at 10 cm depth) recorded at Myall Vale (Lower Namoi) throughout 2002-13 surpassed 16°C consistently by week 14 (early October). The estimated average minimum soil temperatures at Dalby (Darling Downs) and Emerald surpassed 16°C in weeks 14 and 8 (late August) respectively. For Myall Vale and Dalby, these timings were just prior to cotton planting, but for Emerald soil minimum temperature reached 16°C several weeks before planting commenced on many farms.

Season lengths

Season lengths (number of weeks between sowing and harvest) were calculated for individual TUAs at Emerald using the Monsanto database. Across all years, the mean season length was 28.5 weeks, but the distribution of season lengths was highly skewed with a substantial number of seasons being > 30 weeks long. Season lengths also varied between years at Emerald, with the 2010-11

season being notably longer than others. There was a weak, but significant, correlation between planting start date (PS) and harvest end date (HE) at Emerald which suggested little influence of delayed sowing on season length (an increase of 0.23 weeks in season length per 1 week delay in sowing). The equivalent relationship between planting end date (PE) and HE was much more strongly correlated, as would be expected. It is not possible to calculate average season lengths from the Monsanto database for regions outside central Qld, but using the more limited CSD Variety Trials data mean season lengths throughout 2002-13 for the Darling Downs and Namoi were 27.7 and 29.7 respectively, i.e. quite similar to the season length in Emerald. There was no correlation between planting date (PD) and harvest date (HD) for CSD Trial sites in the Namoi, but there was weak correlation between the same dates in the Darling Downs. Whilst harvest date was slightly later when planting date was delayed, season length was actually marginally shorter.

However, these calculations ignore the collective aspects of season lengths amongst fields within a region such as Emerald. e.g. whilst one field might be planted earlier than another and harvested earlier, the season length overall across both fields will be greater than simply the average of the season lengths per se for the two fields. Likewise, if one field is planted earlier than another and harvested later, the overall season length will again be greater. In order to build in some measure of this collective aspect to season length, the mean planting start date less 1 standard error was subtracted from the mean harvest end date plus 1 standard error for each of the 11 seasons (2002-13) at Emerald. The resultant data were then related to each season's mean planting start and end dates, but this did not provide significant correlations. There was thus no significant relationship in either case between planting time and season length, but perhaps particular attention might be paid to the likely influence of planting end date on season length (and Bt exposure) if relaxation of planting windows were to be allowed in future and the range of planting dates became larger.

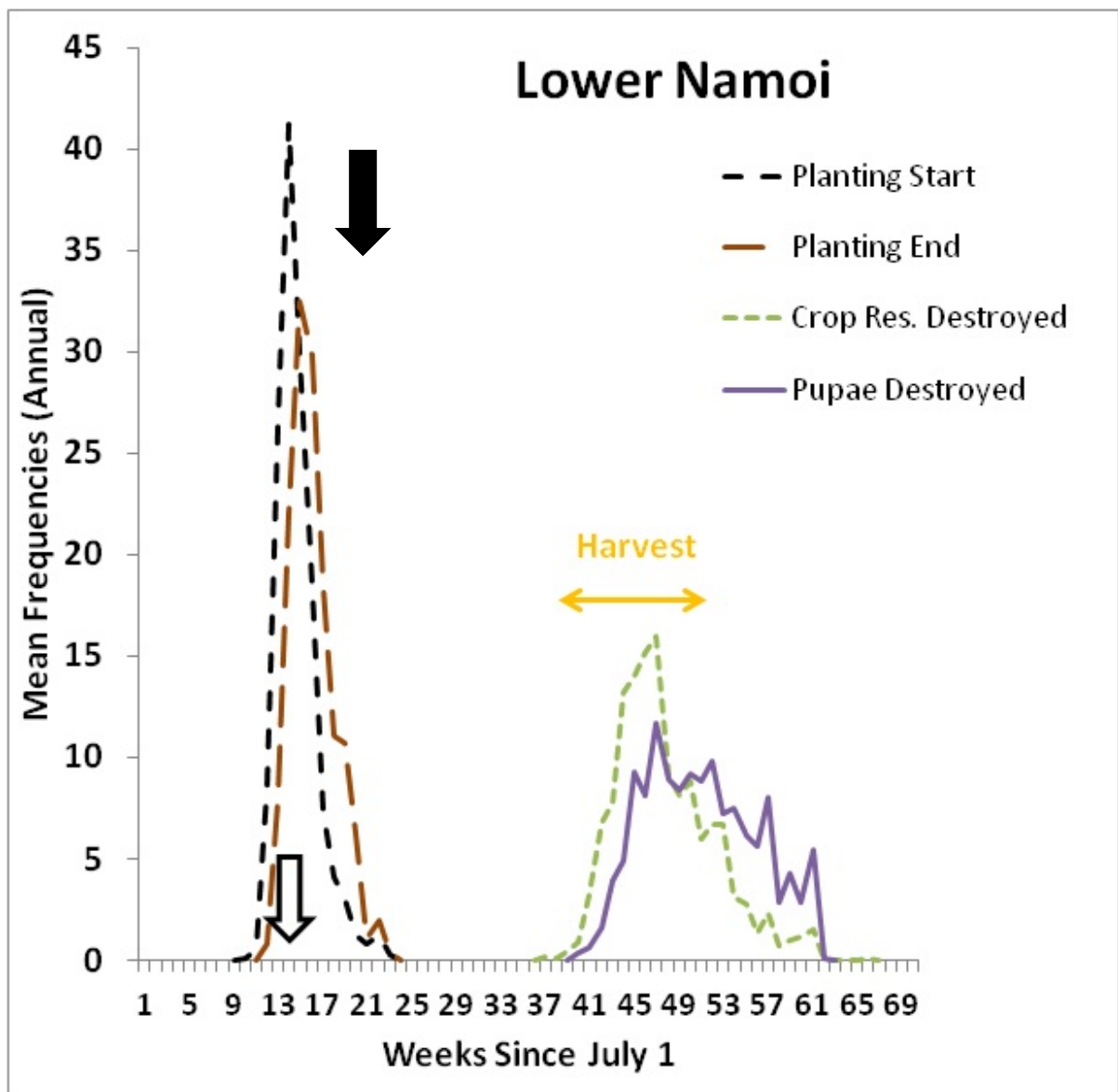


Fig. 17. Average annual frequencies of dates for start of planting, end of planting, crop residue destruction and *Helicoverpa* pupal destruction for Bt cotton crops growing in the Lower Namoi Valley, New South Wales over 11 cropping seasons (2002-2013). Extremities of harvest dates (CSD Variety Trial sourced data) are also included. Vertical black arrow indicates November 15 (planting windows end date). Vertical white arrow indicates when soil minimum temperature was estimated to consistently surpass 16 °C.

