Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2017

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: UNE1502

Project Title: *Helicoverpa punctigera* in inland Australia – what has changed?

Project Commencement Date: 1/07/2014 **Project Completion Date:** 31/12/2017

CRDC Research Program: 1 Farmers

Part 2 – Contact Details

Administrator: Dr. Kath Dougall, Research Grants Officer
Organisation: University of New England
Postal Address: Research Services, University of New England, Armidale, NSW 2351
Ph: 02-67733262 **Fax:** 02-67733543 **E-mail:** grants@une.edu.au

Principal Researcher: Adjunct Professor Peter Gregg
Organisation: University of New England
Postal Address: School of Environmental & Rural Science, University of New England, Armidale NSW 2351
Ph: 02-67732665 **Fax:** 02-67733238 **E-mail:** pgregg@une.edu.au

Supervisor: Professor. Aron Murphy
Organisation: University of New England
Postal Address: Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, NSW 2351
Ph: 02-67732323 **Fax:** 02-67732769 **E-mail:** ers@une.edu.au

Signature of Research Provider Representative: _____

Date Submitted: **19 March 2018**

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.

The cotton industry relies on Bt, now in Bollgard III® varieties. Bt provides security to growers, enables new farming systems and reduces pesticide use, ensuring continued social license to farm. At the time the project began, these benefits were threatened by resistance to Bt, especially to the Cry2Ab toxin in *H. punctigera*.

The rise in resistance frequency in *H. punctigera* experienced in the years immediately prior to the project was surprising and disturbing. For decades *H. punctigera* never sustained resistance to pesticides, unlike *H. armigera*. This was attributed to differences in their ecology, and it was assumed that *H. punctigera* would never pose a threat for Bt resistance. Current RMPs were designed for *H. armigera*, but now they also had to consider *H. punctigera*. Updating our understanding of *H. punctigera* was therefore crucial. We needed to revisit key questions such as immigration from the remote inland, previously thought to be extensive.

For the past three years, we had studied the ecology of *H. punctigera* in western Queensland, and compared results to the work we and others did in the 1980s and 90s. Pheromone trap catches in Narrabri (G. Baker/C. Tann, Project CRC1005) indicate that migration from the inland had failed during the great drought of 2001-2009, and had not revived following rains in 2010 to 2011. Yet we had found that many remote inland areas still regularly produced many *H. punctigera*. We speculated that the drought may have changed the distribution and abundance of key host plants, especially in mulga regions which act as a bridge to enable migration from the floodplains and sandy deserts of the far west. However, in such a variable environment as inland Australia, we needed more than the three years of data provided by our earlier project to understand these changes. This project aimed to extend that work to provide better understanding of long term changes in *H. punctigera* populations that might affect pest impacts on cotton and other crops, and management of resistance to Bt in cotton.

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 Determine patterns of adult abundance using pheromone traps

Milestone: 1.1 Establish new pheromone trap sites (At least two new sites established)

Achieved: In the first year of the project we established four new pheromone trapping sites in South Australia, at Minnipa, Yardea, Kingoonya and Roxby Downs. We also maintained the six trapping sites in western Queensland that had been established in Project UNE1109.

Milestone 1.2 Collect data from pheromone networks (Reliable annual data sets from most inland sites, to compare with cropping sites)

Achieved: Detailed results from all 10 trapping sites for the 3.5 years of the project are presented in Appendix 1 to this report. The data have not yet been analysed in comparison with results from cropping areas – we plan to do this in a paper which will cover long-term trapping results from 1987 to 2017.

Milestone 1.3 Continue collaboration with inland schools (At least one insect session with school children at each site, per year)

Partially achieved: This milestone was carried over from Project UNE1109, which was established during the previous Cotton Catchment Communities CRC. In this project many of the trapping sites were based at inland schools and a component of the CRC school outreach program involved us doing insect collection and identification sessions with the school children. However, following the winding up of the CRC we decided to revert to paid collaborators because the school staff turnover and breaks during the school holidays were affecting the integrity of the data set. With the approval of CRDC we dropped the school visits, although we continued working at two schools, Bedourie and Eromanga where there were local residents involved, and we continued to give informal student sessions.

Objective 2 Determine inland host plant distribution and abundance, and seed bank status

Milestone 2.1 Conduct field trips with quantitative vegetation monitoring (two field trips per year, with at least 200 vegetation records made)

Achieved: During the 3.5 years of the project we made 11 field trips (two in 2014, three in 2015, four in 2016 and two in 2017). Reports from all these field trips are presented in Appendix 2. During these trips we made a total of 876 vegetation records, which have now been databased with records from Project UNE1109 and earlier records from the 1980s and 90s. This has produced a database totalling 3556 records which will provide a unique record of the way in which host plant distribution and abundance has changed over the 30 years of the extended study.

Milestone 2.2 Develop techniques to study seed bank dynamics (Small-scale trials of irrigation and seed bags to demonstrate feasibility)

Milestone dropped: The aim of this milestone was to develop techniques that would enable us to assess whether there had been any long-term depletion of the seed banks of major host plants as a result of the Millennium Drought (2000 – 2009). This would have been necessary if sufficient winter rainfall to produce natural germination had not occurred. In the event, rain during 2015 and especially in 2016 made it unnecessary, and with approval of CRDC we dropped this milestone.

Milestone 2.3 Wider scale seed bank dynamics studies (only if natural rainfall has been inadequate, if enough winter rain has not fallen by 31/5/2017, conduct irrigation/seed bag studies in three mulga regions)

Milestone dropped: This milestone was conditional on lack of natural rainfall, and proved unnecessary (see Milestone 2.2 above).

Milestone 2.4 Determine potential of hosts with the C4 photosynthetic pathway to contribute to inland populations (At least 20 samples on inland C4 species in conjunction with field trips)

Achieved: This milestone was inserted in 2016 following reports by Geoff Baker and Colin Tann (Project CRC1005) that some *H. punctigera* moths from cotton areas exhibited carbon isotope profiles consistent with feeding on host plants that had the C4 photosynthetic pathway. This was surprising because all previously known hosts for *H. punctigera* have the C3 pathway. C4 plants include sorghum and maize which were thought not to be hosts. Many chenopods and succulents from inland regions were C4 plants. It was thought that the C4 plants seen in cotton areas could have come from larvae reared on these hosts in the inland. In previous projects we had conducted some limited sweep net sampling on such plants with negative results, but the Baker/Tann data prompted us to re-investigate the question. During the last two seasons of field trips we conducted an additional 37 samples on chenopods and succulents, making a total of 63 when all the earlier projects are included. All samples were negative, suggesting that these plants are not hosts. Details are provided in Appendix 3 to this report.

However, we have since grown several species of chenopods in the laboratory, and shown that when the plants are young, larvae will survive on them. All field samples have been done in mature plants because it is necessary to have fruiting bodies for identification of the plant species. It is possible that young chenopods will support larvae because the foliage is less tough and has not accumulated high levels of salt. It is also possible that there are hosts among prostrate succulent species which cannot be sampled by sweep netting. At least one of these species supports larvae in laboratory studies. We plan on further investigating these possibilities through the work of a postgraduate student, Samuel Abukari, who is enrolling in April 2018.