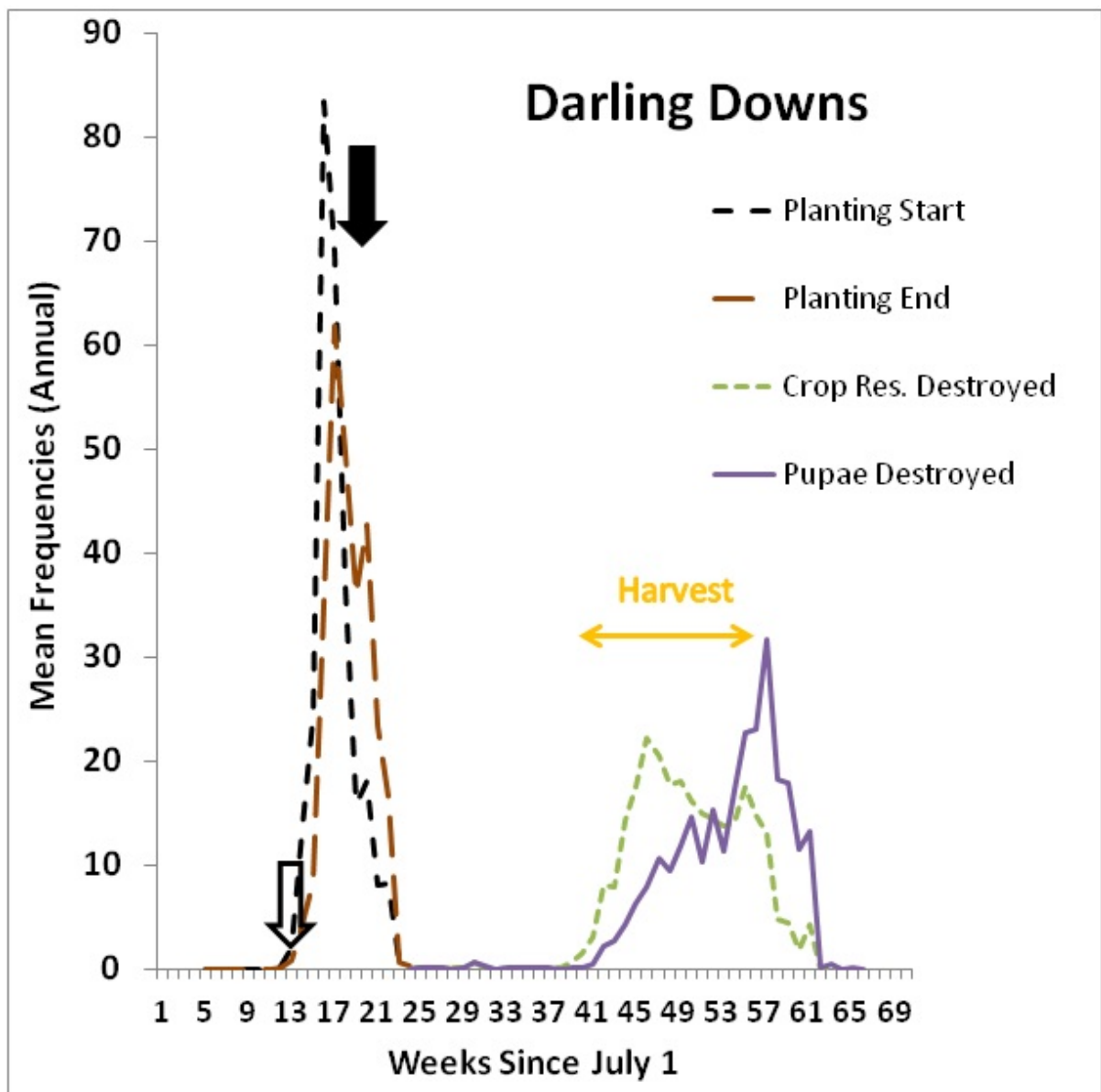


Fig. 18. Average annual frequencies of dates for start of planting, end of planting, crop residue destruction and *Helicoverpa* pupal destruction for Bt cotton crops growing in the Darling Downs, southern Queensland over 11 cropping seasons (2002-2013). Extremities of harvest dates (CSD Variety Trial sourced data) are also included. Vertical black arrow indicates November 15 (planting windows end date). Vertical white arrow indicates when soil minimum temperature was estimated to consistently surpass 16 °C.

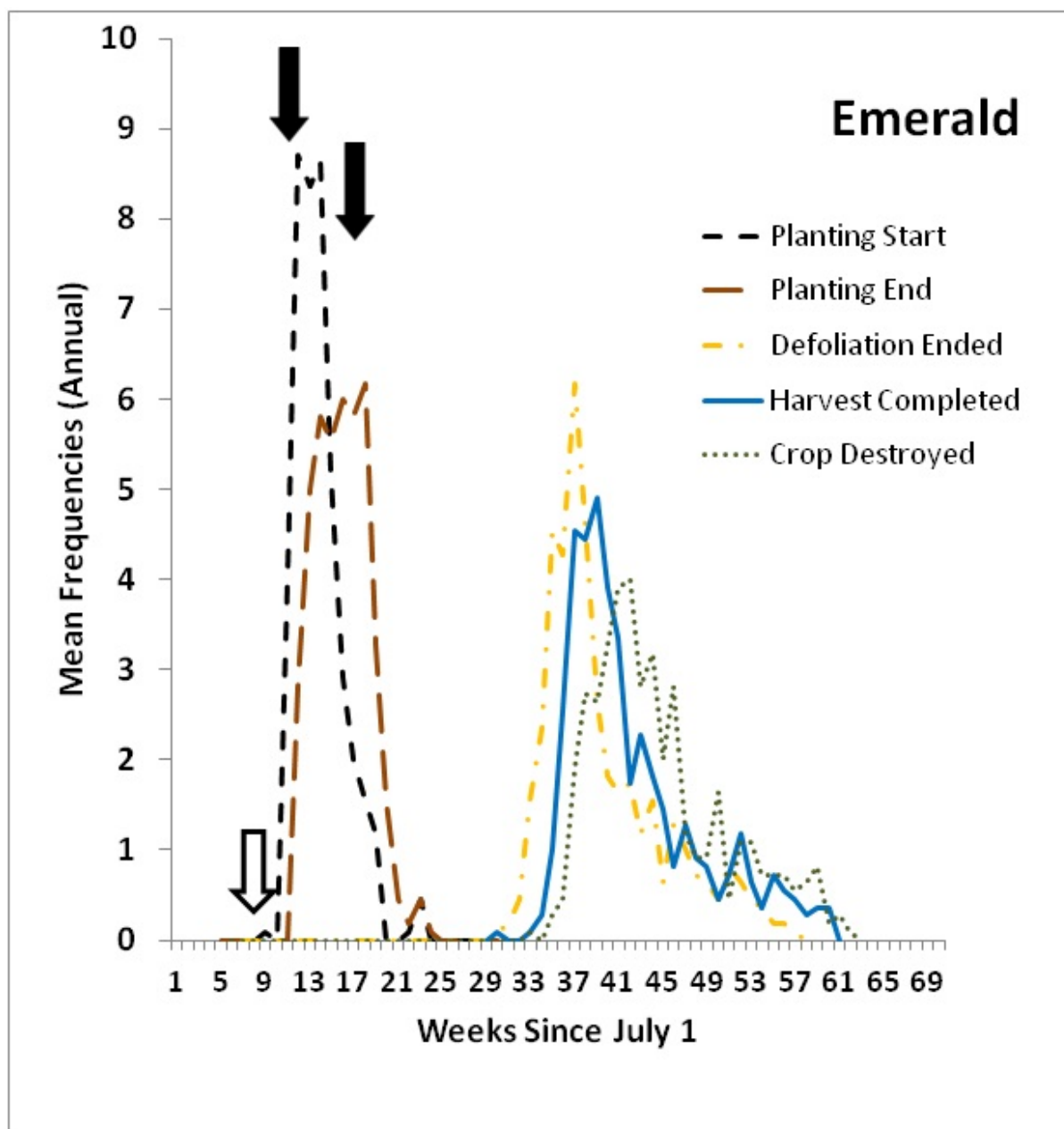


Fig. 19. Average annual frequencies of dates for start of planting, end of planting, completion of defoliation, completion of harvest and crop destruction by week for Bt cotton crops growing in the Emerald Irrigation Area, central Queensland over 11 cropping seasons (2002-2013). Vertical black arrows indicate September 15 and October 26 (planting windows start and end dates). Vertical white arrow indicates when soil minimum temperature was estimated to consistently surpass 16 °C.