Milestone 2.5 Determine C3/C4 status of inland host plants (at least 30 host plants collected and analysed for carbon isotopes)

Achieved: This milestone was also inserted in 2016, and was aimed at determining whether known inland host plants had C3 or C4 pathways. Most of these hosts were daisies and legumes, and while the majority of species in these families have the C3 pathway, there are some exceptions, especially among species growing in hot regions. We collected and performed carbon isotope analysis on 15 species of major host plants (two samples for each) in the following families: Asteraceae (7 species), Fabaceae (3 species), Goodeniaceae (2 species), Solanaceae, Geraniaceae and Portulaceae (1 species each). All except *Portulacca intraterranea* (large pigweed) proved to have the C3 pathway. This indicates that the source of C4 *H. punctigera* moths is not well-known hosts which, unusually, might have the C4 pathway. We are also conducting laboratory rearing on several inland chenopods (see Milestone 2.4) and will do carbon analysis on these; they are expected to be C4.

Milestone 2.6 Determine C3/C4 status of moths collected in the inland (At least 300 moths collected from collaborator-operated pheromone traps or inland field trips, analysed for carbon isotopes)
Achieved: Starting in 2016 we analysed 445 moths collected from pheromone traps at five inland sites. Overall 22.5% had the isotope signature of C4 host plants, but this percentage was quite variable. C4 moths were in the minority in all samples except for one from Bedourie in June 2016, which had 68% C4 moths. Details are provided in Appendix 3.

Milestone 2.7 Determine C3/C4 status of moths collected in southern NSW (At least 400 moths collected during trials of moth busting with Magnet in southern NSW analysed for carbon isotopes)
Achieved: This milestone was inserted in 2017, and relates to a different project undertaken primarily with funding from AgBitech Pty Ltd and Monsanto, to investigate the feasibility of moth busting as an option in RMPs for southern NSW. It was added to the inland project for administrative convenience. A detailed report of the southern moth busting trials has been supplied to AgBitech and Monsanto. It includes data from 383 *H. punctigera* moths, mostly killed by Magnet® applications, of which all but three had the C3 profile. We also analysed 191 *H. armigera* moths, mainly from pheromone traps, and 80% had the C4 profile. This indicates that the *H. punctigera* that were targeted by Magnet® had come from crop or non-crop C3 hosts, but probably not cotton, since of 100 analysed for gossypol by Monsanto, only one was positive. On the other hand, most *H. armigera* had probably come from nearby corn and sorghum crops.

Objective 3 Determine inland larval abundance patterns
Milestone 3.1 Conduct field trips with quantitative larval sampling (Two field trips per year, sampling up to 20 sites per trip if vegetation conditions permit)
Achieved: During the 11 field trips we sampled for larvae at a total of 245 sites. Results are included in the field trip reports (Appendix 2).

Objective 4 Determine emergence times and compare with local and cropping region pheromone catches
Milestone 4.1 Establish inland emergence cages each winter with local collaborators (At least one site with at least 4 cages per year, provided larvae are present).
Achieved: This work was actually done during Project UNE1109, as part of Kris le Mottee's PhD project, but was analysed and written up during the period of his employment as a research technician in UNE1502 (2016/17). Emergence cages were operated at three sites in western Queensland. Results suggested that winter diapause played little role in the timing of spring emergence in this area, though soil temperature readings indicated that diapause induction would be possible in the heavy clay soils of the floodplains, if not in lighter soils of the sandy deserts. Comparisons with pheromone catches locally and in the Namoi valley suggested that migration from the inland was an important source of moths in early spring. Detailed results are given in Appendix 4, which is an extract form Kris Le Mottee's thesis.

Objective 5 Develop GIS-based data system to enable comparisons with early inland data
Milestone 5.1 Complete GIS system for vegetation and larval data (ArcGIS system up and running, including all old data from 1980's-90s).
Achieved: This work was also done during Kris Le Mottee's employment within the project. Kris developed a GIS using ArcInfo, and including layers of vegetation mapping derived from digital State-based vegetation maps in NSW, South Australia and Northern Territory. For western Queensland older paper-based maps, digitised by Kris, were used for consistency, because they had been used in the old Heliothis Inland Research Group (HIRG). Other layers in the GIS were based on our survey data for larval populations and vegetation records. A major breakthrough was the rescue of all the old HIRG

data from archaic database programs and their conversion to Excel spreadsheets which could be read directly into ArcInfo.

Milestone 5.2 Addition of new data from field trips, annually (new data in system)
Achieved: All data from the 11 field trips in this project, in addition to the 71 trips made during earlier projects, have now been incorporated into the GIS and have formed the basis for recent publications. The GIS has also been adapted to run on a high-end Windows based tablet which can be used in a vehicle to provide real-time information on vegetation associations and previous larval or vegetation data.

Objective 6 Analyse data and publish results
Milestone 6.1 Publish in the scientific literature and in industry publications (At least two scientific journal papers and at least two industry publications)
Achieved. We have published one paper in the scientific literature and another has been submitted. We have presented nine papers to major national and international scientific conferences, and written two articles for *The Australian Cottongrower* (see Section 9 for details). We plan to publish several more papers, on changes in host plant abundance since 1987, on the pheromone catches, on possible C4 host plants, and on diapause and emergence in the inland compared to cropping areas. The data sets for this publication are very large, and analysis will require time.

Objective 7. Contribute to formulation of RMPs and industry discussions on resistance management
Milestone 7.1 Participation in REFCOM and TIMS meetings (Changes to RMPs based on evidence from the project, if needed).
Achieved: Peter Gregg attended all meetings of the Biotechnology Panel of TIMS during the project and he or other project participants attended all REFCOM meetings, giving presentations on topics such as late season options for resistance management, moth movement and gene flow in the two *Helicoverpa* species, the risk status of *H. punctigera* vs *H. armigera* and the ecology of the two species in southern NSW. Peter also discussed the potential for moth busting in RMPs and RRMPs, as part of his work on Magnet® in other projects. These presentations contributed to discussions on refuge requirements, pupae busting and other changes for the Bollgard III RMP as well as more general discussions on resistance management.

Methods

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

The methodology used in the project was generally well established, and sampling and vegetation assessment techniques used were the same as those that had been used in previous projects, going back to the 1980s. This was a deliberate choice to ensure that data were compatible across a long time period.

Since there were several different aspects to this project, and the methodology for each is written up in detail in the four Appendices describing the work, it will not be further discussed here.

Results

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

Detailed results for the major activities of the project are also presented in the four Appendices attached to this report. Analysis is still underway for the data in the larval survey and vegetation record databases. Complex analyses will be required given the sheer volume of data. For example, the larval survey database contains over 118,000 data points and the vegetation assessment database contains over 71,000. Statistical exploration of these data is likely to be a long-term effort!

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.**

The outcomes for industry that were identified in the application were (1) better understanding of pest population dynamics in cotton and other crops, and (2) improved RMPs which better take *H. punctigera into* account.

The project has clarified the role of migration from the inland in the population dynamics of *H. punctigera*, providing additional information on the habitats and regions that contribute most to immigration, and the most important host plants. This has added to the knowledge that would be used in the development of regional forecasting systems for crops other than cotton. For cotton it has contributed to the ecological understanding on which resistance management plans are based. Recent changes in RMPs including the dropping of sorghum and corn as refuge options (because they do not support *H. punctigera* larvae), and (for Bollgard III) the elimination of pupae busting where defoliation has occurred by 31 March have been informed by results from this project and its predecessors.