Pheromone trapping of Helicoverpa moths in the Lower Namoi and St George

The average numbers of *Helicoverpa* moths trapped in the grid near Narrabri throughout 2002-13 were graphed, along with the Lower Namoi records for Bt cotton planting dates (from Monsanto database), as well as the range of harvest dates (from CSD Variety Trials booklets) (Fig. 20). The first generations of *H. armigera* and *H. punctigera* moths were clearly apparent and peaked in numbers in the trap catches close to the time of Bt cotton planting. Such moths could only have been exposed to Bt toxins if they fed as larvae on Bt cotton late in the previous season and overwintered as pupae. Subsequent peaks in trap catches were evident, but the number of these peaks (i.e. likely generations) was not particularly clear, especially for *H. armigera*, in part because averaging catches across 11 years, within which the timings of generations shifted slightly between years, tended to obscure detail. Catches for both species were negligible by the time the cotton harvest was nearing completion.

When catch data are graphed at single season level, the separate generations of moths are more apparent. For example, in 2005-06, four generations of *H. armigera* and two generations of *H. punctigera* were evident; in 2009-10, four generations of *H. armigera* were again seen and possibly

four generations of *H. punctigera* as well (Figs 21-22). Maxima of 4 generations for the two species were also seen in the other individual seasons, with the first generation occurring (as moths) prior to or concurrently with cotton planting in all cases.

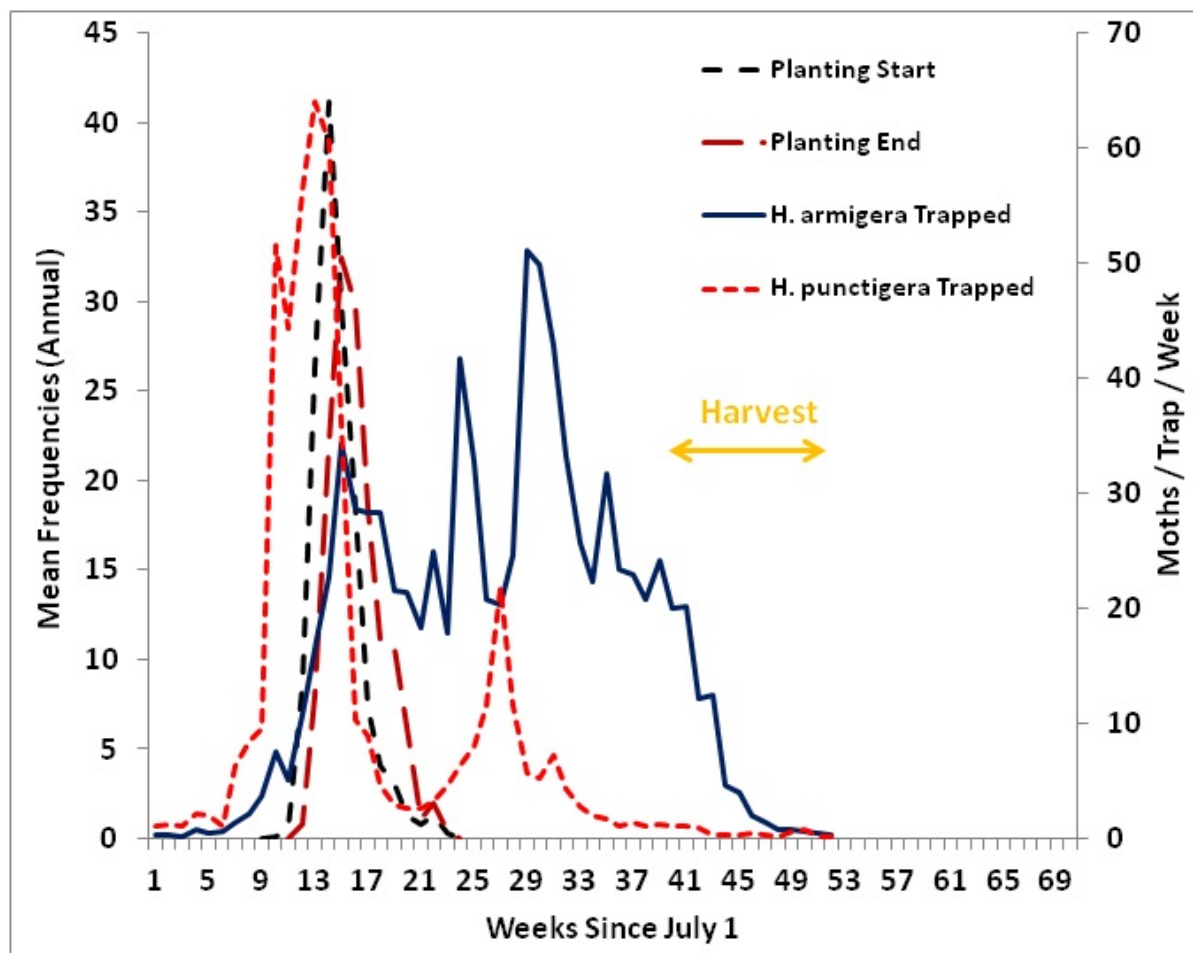


Fig. 20. Average annual frequencies of dates for start of planting and end of planting for Bt cotton crops growing in the Lower Namoi Valley, New South Wales over 11 cropping seasons (2002-2013) and the average pheromone trap catches for *Helicoverpa* within the ACRI, Narrabri trap grid over the same period. Extremities of harvest dates (CSD Variety Trial sourced data) are also included.

The pheromone trap catches in the St George region, whilst more limited in scope than those from the Lower Namoi, were quite similar in seasonal pattern, over the 12 years when the two trapping grids were run concurrently. Whether or not there was a generation of *H. armigera* present at St George similar to the first generation that occurred in the Lower Namoi in late September-early October (weeks 14-16) is impossible to determine. The St George trapping regime did not start until week 16 in most years. In one year (2002-03), traps were however run in all months, but this year was, unfortunately, one of low *Helicoverpa* activity. It was not possible to recognise a spring generation in the limited data available at that time.

Moth emergence and pupal diapause induction

The predicted patterns of moth emergence (note : based on *H. armigera* – see above) varied amongst the selected sites within the Australian cotton industry during 2002-13, with moths emerging earliest in the warmer conditions in the north (e.g. Emerald) and latest in the cooler south (e.g. Lachlan). In contrast, *Helicoverpa* entered winter pupal diapause earliest in the south and latest in the north.

More specifically, moths were predicted to have emerged in the Lower Namoi just after cotton planting started and well after the pupae busting was completed in response to the previous season's requirements (Fig. 23). Many moths, especially *H. punctigera*, were trapped in the Lower Namoi prior to predicted emergence. This may reflect immigrant moths arriving from outside the Lower Namoi and / or a poor fit of the emergence model to *H. punctigera*'s biology. The predicted

pattern for pupal diapause induction in the Lower Namoi during the 2002-13 seasons suggests that most *Helicoverpa* that were going to enter diapause would have already done so before the majority of cotton was harvested (mean harvest completion = week 44.4) and before pupae busting activities began.

Near Emerald, during 2002-13, moths are likely to have begun emerging at the end of cotton harvest and just prior to cotton planting for the new season. Most pupal diapause induction was likely to have occurred after the majority of harvests (mean harvest completion = 42.3).