6. **Please describe any:-**
   a) **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**

   The project was not expected to produce commercially valuable IP, rather it was intended to add understanding of the basic ecology of *H. punctigera*, so all of the IP will be in the public domain.

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

   On several field trips we collected many *H. punctigera* larvae into absolute alcohol for genetic analyses by T. Walsh and W. Tek Tay (CSIRO). This work will contribute to a better understanding of gene flow and population structure in *H. punctigera*, which may have implications for Bt resistance management.

   In the last two years of the project we also contributed data to the *PestFacts* group including the Centre for Environmental Stress and Adaptation Research (CESAR) and South Australian Research & Development Institute (SARDI). This facilitated forecasts of *H. punctigera* abundance for the southern grain legumes and canola industries.

   c) **required changes to the Intellectual Property register.**

   There are no changes required to the IP register

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?**

The main implications of our results are for resistance management plans for Bt cotton. In the past, *H. punctigera* was not considered to be a threat for resistance, because it had not developed resistance to conventional insecticides. This was thought to be because of gene flow resulting from frequent immigrations of unselected moths from the inland. That is, for *H. punctigera* there was a very large, distant, unstructured refuge.

However, from 2005 to 2009 there were indications of increasing resistance frequency in *H. punctigera*, and resistance management plans were adapted to include this species as well as *H. armigera*. Baker & Tann (2018, *Bulletin of Entomological Research* 107, 174–187) provided evidence of fewer immigrant moths arriving in cotton areas following the Millennium Drought (2001 – 2009). An important question is whether this reduction is permanent and therefore whether additional requirements are needed for RMPs.

Our results indicate that the reduction in immigration from the inland is likely to be long term, but not consistent and probably not permanent. A major finding was the reduction in good host abundance in the mulga regions of western Queensland. These form a bridge between the more highly productive habitats of the floodplains and sandy deserts in the far inland, which were less affected in the Millennium Drought. We believe late winter and early spring migrations from the far inland often do not reach cropping areas directly, but there is an intervening generation in the mulga regions which gives rise to moths which invade cropping areas in mid-late spring. Our results indicate fewer hosts and probably reduced seed banks in the mulga since the drought, which means that in the short term, even in very favourable springs such as 2016, the mulga is unlikely to contribute the unselected moths it once did.

However, we saw evidence in 2016 of patchy re-colonisation of these regions, and given that the major hosts have seed dormancy mechanisms, this re-colonisation is likely to continue, possibly sporadically, whenever favourable winter rainfall occurs. Also, when rainfall such as in 2016 does occur, such a wide area of the inland can be affected that some immigrants are almost certain to reach cropping areas, even though they are distant. Finally, our work in South Australia indicates that the winter grain legume and canola areas of South Australia and Victoria may receive moths in spring from different areas of the inland than those which colonise cropping areas. This includes the Great Victoria Desert and adjacent areas of Western Australia, which were not affected by the Millennium Drought, and which appear to be generally unaffected by El Nino periods. It is possible that, later in spring and summer, the next generation of moths from the southern grain areas may migrate north. This could explain the mid and late summer influxes we saw in the Namoi in 2014 and 2016.

For all these reasons we see potential for continued occasional immigrations of unselected *H. punctigera* moths to cotton areas. While the industry should be prepared for increases in resistance frequency from time to time which may suggest a developing problem, we see these as likely to be associated with prolonged drought but not being maintained after the droughts end, at least, not to the point where resistance in *H. punctigera* threatens the industry. We continue to see *H. armigera* as a more pressing threat. Our work has conclusively shown that very few *H. armigera* originate from the inland, and the large unstructured refuge that exists there for *H. punctigera* does not exist for *H. armigera*.

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.

Most of the project technology we developed was not novel and is readily available to researchers who may conduct similar broad scale insect monitoring work. We did however develop a portable 12 volt UV LED light trap suitable for use on field trips and may publish the design for it.

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

We have already extensively disseminated our findings through the cotton industry via the two *Australian Cottongrower* articles, presentations at conferences, and discussions in TIMS and REFCOM. We think the main opportunities in the future will be further publication in the scientific literature of papers based on the extended data set.

(c) for future research.

We see further research needs in:

1. Future occasional inland surveys to determine whether our predictions of sporadic but slowly increasing host abundance in the mulga country of southwestern Queensland are realised.

2. Population genetic studies using modern techniques which might show whether our postulated migration pathways from South Australia and Victoria to the north in late spring and summer exist, and whether there is back migration in autumn from cotton areas to the inland, which might lead to contamination of the inland refugia.

3. Identification of potential C4 hosts in the inland, with further studies to quantify their contribution to inland populations, and laboratory host preference studies which might indicate whether our hypothesis that C4 hosts are only utilised when good C3 hosts are not available is correct. We are in the process of enrolling a PhD student who will initiate some of this work.

4. We are aware of suggestions that broad scale pest monitoring approaches such as *Pestfacts* should be extended to cropping areas in northern NSW and southern Queensland. We believe it would be a mistake to confine these monitoring programs to cropping regions when pests such as *H. punctigera*, some armyworms, mirids, locusts etc can originate in non-cropping areas and migrate to infest crops. Remote monitoring is possible using smart traps such as the Trapview® designs we are evaluating as part of Project UNE1404, but there may also be a need for occasional inland surveys. We strongly recommend that any attempts at developing cross-industry area-wide monitoring and forecasting systems for these pests should include work in non-cropping regions in the inland.

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)

Refereed journal publications:

Gregg PC, Henderson GS, Del Socorro AP, Le Mottee K & Birchall C (2016). Polyphagy in an uncertain environment: *Helicoverpa punctigera* in inland Australia. *Austral Ecology*, 41, 819 - 828.

Gregg PC, Del Socorro AP, Le Mottee K, Tann CR, Fitt GP & Zalucki MP (2018). Host plants and habitats of *Helicoverpa punctigera* (Wallengren) and *Helicoverpa armigera* (Hübner) in inland Australia. Submitted to *Austral Entomology*.

Industry publications

Gregg P. (2017) Thirty years of *Helicoverpa* research in inland Australia. Part 1. The insects and their hosts. *Australian Cottongrower* **38** (4) 29-33.

Gregg P. (2017) Thirty years of *Helicoverpa* research in inland Australia. Part 2. The Millennium Drought. *Australian Cottongrower* **38** (5) 12-18.

Conference presentations

Binns M, Le Mottee K, **Gregg P** & Del Socorro, A. (2014) *Helicoverpa punctigera*: Local and inland overwintering biology. *REFCOM meeting, Goondiwindi, 17-18 September 2014, CRDC, Narrabri.*

Fitt, G, Downes S & **Gregg P.** (2014) The Australian experience: from on farm IPM to landscape level change. *62nd Annual Meeting of the Entomological Society of America, 16-19 November 2014, Portland, Oregon* A1205.

Le Mottee K, Gregg P & Del Socorro A (2015) Summer and Winter Diapause in *Helicoverpa punctigera*: adaptations for a changeable climate. *Australian Entomological Society 44th Annual General Meeting, Cairns, Queensland, 27-30 September.*

Le Mottee K, Gregg PC & Del Socorro AP (2016) Long term changes in host plants of *Helicoverpa punctigera* in inland Australia: Effects on migration patterns. *International Congress of Entomology*, Orlando, Florida, 26 -30 September 2016.

Del Socorro AP, Gregg PC & Le Mottee K (2016) Ecology and management of migratory *Helicoverpa punctigera* in Australia. *International Congress of Entomology*, Orlando, Florida, 26 -30 September 2016.

Le Mottee K, Del Socorro A & Gregg P. (2017) Host plants for *Helicoverpa* spp. in inland Australia: Impacts of the Millennium Drought. *Biosecurity: A Partnership Approach*. 48th AGM and Scientific Conference of the Australian Entomological Society, Terrigal NSW, 17-20 September p.12.

Gregg P, Del Socorro A & Le Mottee K. (2017) Long term studies of the ecology of *Helicoverpa* spp. in inland Australia. *Biosecurity: A Partnership Approach*. 48th AGM and Scientific Conference of the Australian Entomological Society, Terrigal NSW, 17-20 September p.9.

Del Socorro A, Gregg P & Le Mottee K. (2017) Abundance of *Helicoverpa* host plants in inland Australia before and after the Millennium Drought. *Cotton Science Delivering Impact.* Proceedings of the AACS Cotton Research Conference, Canberra, 5-7 September. Abstract S4S15.

Gregg P. (2017) Thirty years of chasing moths in the bush, and what have we learned? *Cotton Science Delivering Impact.* Proceedings of the AACS Cotton Research Conference, Canberra, 5-7 September. Abstract S4S11.

We have attached copies of the two refereed journal papers and two industry publications. Note that the 2018 manuscript is currently under review and should not be published on the CRDC website yet – we will replace it with the final version when it is published.

Future publication plans: The compilation of all our old data into the two databases on larval numbers and host abundance provides a unique record of long-term ecological changes that need to be written up, and we expect to publish at least another four refereed journal papers over the next 1-2 years.

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

No.

Part 4 – Final Report Executive Summary

This project aimed to provide ecological information on an important pest species to underpin strategies for resistance management in Bt cotton. The cotton industry relies on Bt, now in Bollgard III® varieties, which provide security to growers, enable new farming systems and reduce pesticide use, ensuring continued social license to farm. At the time the project began, these benefits were threatened by rising frequencies of alleles giving resistance to Bt, especially to the Cry2Ab toxin in *H. punctigera*. Current resistance management plans were designed for *H. armigera*, but now they also had to consider *H. punctigera*. Updating our understanding of *H. punctigera* was therefore crucial. We needed to revisit key questions such as immigration from the remote inland, which was previously thought to be extensive

and a valuable asset for resistance management, because it brought genes from unselected populations into cropping areas, thus diluting resistance.

For the previous three years, we had studied the ecology of *H. punctigera* in western Queensland, and compared results to the work we and others did in the 1980s and 90s. We had found that many remote inland areas still regularly produced many *H. punctigera,* but there was evidence of decreasing immigration to cropping areas. We speculated that the Millennium Drought of 2001 -2009 may have changed the distribution and abundance of key host plants, especially in mulga regions of western Queensland which act as a bridge to enable migration from the floodplains and sandy deserts of central Australia to cropping regions. However, in such a variable environment as inland Australia, we needed more data. This project aimed to provide better understanding of long term changes in *H. punctigera* populations that might affect pest impacts on cotton and other crops, and management of resistance to Bt in cotton.

We established ten pheromone trapping sites, six in western Queensland and four in non-cropping regions of South Australia, to monitor moth populations. Results showed fewer moths than before the Millennium Drought in parts of western Queensland, but substantial numbers in some South Australian sites, suggesting alternative migration routes from the inland. Eleven survey trips were made to inland regions, during which larval populations were sampled by sweep netting, and vegetation conditions and the presence of host plants were recorded. These results indicated a depletion of good host plants in the mulga regions since the drought, which probably contributed to reduced migration. We also studied diapause induction and termination and the timing of spring emergence in inland populations, and the potential for host plants with the C4 photosynthetic pathway to contribute to inland populations.

We added results from this project to data from earlier projects in a geographic information system which provides a long-term record that is unique in the study of insect pest ecology in Australia. The information will be crucial for the development of pest forecasting systems for a range of crops affected by *H. punctigera*. For cotton it has provided a long-term perspective of changes in resistance to Bt which indicates that increases in the frequency of resistance alleles in *H. punctigera* may occur from time to time due to prolonged droughts. However such increases are unlikely to be sustained, and the greatest resistance risk continues to be posed by *Helicoverpa armigera* rather than *H. punctigera*, because this species does not develop large populations in the inland.

Appendix 1. Pheromone trapping at inland sites, 2014 – 2017

Project UNE1502, for Final Report

Introduction

Pheromone trapping has been a critical component of Project UNE1502 and previous similar projects, both before and after the Millennium Drought. The aim is to obtain long-term trends in moth populations in key inland areas. In UNE1502 we continued traps in western Queensland sites that were established during Project UNE1109 (Bedourie, Birdsville, Windorah, Eromanga, Thargomindah and Eulo), and established four new sites in inland South Australia (Minnipa, Yardea, Kingoonya and Roxby Downs). These locations are shown in Fig. 1. At each of these sites we set up two AgriSense pheromone traps 50 m apart, both baited with AgriSense laminate lures for H. punctigera. They were serviced weekly by local collaborators who were paid $500 per year. Trap catches have been converted to average moths per day per trap.