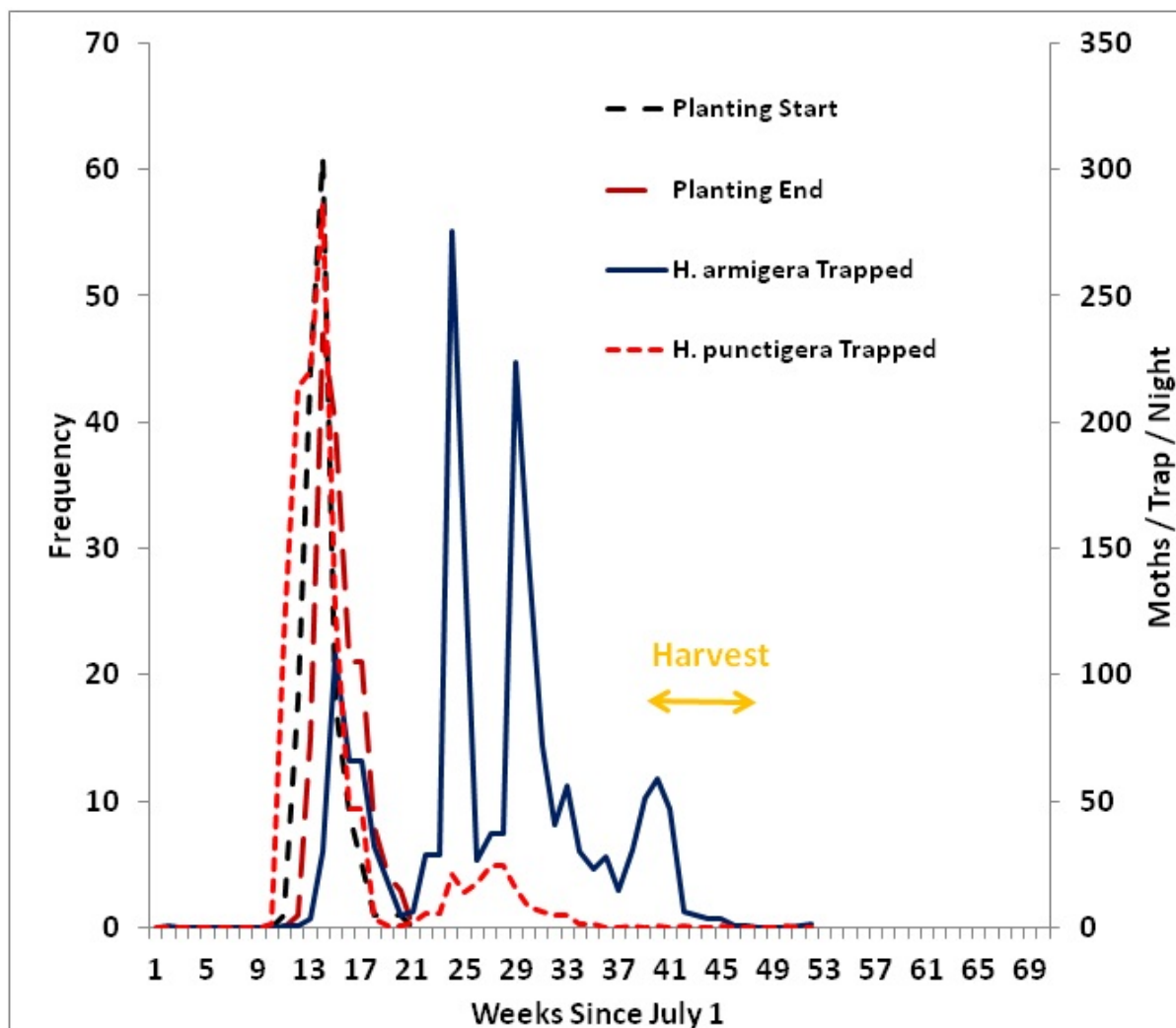


Fig. 21. Frequencies of dates for start of planting and end of planting for Bt cotton crops growing in the Lower Namoi Valley, New South Wales during the 2005-2006 cropping season and the average pheromone trap catches for *Helicoverpa* within the ACRI, Narrabri trap grid during the same period. Extremities of harvest dates (CSD Variety Trial sourced data) are also included.

Brief Overview

This aspect of the project evaluated the planting window strategy of the RMP by utilising industry-wide databases of planting dates and other cropping / pest management events throughout 11 growing seasons. The analysis included model predictions of when *Helicoverpa* is likely to enter winter (pupal) diapause and emerge as moths in spring, and the long-term pheromone trapping records of moths. In N.S.W. and southern Qld, most 1st generation moths were caught at the same time, or before, cotton was planted (mostly in October). This generation was, therefore, unlikely to have been exposed significantly to Bt toxins as feeding larvae in spring, but some individuals (especially *H. armigera*) may have been exposed to Bt prior to pupating in the previous autumn. Three subsequent generations, which could have been exposed to Bt during summer-autumn, were recognisable in trap catches before the moths became very rare in early April. By then, the majority of *Helicoverpa* had entered winter diapause as pupae and cotton harvest was well underway. We concluded that the current planting window (pre-mid November in these regions) achieves little in limiting the exposure of *Helicoverpa* to

Bt. However, insufficient is known of the population dynamics of *Helicoverpa* in central Qld to be as definitive about the need for planting windows there. Importantly, *Helicoverpa* are active all year round in central Qld and cotton can be grown over a longer period, thus increasing the risks of greater exposure to Bt.

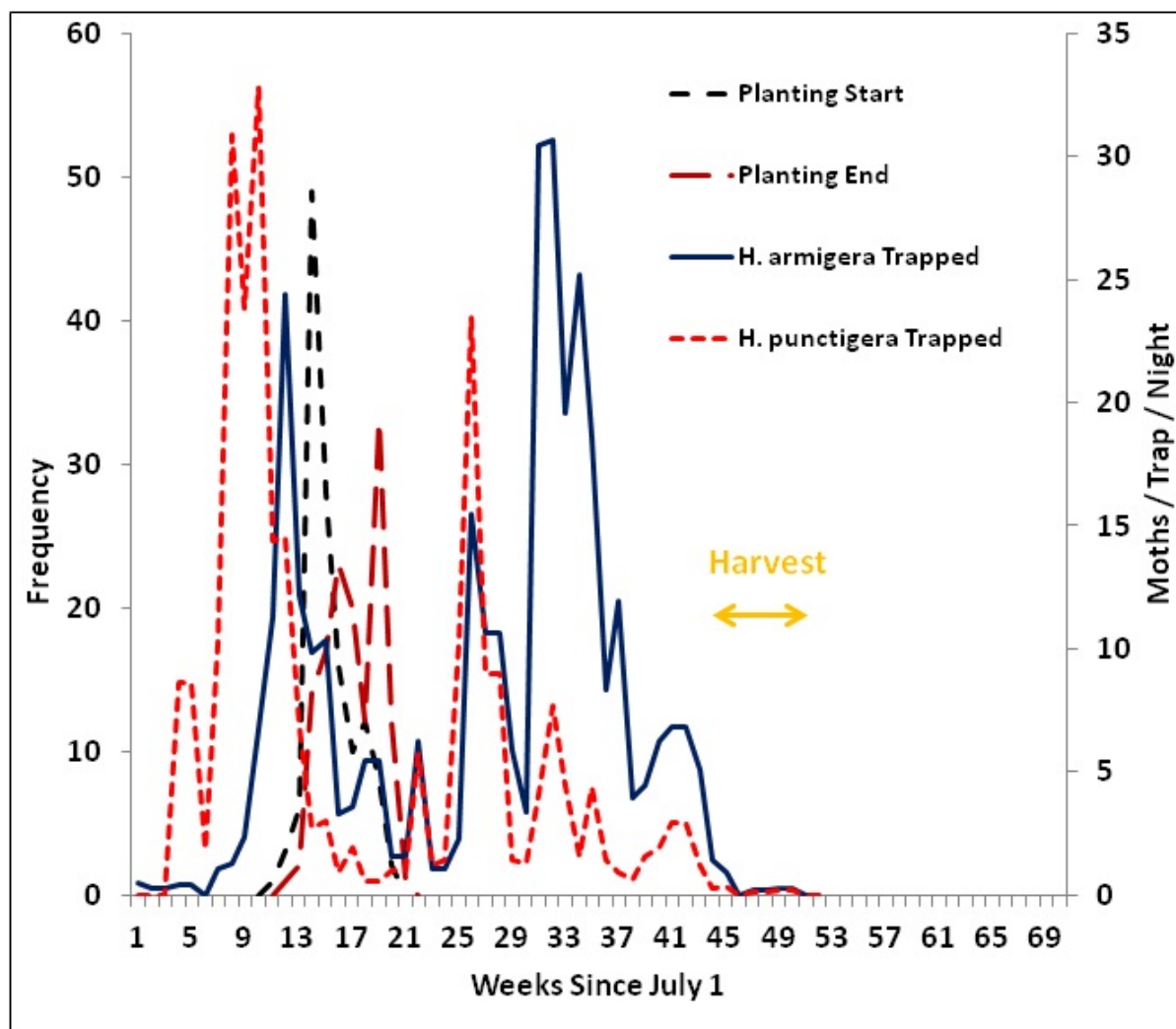


Fig. 22. Frequencies of dates for start of planting and end of planting for Bt cotton crops growing in the Lower Namoi Valley, New South Wales during the 2009-2010 cropping season and the average pheromone trap catches for *Helicoverpa* within the ACRI, Narrabri trap grid during the same period. Extremities of harvest dates (CSD Variety Trial sourced data) are also included.