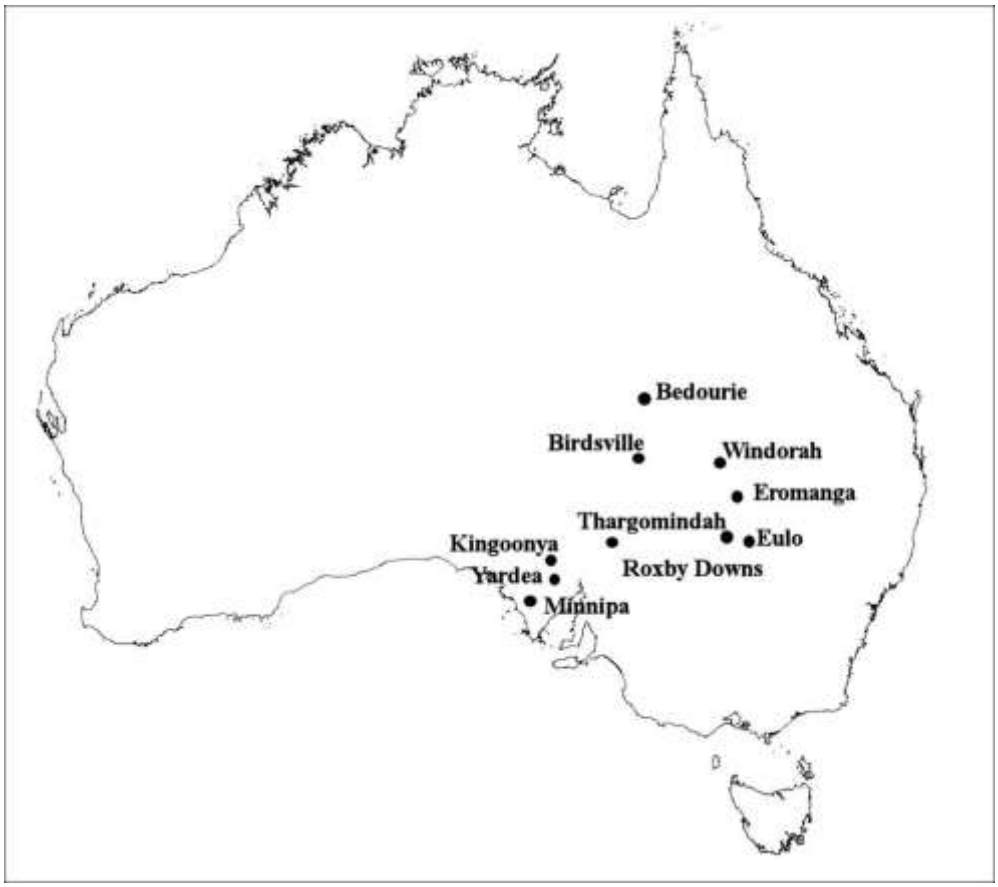


Fig. 1. Sites of pheromone traps in Project UNE1502

Results and discussion

Trap catches at each site are shown in the following ten figures, standardised to a maximum of 15 moths/trap/day except for Minnipa, where higher catches necessitated a maximum of 75 per day.

While the numbers varied considerably between sites, the patterns of catches fell into two categories:

1. More inland sites which are presumably source areas (Bedourie, Birdsville, Windorah, Roxby Downs). These sites are all in either floodplains or sandy deserts, habitats known to produce high numbers of H. punctigera if rainfall or flooding has produced favourable host growth. In these sites, while there was often a peak in trap catches in late winter/early spring, in some years but not others there were catches at other times of the year, including mid-winter and summer.

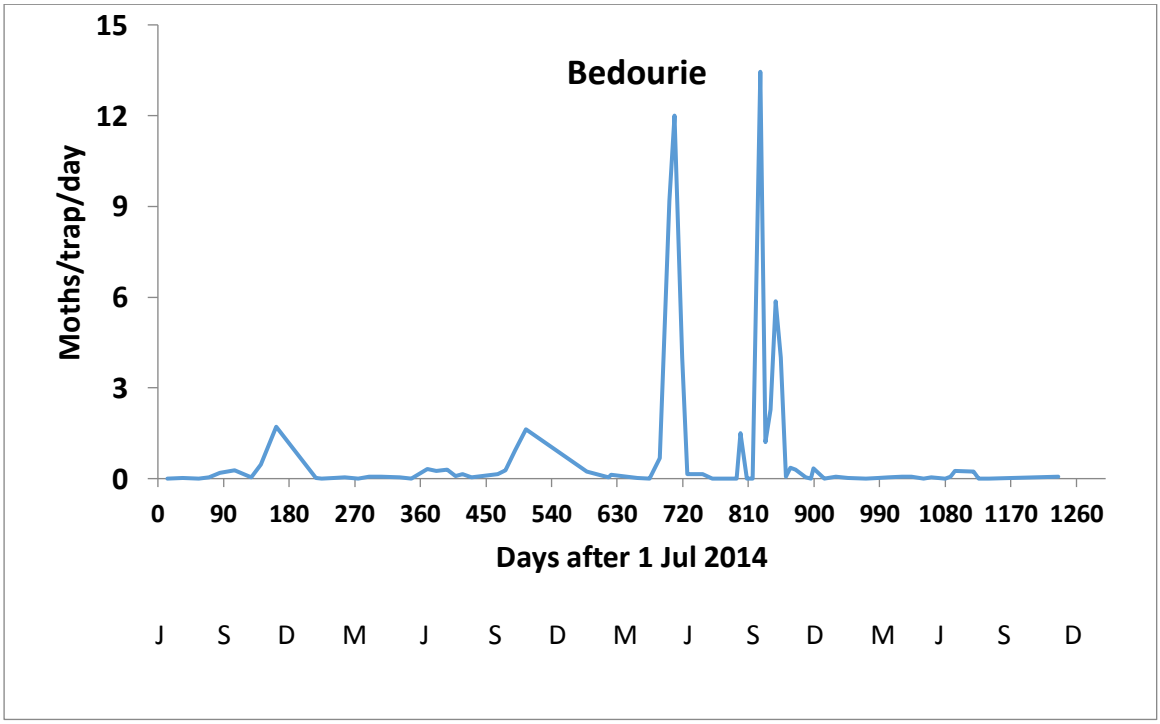
2. More peripheral areas (Eromanga, Thargomindah, Eulo, Kingoonya, Yardea and Minnipa) where peaks in trap catches were either absent or restricted to late winter/early spring. At Minnipa which is on the northern edge of the South Australian wheat belt these peaks occurred every year, but in sites in the mulga country of western Queensland (Eromanga, Thargomindah and Eulo) they occurred mostly in 2016, with a smaller peak in 2015 and little or no activity in 2014 and 2017. At Yardea and Kingoonya, in the saltbush country of South Australia, there were only low peaks, mostly in the spring of 2015.

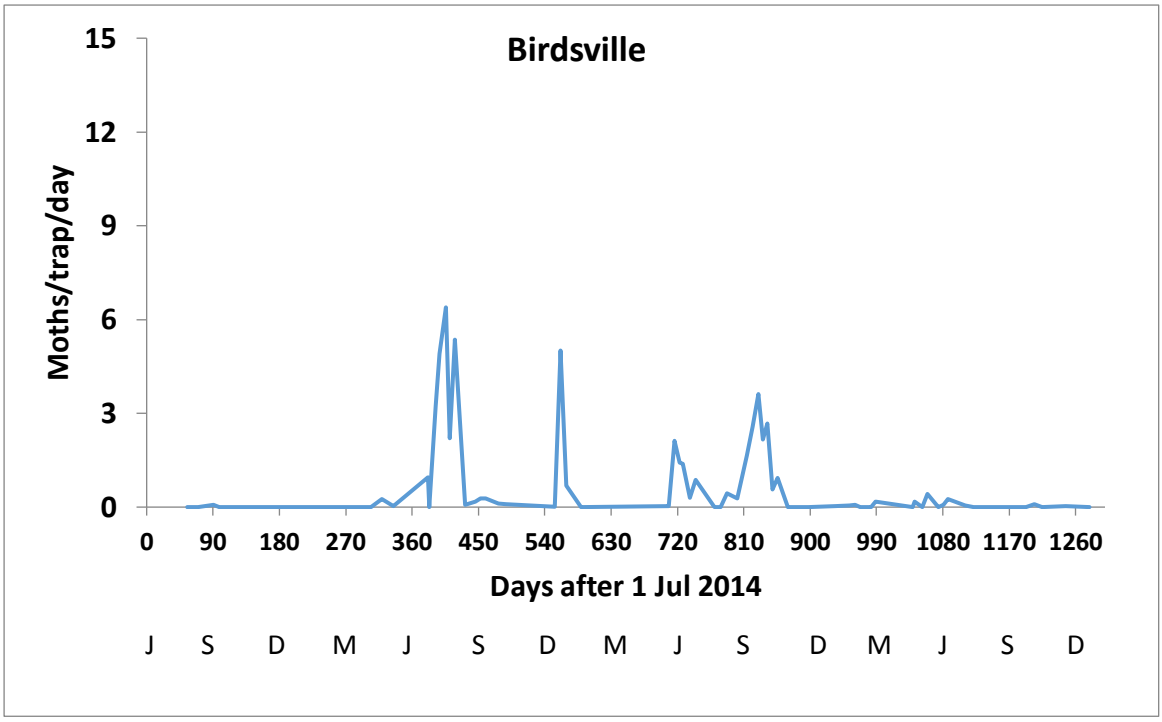
These results suggest that moths originated from the inland areas and migrated to the south and east, and in some years (eg 2016) these migrations were large, but in others they were not. The large catches at Minnipa suggest that immigrants were supplemented by local overwintering. While it is difficult to discern populations in autumn which may have given rise to such overwintering moths, there are difficulties with the pheromone for H. punctigera in summer and autumn which can lead to under-estimation of populations at this time (Baker *et al.* 2011, *Bulletin of Entomological Research* 101, 9–23