Extend research results to industry & scientific colleagues.

During the tenure of this research grant, several papers were published or accepted for publication in international scientific journals, following the write up of work done in this project or other cotton related projects. These are listed in Section 9 below, along with several articles written for industry-related publications.

Many presentations were also made at various Bt Tech Panel meetings, REFCOM meetings, Cotton Research Conference, Cotton Conference, Australian Entomological Soc. Ann. Conferences, IPM Forum meetings & field days (see list given below).

There is little doubt that the success of Geoff Baker and Colin Tann in receiving the 2015 CSD Researchers of the Year Award also helped transfer outcomes from the research in the project more broadly to the cotton industry.

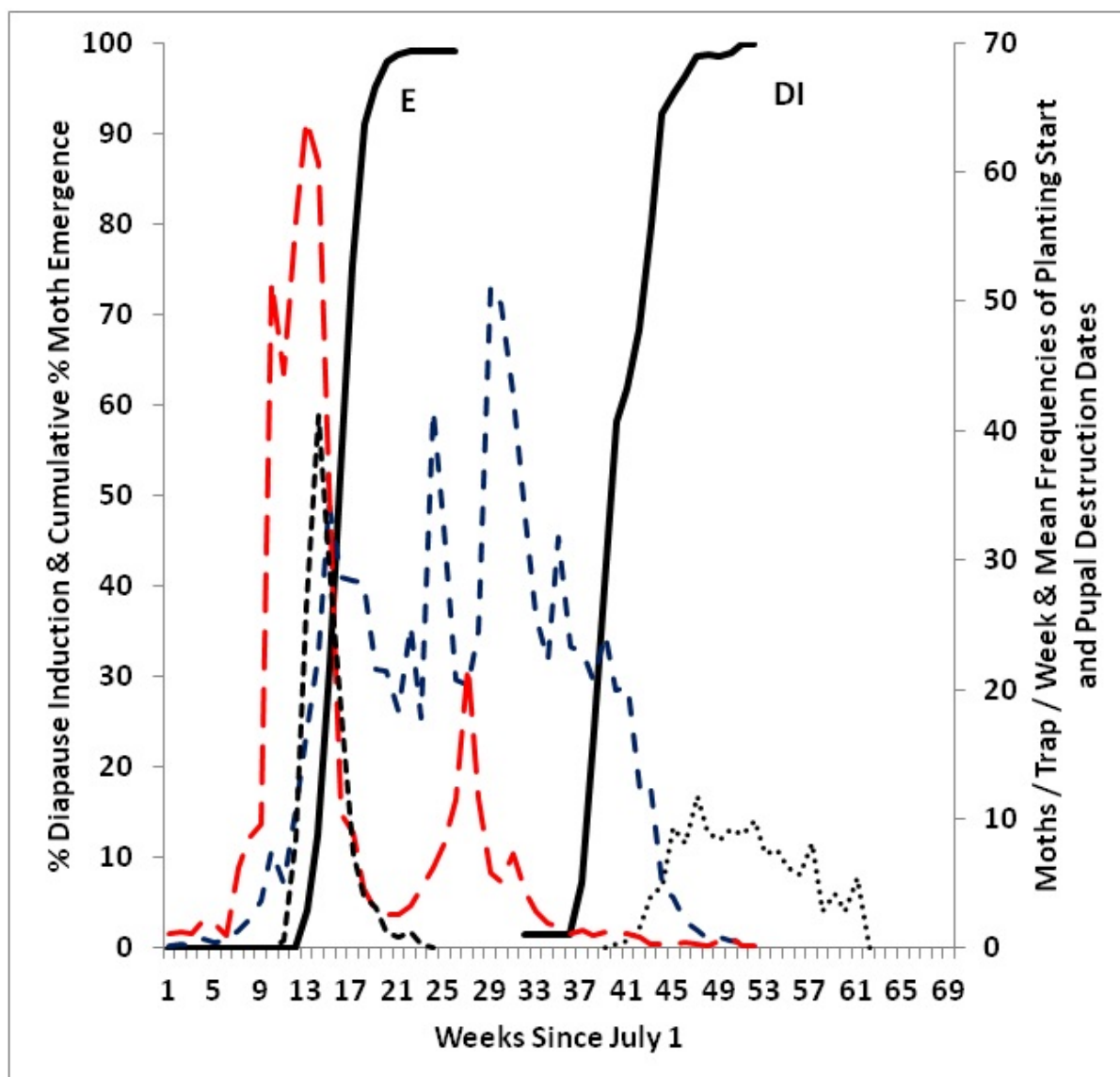


Fig. 23. Average pheromone trap catches for *Helicoverpa armigera* (large dashes) and *H. punctigera* (medium dashes) within the ACRI, Narrabri trap grid during 2002-13, mean frequencies of planting start dates (small dashes) for Bt cotton crops growing in the surrounding Lower Namoi Valley and associated pupal destructions (dots) during the same period, and modelled predictions (averages) for % diapause induction (DI) of *Helicoverpa* pupae and % moth emergence (E), also throughout 2002-13. [Note week 62, when pupae destruction was complete, is equivalent to week 10 in a subsequent year]

Provide a review of historic research outputs on the biology and management of *Helicoverpa* pupae, of relevance to the efficacy of “pupae busting”.

The review of ‘pupae busting’, as well as summarising the efficacy of various methods in destroying diapausing pupae in the soil, provided a reminder of the significant changes that have taken place since Bt cotton first arrived on the Australian cotton scene in 1996. The review illustrated changing agricultural practices, improving technology, and shifts in the ecology of *Helicoverpa* spp. over time.

Importantly there is no longer the same emphasis on insecticide resistance in the industry compared to the 1980’s and early 1990’s ; the *Helicoverpa* spp. populations under today’s Bt cotton are not considered significantly large. This is quite different to the pre-Bt cotton period when cotton was found to be the main contributor to over-wintering populations in most cotton-growing valleys, and hence a significant contributor to the following spring’s emergence, particularly of *H.armigera*. Insecticide resistance was considered to be the main focus during that period and pupae busting, or targeted tillage worked very well at destroying potentially resistant survivors.

The success of pupae busting during the insecticide-focused era and the confirmation of the tactic’s validity through various research efforts over many years, led naturally to its inclusion in the

RMP for Bt cotton. We are now targeting far fewer pupae, but importantly there remains the risk of some individuals over-wintering, which are the section of the population with the highest probability of carrying resistance into the following season. Because most of the cotton grown in Australia is Bt (>90%) the industry cannot afford to lapse in their RMP, as the selection pressure to the technology continues to be high.

However, in the presence of new Bt technologies that are more robust (as we embrace a three stacked product in Bollgard 3®), it may be possible to refine our targeted over-wintering pupae tillage operation and use our science base to determine the appropriate means to do that. Using tillage to destroy pupae in the soil, the key requirement has always been to cultivate before diapause emergence using science-based tools that characterize when diapause is induced and hence which fields are at risk of harbouring diapausing pupae. There is also tools that predict when diapause ends, and therefore the need for pupae busting effectively before then is quite simply all that is required. But, the practical application of this targeted tillage operation can be a problem for growers and become a challenge for the industry to administer.

There have been a few cultivation experiments demonstrating different tillage machinery to determine pupae survival which are helpful in guiding what works best. But because conditions can be so variable with regard to soil type, soil moisture, general on farm management practices and machinery used, there cannot be a one-size fits-all approach to this task. The pupae busting operation therefore needs to be conservatively managed, and flexibility according to the conditions needs to be factored in.

There has been talk of applying thresholds to help determine the need to pupae bust, but realistically, just finding one over-wintering pupae surviving in a Bt cotton crop is probably cause to undertake the tillage operation, when weighing up the risks. Therefore identifying the Bt crops that have potential to contain over-wintering pupae would seem to be of paramount importance, and a comprehensive cultivation, as deemed most appropriate for the soil conditions facing the operation, should be essential under these circumstances and remain mandatory under the RMP.

Our review document is attached for further consideration (Attachment 5).

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

1 (a) Expected Science Outcome

*Contribution to improving the RMP, and the future development of an improved tool kit for understanding the dynamics, in particular movements, of key insect pests in cotton (& other crop) production landscapes. Recognition of the influence local landscape design is likely to play in the movement / mixing of sub-populations of *Helicoverpa* in landscapes of different designs, thus better informing the development of models (based mostly hitherto on spatial abundance data per se) of the factors influencing the development of Bt resistance.*

1 (b) Expected Industry / Applied Outcome

*Recognition of the influence (or lack thereof) of local landscape complexity in the mixing of subpopulations, and hence potential Bt susceptible and Bt resistance alleles, within both *Helicoverpa* spp. This will be highly relevant to confidence in outcomes from the Bt RMP, and will advise on the need (or otherwise) for more rigour in landscape design (e.g. better informed placement of refuges in some systems). The work will also facilitate optimal selection of sites in Bt resistance monitoring programs (early-warning) where risks of emergence are greatest. In addition, improved appreciation of the role of unstructured refuges in Bt cotton production systems is likely to gain greater profile as the economics of future GM cotton deployment and resistance management requirements become more critical.*

The cotton industry has operationalised its Bt cotton RMP at farm scale (e.g. planting windows, refuge crops, pupae busting), and necessarily so. However, the population dynamics (in particular

mobility) of *Helicoverpa* beyond such scale has long been recognised. Recent research has focussed on cropping at landscape scale to provide understanding of Bt resistance development (via models) and mostly relied on abundance data (especially of eggs). To test, in part, the reality of this landscape-scale thinking, we recorded the plant host origins of *H. armigera* and *H. punctigera* moth populations at various scales in two primary regions (Maranoa & Darling Downs) which have contrasting cropping designs (simple & complex respectively). We supplemented this work with a third region (Namoi, intermediate in cropping complexity) in one year of the three year study. We argued that moth populations should reflect the landscapes they are found within to merit modelling for Bt resistance development at such a scale.

The research found no evidence of differences in moth population structure at single trap and sub-regional scale. Differences in population structure were only evident at regional scale. In addition, many more moths with C₄ plant host origins (typified by crops such as sorghum and maize) were found in the Maranoa than this region's cropping statistics would lead one to expect. We suggest these results most likely reflect large (inter-regional) movements, which we know these moths are quite capable of, and that these could over-ride any impact that more local (e.g. landscape) dynamics can instil in population structure. That in turn suggests that Bt resistance modelling might be better pitched at a larger scale than it currently is? However, until better plant host origin analyses (i.e. more discerning than C₃/C₄ level) are developed we need to be cautious with our conclusions. We still remain ignorant of where these C₄ moths are coming from (local, as yet unidentified host plants or well-known host crops from far afield?).

The research also illustrated the numerically important contribution that "unstructured" refuges (i.e. host plants other than pigeon pea and unsprayed cotton) are making in cotton production regions (e.g. 50-70% for *H. armigera*). This is in accord with other data (approximately 50%) we have reported in previous years across several cotton production regions.

A paper related to the above work is in an advanced draft and will be submitted in coming months. A separate paper, based on our work prior to CSE1306 but written recently, has been published in Austral Entomology (see publication list below) which indicates the likely patchy dispersal of moths away from mandatory refuges to cotton crops within surrounding landscapes. The paper discusses the implications of such limited delivery within the context of the current Bt RMP.

2 (a) Expected Science Outcome

A high profile scientific publication (or two) on major drivers of insect pest abundance at agricultural landscape scale, in particular with relevance to the deployment of transgenic plants.

2 (b) Expected Industry / Applied Outcome

Industry appreciation of ongoing variations in large-scale abundance of key pests, with implications for adoption and alterations of management strategies to control them.

This project, and its predecessors, tracked the abundance of *H. armigera* and *H. punctigera* at landscape-scale for 23 years. The study covered the advents of both Ingard[®] and Bollgard II[®] cotton. It showed primarily that Bollgard II[®], but not Ingard[®], has suppressed the abundance of *H. armigera* in time. A temporal decline in the abundance of *H. punctigera* seems better explained by poor recruitment of spring migrants into cotton production regions from inland Australia rather than impact from Bt cotton. The study also illustrated positive associations between pest abundance and the incidence of other crops within production regions (e.g. chick pea in spring and sorghum in summer). Two publications arising from this work are now in press in the Cambridge University Press journal, Bulletin of Entomological Research. The research and its implications have been presented to / published in various cotton industry meetings and publications many times.

3 (a) Expected Science Outcome

*A framework upon which to model seasonal exposure of *Helicoverpa* spp. to Bt toxins, at individual valley scale.*

3 (b) Expected Industry / Applied Outcome

A framework upon which key committees, such as TIMS, can identify general patterns of use re planting windows & defoliation dates and better assess their likely utility in Bt RMP.

CSIRO partnered with Monsanto and CRDC staff to analyse industry-wide patterns of use in planting windows and other agronomic practices in cotton fields throughout 2002-13 and associated

these with concurrent patterns in *Helicoverpa* abundance and modelled likely pupal diapause / moth emergence dates. This work has been published (*Austral Entomology* **55**, 32-42). Findings were presented to several industry meetings, most notably TIMS Bt Tech Panel, and formed a basis for planning of the Bt RMP for Bollgard 3[®].

4 (a) Expected Science Outcome

A review of existing information re Helicoverpa pupae ecology and busting, to inform what is well-based fact and where the gaps in knowledge lie and further experimentation is needed and achievable.

4 (b) Expected Industry / Applied Outcome

A review to inform cotton industry committees, such as TIMS, of the status of knowledge on Helicoverpa pupae busting, to enable sound decision making within the Bt RMP.

The need for a better understanding within the cotton industry of what has previously been researched and documented on the practice of “pupae busting”, and allied aspects of *Helicoverpa* ecology, became apparent at TIMS Bt Tech Panel meetings during the tenure of CSE1306. As a result, CRDC requested that CSIRO provide a review on this during a 4th year extension to the project. The review has recently been completed and is attached to this report.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

No technical advances with commercial implications were generated in this project.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

No novel methods or equipment design were generated in this project.

c) required changes to the Intellectual Property register.

There are no major changes to the IP register although the section on potential IP to be generated or created has been updated to include specific mention of Extension Material and Data separately to Reports and Articles.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

This research focussed primarily on providing rigorous understanding of current *Helicoverpa* spp. ecology and evaluations of some of the core assumptions / expectations of the Bt Resistance Management Plan (RMP). In particular the research investigated 1) long-term trends in the abundance of the two key *Helicoverpa* spp. and the causative factors driving such trends, 2) the capacity of different cotton production regions and landscapes therein (i.e. different spatial scales) to influence moth communities, with implications for the development of Bt resistance in these pests, 3) the efficacy of planting windows as a strategy to limit exposure of *Helicoverpa* to Bt toxins, in contrasting production regions, and 4) the state of knowledge of the efficacy of previous and current pupae busting techniques as tools to limit the survival of *Helicoverpa* between cotton production seasons.