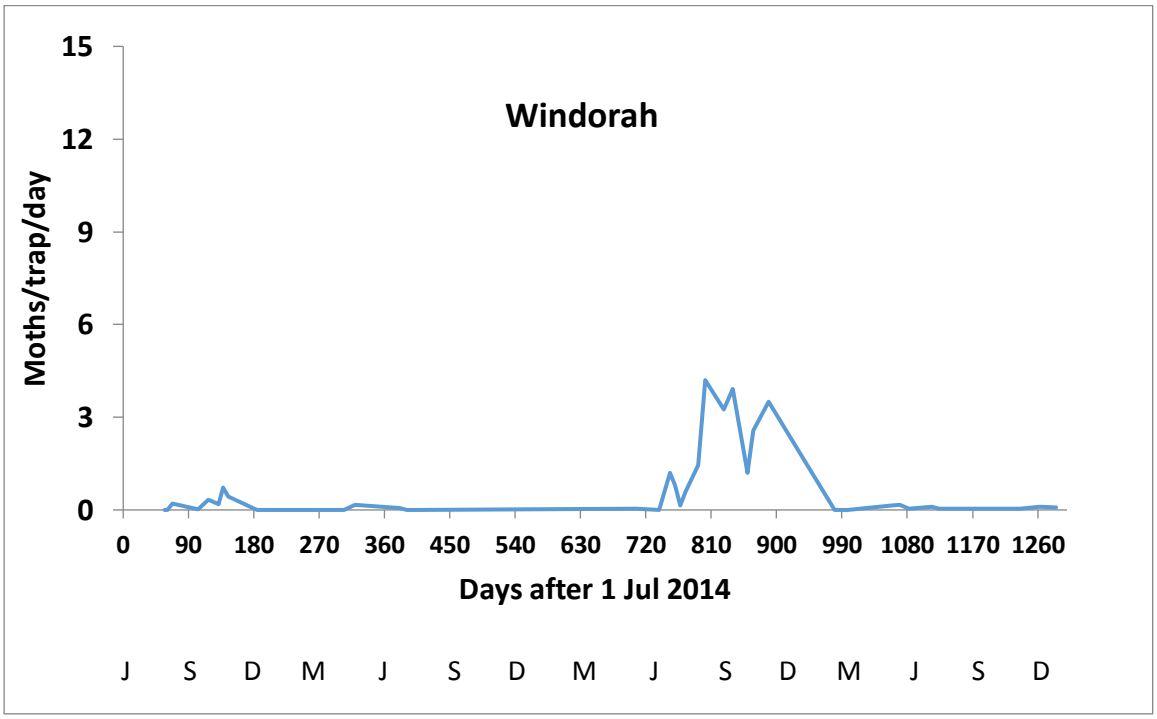
The pheromone trap data will be analysed along with similar data from Project UNE1109 (2011-2014) and earlier work from the Heliothis Inland Research group (1987-1993) with the aim of establishing patterns of moth numbers over an extended time frame in these areas.

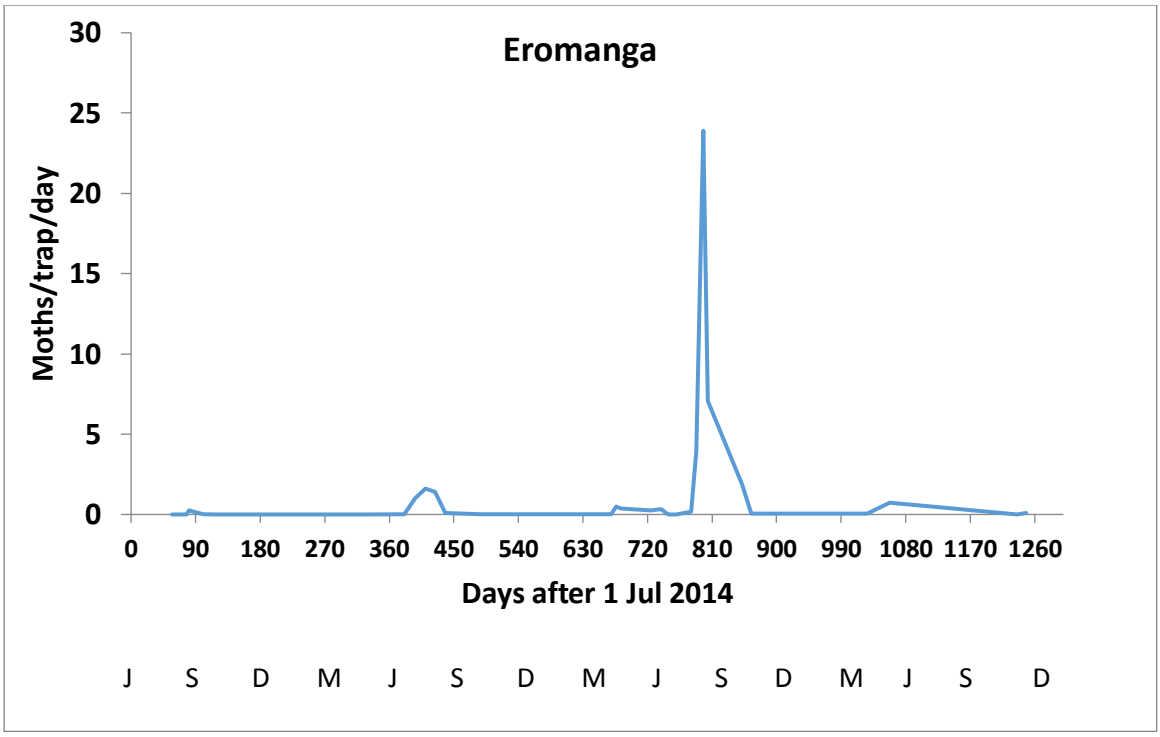
Graphs of pheromone catches for each site follow:

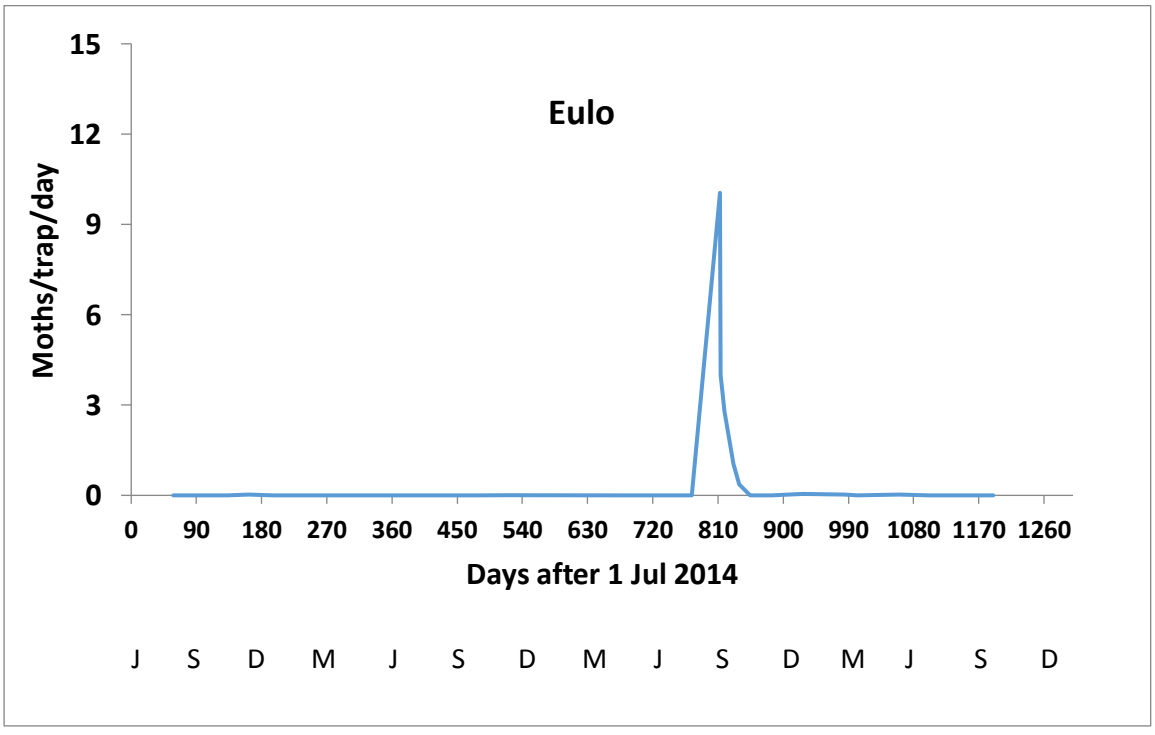
Western Queensland

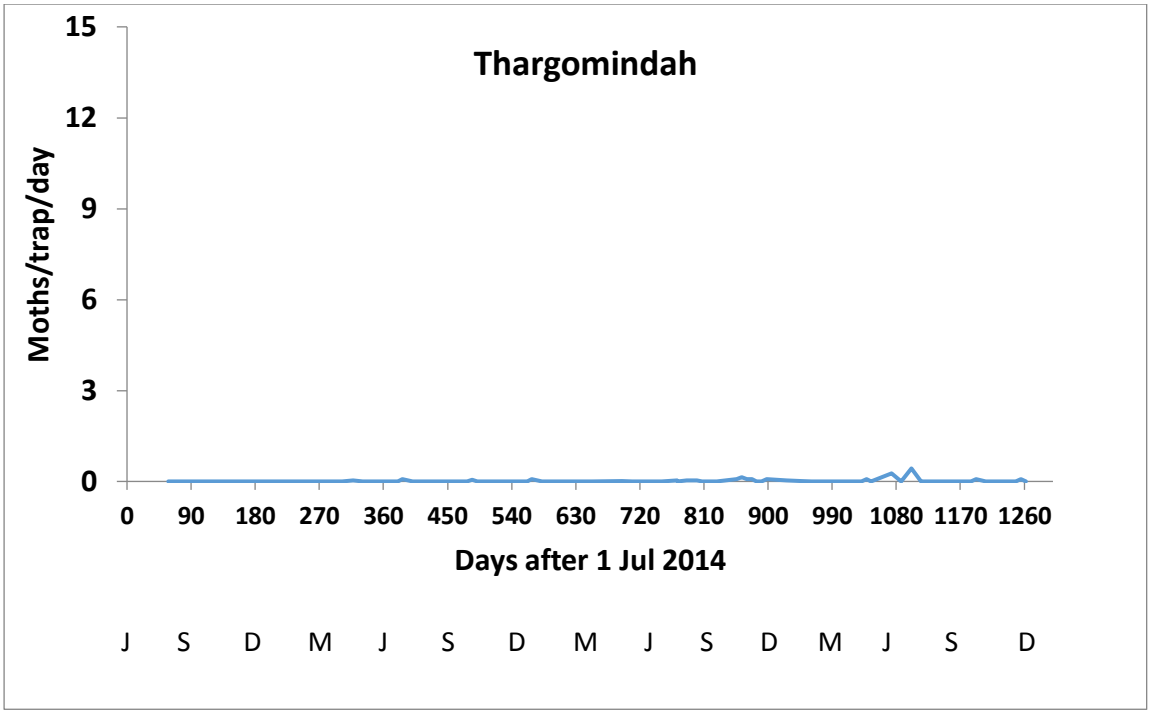



Birdsville
Moths/trap/day
15
12
9
6
3
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days after 1 Jul 2014
J S D M J S D M J S D M J S D