In essence, the research demonstrated that the advent of Bt cotton, in particular Bollgard II[®], has led to the (numerical) suppression of *H. armigera* at a wide scale (which is different from a reduction in pest status *per se*). The abundance of *H. punctigera* has declined in recent years, but it seems unlikely that this decline can be attributed much to Bt cotton. Rather, the reduction in numbers seems attributable to reduced annual (spring) recruitment of *H. punctigera* from its hitherto primarily important inland

habitats. The research further suggested that the plant host origins of populations of *Helicoverpa* moths do not necessarily reflect the landscapes / regions (crop compositions) they are caught within. This implies that the moths are indeed highly mobile, and that gene flow can occur at large (inter-regional) scales. The findings call into question, or at very least advise caution in interpreting, the modelling of Bt resistance development at smaller (e.g. landscape) scales using abundance data only. In addition, the importance on non-structured refuges (i.e. plant hosts outside the cotton production system *per se*) in contributing to moth communities was high-lighted. The research also demonstrated that planting windows, used as resistance management tools, are likely to have little efficacy, at least in southern production systems. This finding has already had bite in developing a revised RMP for the arrival of Bollgard 3[®] cotton. The review of pupae busting is intended to improve awareness of the state of the art in this mandatory requirement associated with Bt cotton production, thus assisting better informed discussion of its efficacy and need. We have also expressed our concern with the industry's reduction of mandatory refuge crop sizes through our publication on refuge efficacy, in particular the limited capacity of previous, let alone new, refuge crop sizes to deliver Bt susceptible moths throughout a region. All these observations have major relevance to the Australian cotton industry's understanding and capacity to manage *Helicoverpa* as key pests going forward into coming production seasons, in particular the control of emergent Bt resistance.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

No new technology *per se* was developed in this project. We are however pleased to see a recent, significant expansion in interest in tracing plant-host origins of *Helicoverpa* amongst other researchers, in particular their recognition of the use of stable isotope signatures. More research support could be profitably invested in further development of this and more efficient techniques.

(b) for the future presentation and dissemination of the project outcomes.

Dissemination (& adoption) of the outputs of the research (e.g. recognition of long-term changes in the abundance of *Helicoverpa* spp. and the influence of Bt cotton, relevance of habitat heterogeneity in *Helicoverpa* spp. dynamics, better understanding of *Helicoverpa* movement at various scales and resultant implications for resistance management, importance of planting windows and "pupae busting" in the RMP, etc) is envisaged via future inputs to key industry committees / workshops / conferences etc such as REFCOM and TIMS Bt Panel meetings. Colin Tann will continue to attend these meetings; Geoff Baker (now retired) will attend, if available and requested.

Most of the project outcomes have already been published, or are in press, through both international scientific journals and various industry publications (e.g. Aust Cottongrower, CSD Variety Trials booklets, Spotlight). Additional journal papers are planned, most notably submission of the draft manuscript referred to above concerning the comparative study (D Downs vs Maranoa) of population structures (indicated by stable isotope assessments) in relation to landscapes / regions of different complexity. The next paper in the queue will consider long-term temporal shifts in the abundance of *Helicoverpa* parasites. Following that, other planned papers include e.g. the assessment of spatial pattern in the performance of refuges in the Goondiwindi region (a partner to the already published paper re St George). Many talks have already been given (see list below); additional talks will be offered, as opportunity arises.

(c) for future research.

We see little point in continuing the pheromone trapping grid near ACRI into further years (see comment above as well), at the level of intensity with which it has been monitored in the past. GPS coordinates are documented (see latest trapping publications) which means traps can be reset at exactly the same sites for specific purposes, should the need arise (e.g. if there is a suggestion that population numbers have shifted from the current, relatively low levels).

This project has demonstrated, more clearly than any other before, the scale of importance of unstructured refuges. But we know very little about the nature of such (i.e. the host-plant

origins within this generality, for both species, but particularly *H. punctigera*). A further project, to tease out where most moths are coming from (which should also consider some improvement in understanding the contributions made by the “cotton system” per se – i.e. cotton and pigeon pea) is needed, in particular to guide Bt resistance modelling. Such work will necessitate development of new, more specific tools for recognising plant host origins. Stable isotope research, whilst very useful, is still a much blunter instrument than we really need.

There are, however, some quite small loose ends in the research reported here that could be tied off quickly with minimal investment. e.g. we strongly suspect that the C₃ *H. armigera* and *H. punctigera* moths caught relatively early in the cotton season (see the Results section above) reflect origins in chick pea, but we do not have data to nail that idea that are similar to that presented in the figures re moths of other known host origins (e.g. sorghum, maize, cotton, pigeon pea). It would also be useful to have an extra replicate of *H. armigera* moths known to have come from sorghum.

Given that the modelling of diapause induction / emergence was done many years ago, many factors have changed in the cotton industry since then, it is well accepted that the original modelling was faulty anyway for southern regions (where the cotton industry has greatly expanded in recent years), and in the face of climate change, it would seem prudent that this area of diapause research be revisited.

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

Scientific Journal Papers

Papers published / submitted during the tenure of CSE1306, or that are in advanced draft form are listed here. Copies of those directly related to the project per se are attached with the Final Report.

Baker, G.H. & Tann, C.R. 2013. Mating of *Helicoverpa armigera* (Lepidoptera : Noctuidae) moths and their host plant origins as larvae within Australian cotton farming systems. *Bull. Ent. Res.* **103**, 171-181.

Lu, Z.Z. & Baker, G. 2013. Spatial and temporal dynamics of *Helicoverpa armigera* (Lepidoptera, Noctuidae) in contrasting agricultural landscapes in northwestern China. *Int. J. Pest Manag.* **59**, 25-34.

Wilson, L., Downes, S., Khan, M., Whitehouse, M., Baker, G., Grundy, P. & Maas, S. 2013. IPM in the transgenic era : a review of the challenges from emerging pests in Australian cotton systems. *Crop & Past. Sci.* **64**, 737-749.

Whitehouse, M.E.A., Wilson, L.J., Davies, A.P., Cross, D., Goldsmith, P., Thompson, A., Harden, S. & Baker, G. 2014. Target and nontarget effects of novel “triple-stacked” *Bt*- transgenic cotton 1 : canopy arthropod communities. *Environ. Entomol.* **43**, 218-241.

Baker, G.H. & Tann, C.R. 2014. Refuge crop performance as part of the Bt resistance management strategy for *Helicoverpa* spp. (Lepidoptera : Noctuidae) in Australia cotton production systems. *Austral Entomol.* **53**, 240-247.

Baker, G.H., Leven, T., May, A. & Tann, C.R. 2016. Planting window requirements for Bt cotton in Australia : do they limit the exposure of *Helicoverpa* spp. (Lepidoptera : Noctuidae) to Bt toxins ? *Austral Entomol.* **55**, 32-42.

Baker, G.H., Parry, H. & Tann, C.R. 2016. *Helicoverpa* (Lepidoptera: Noctuidae) abundance in and modelled dispersal from pigeon pea refuge crops within a Bt cotton production region in eastern Australia. *Austral Entomol.* In Press (available on-line in Early View).

Baker, G.H. & Tann, C.R. Long-term changes in the numbers of *Helicoverpa punctigera* (Lepidoptera : Noctuidae) in a cotton production landscape in northern New South Wales, Australia. *Bull. Ent. Res.* In Press.

Baker, G.H. & Tann, C.R. Broad-scale suppression of cotton bollworm, *Helicoverpa armigera* (Lepidoptera : Noctuidae), associated with cotton crops in northern New South Wales, Australia. *Bull. Ent. Res.* In Press.

Baker, G.H., Tann, C.R., Verwey, P. & Lisle, L. Do the plant host origins of *Helicoverpa* (Lepidoptera : Noctuidae) moth populations reflect the agricultural landscapes within which they are caught ? In Prep.

Other articles :

Ceeney, S., Baker, G., Whitehouse, M., Gregg, P., Tann, C., Leven, T., Downes, S. & Wilson, L. (2012). Refuge crops – investing in cotton’s future. *The Australian Cottongrower*. **33** : 14-16.

Baker, G., Ceeney, S., Downes, S. & Tann, C. (2013). Pigeon pea refuges – a smart choice for resistance management. *The Australian Cottongrower*. **34** : 12-14.

Tann, C., Downes, S. & Baker, G. (2012). Entomology. In : “2012 Variety Trial Results”. Cotton Seed Distributors, Wee Waa, pp. 99-101.

Tann, C., Downes, S. & Baker, G. (2013). Entomology. In : “2013 Variety Trial Results”. Cotton Seed Distributors, Wee Waa, pp. 79-81.

Tann, C., Downes, S. & Baker, G. (2014). Entomology. In : “2014 Variety Trial Results”. Cotton Seed Distributors, Wee Waa, pp. 86-88.

Tann, C., Whitehouse, M., Downes, S. & Baker, G. (2015). Entomology. In : “2015 Variety Trial Results”. Cotton Seed Distributors, Wee Waa, pp. 45-48.

Contributions to various other articles e.g.

Spotlight (Autumn 2013). Pupae busting is essential for resistance management.

Spotlight (Spring 2013). Pigeon pea refuges.

Spotlight (Summer 2012-13). Taking refuge against resistance.

Talks given :

REFCOM, Gold Coast (August 2012) ‘Is Bt cotton in NQ a threat for Bt resistance management?’ Presentation jointly by Paul Grundy and Colin Tann

Australian Cotton Conference, Gold Coast (August 2012) ‘Refuge crop performance as part of the Bt Resistance Management Strategy for *Helicoverpa spp.*’ Presented by Geoff Baker

Australian Ent Soc Conference Hobart (Sept 2012) ‘The Landscape assessment of Transgenic (Bt) cotton refuges, as a requirement for resistance management’. Presented by Colin Tann

IPM Forum, Toowoomba (July 2013) ‘Long-term trends in the abundance of *Helicoverpa spp.* and their natural enemies’. –Presented by Geoff Baker.

REFCOM, Narrabri (August 2013) ‘Long-term evaluation of pigeon pea and non-Bt cotton refuges’. – Presented by Geoff Baker.

TIMS Meeting, Sydney (August 2013) 1) ‘Long-term evaluation of pigeon pea and non-Bt cotton refuges, & 2) Planting windows.’ Presentations by Colin Tann & Geoff Baker

Cotton Research Conference, Narrabri (September 2013) ‘Refuge crop performance as part of the Bt resistance management strategy for *Helicoverpa* spp.’ Presented by Geoff Baker.

TIMS Meeting, Sydney (February 2014) ‘Planting Windows’. Presented by Geoff Baker.

REFCOM, Goondiwindi (September 2014) ‘Identifying *Helicoverpa* sp. population mixing from different host plant origins within landscapes of differing habitat complexity’ Presented by Colin Tann.

Moree Field Day (Monsanto) (June 2014). Presented by Colin Tann.

Australian Cotton Conference, Gold Coast (August 2014) ‘How does landscape management influence *Helicoverpa* abundance & the risk of Bt resistance?’ Presented by Geoff Baker.

TIMS Meeting, Narrabri (July 2014) ‘Refuge Performance and Variability’. Presented by Colin Tann.

Australian Ent. Soc. Conference Canberra (September 2014) ‘How does landscape management influence *Helicoverpa* abundance & the risk of Bt resistance?’ Presented by Geoff Baker.

REFCOM, Narrabri (August 2015) ‘Moth populations and landscape patterns’. Presented by Geoff Baker.

Cotton Research Conference, Toowoomba (September 2015) ‘Moth populations and landscape patterns.’ Presented by Geoff Baker.

Australian Ent Soc Conference Cairns (September 2015) ‘Using stable isotopes to determine *Helicoverpa* spp. spatial movement within landscapes’. Presented by Colin Tann.

REFCOM, Gold Coast (Aug 2016) ‘Moth populations and landscape patterns’. Presented by Geoff Baker.

B. Have you developed any online resources and what is the website address?

No.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Transgenic (Bt) cotton has provided improved control of *Helicoverpa* pests and reduced pesticide use within the Australian cotton industry, but the possibility of Bt resistance in these insects is still a major threat. Research prior to the start of this project found increasing frequencies of resistance alleles (especially Cry2Ab) which raised questions of the adequacy of some strategies and assumptions within the Bt Resistance Management Plan (RMP). More recently, the likely release of an improved product (Bollgard 3[®] cotton) also led to queries on potential changes that might be made to the RMP, notably relaxations in the requirements for refuge crops, planting window restrictions and pupae busting.

Firstly, it is fundamental to any pest management program that the abundance of the pest(s) be monitored through time. This project concluded a 23 year monitoring program of the landscape-scale abundance of both *H. armigera* and *H. punctigera* using a grid (10km radius) of pheromone traps near Narrabri in the Lower Namoi. Both species have declined in

numbers in time, but for different reasons. The abundance of *H. armigera* has been suppressed by Bt cotton, in particular Bollgard II®. During the (initial) Ingard® era, the abundance of *H. armigera* was actually higher than it had been in preceding years, fuelled by a greater amount of cotton on the landscape and a precautionary 30% area cap that was applied for Bt cotton to avoid Bt resistance development. The advent of Bollgard II®, brought an improved product (two toxins), no area cap on Bt cotton use, and increasing numbers of natural enemies of *Helicoverpa* (probably due to declining use of insecticides). In the case of *H. punctigera*, the primary driver of reduced numbers in the cropping regions seems to have been a decrease in the spring arrival of immigrant moths originating in inland Australia. The Bt RMP has hitherto assumed a regular influx of these (Bt susceptible) spring immigrants dilutes the development of resistance in this species. The research highlights the fragility of this assumption.

Secondly, whilst our previous research identified e.g. the most productive (although highly variable) refuge crops for *Helicoverpa* production, their likely patchy delivery of susceptible moths throughout landscapes, and that moths from different plant host origins mate at random (an implicit assumption in the RMP), our understanding of the relative contributions of the various plant host origins of these moths, be they Bt cotton crops, mandatory refuge crops or other (“unstructured”) refuges to the total populations of moths throughout cotton production areas, and the degree of spatial mixing of such moths remains limited. Such information is critical to an effective RMP, for example in selecting appropriate scales for modelling Bt resistance development. We argue that population structure (moth origins) should reflect available plant hosts if appropriate spatial scales are being used. Over three years, we surveyed the stable isotope signatures (especially carbon) of both moth species in cotton regions with very different land-uses (in particular, the Maranoa and Darling Downs) to identify the plant hosts being used. Differences in moth population structure were evident at regional scale, but were not at smaller scales (sub-region, single trap). Notably, the abundance of moths with C4 origins (typified by crops which include sorghum & maize) was much greater in the Maranoa than land-use would predict. From these results, we suggest that moth population structure that is driven by local (e.g. landscape) land-use design could be overridden by immigrant moths from further afield, and that modelling will need to be appreciative of this. The work also highlighted the numerical importance of “unstructured” refuges in contributing to overall moth populations within cotton production regions (> 50%).

Thirdly, this project also evaluated the planting window strategy of the RMP by relating industry-wide databases of planting dates and other crop management events to the known biology of the pests. The research concluded that the planting windows set previously for southern production regions achieve little in delivering their aim of limiting the exposure of *Helicoverpa* to Bt. However, insufficient is known of the biology of *Helicoverpa* in central Qld to be as definitive about the need for planting windows there.

Finally, winter pupae busting is intended to reduce the survival of resistant *Helicoverpa* from one season to the next. The efficacy of this tool is being questioned, but the majority of literature available to assist relevant discussion on the topic is dispersed and not well known. The project therefore provided a review of the literature to assist this process.

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