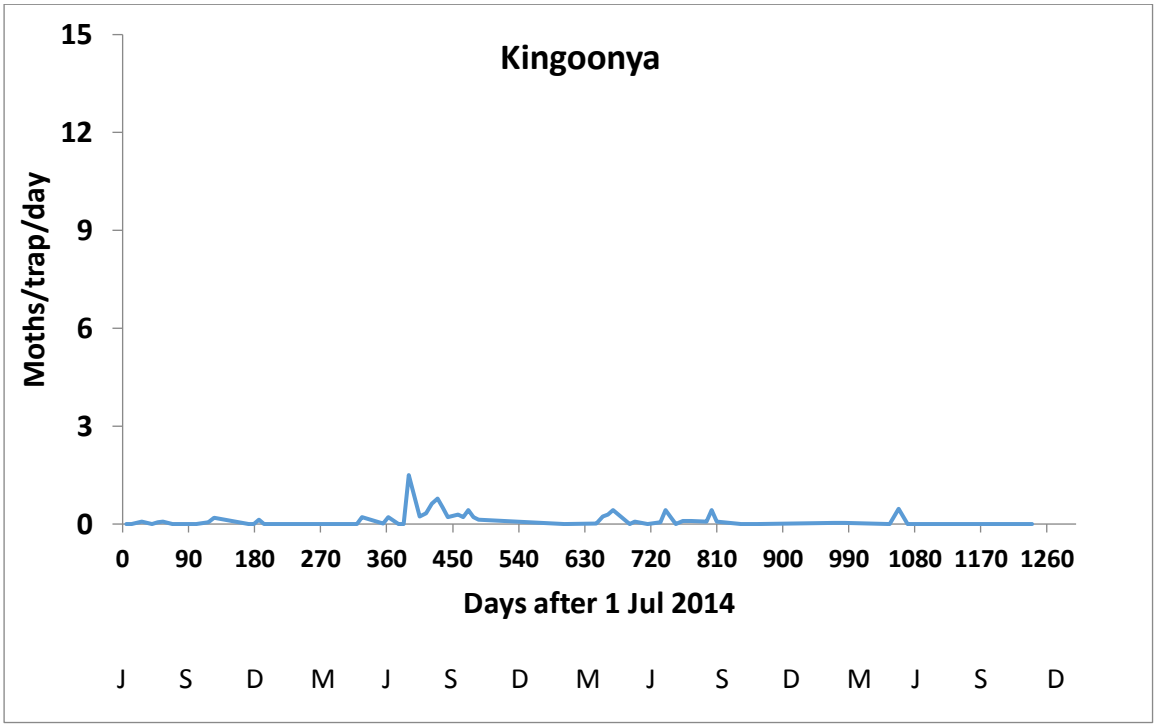

Windorah
Moths/trap/day
15
12
9
6
3
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days after 1 Jul 2014
J S D M J S D M J S D M J S D

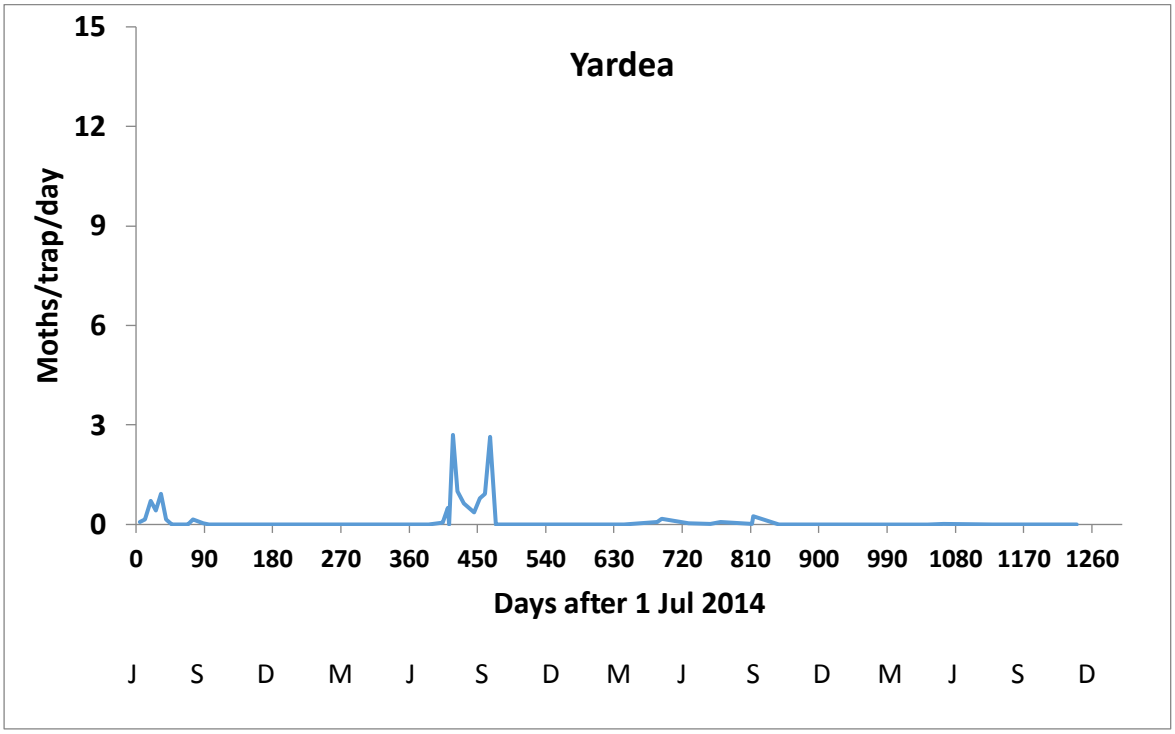

Eromanga
Moths/trap/day
30
25
20
15
10
5
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days after 1 Jul 2014
J S D M J S D M J S D M J S D

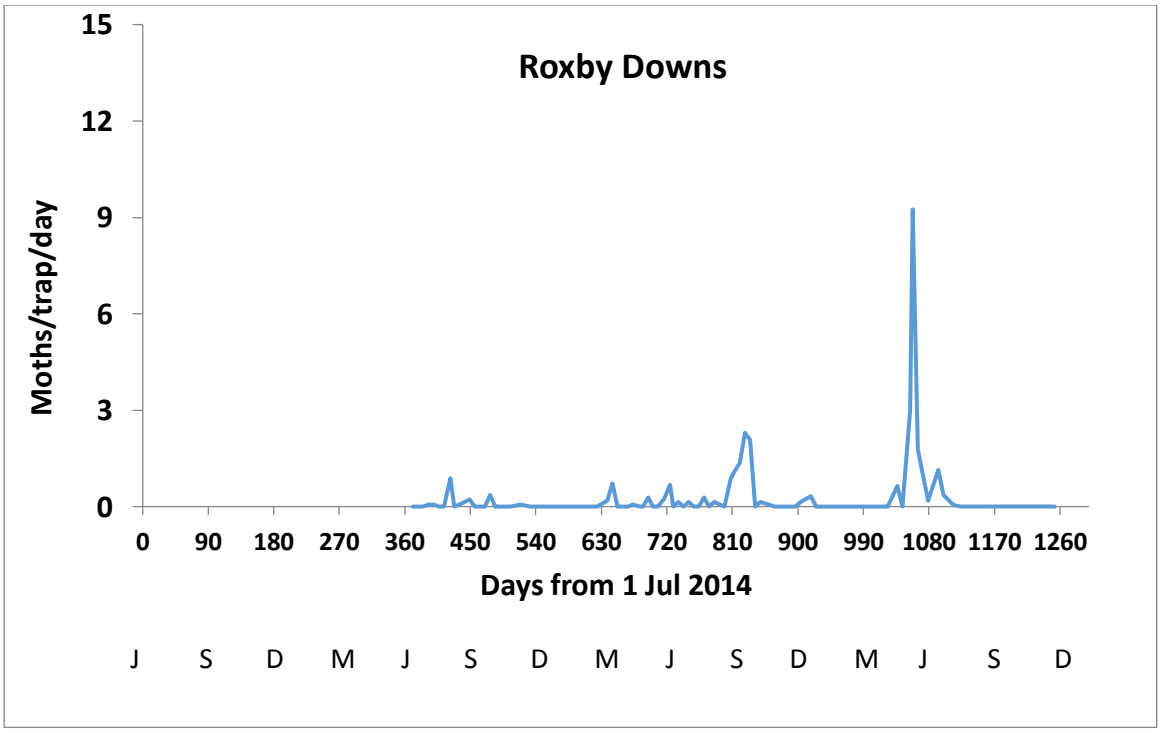

Eulo
Moths/trap/day
15
12
9
6
3
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days after 1 Jul 2014
J S D M J S D M J S D M J S D

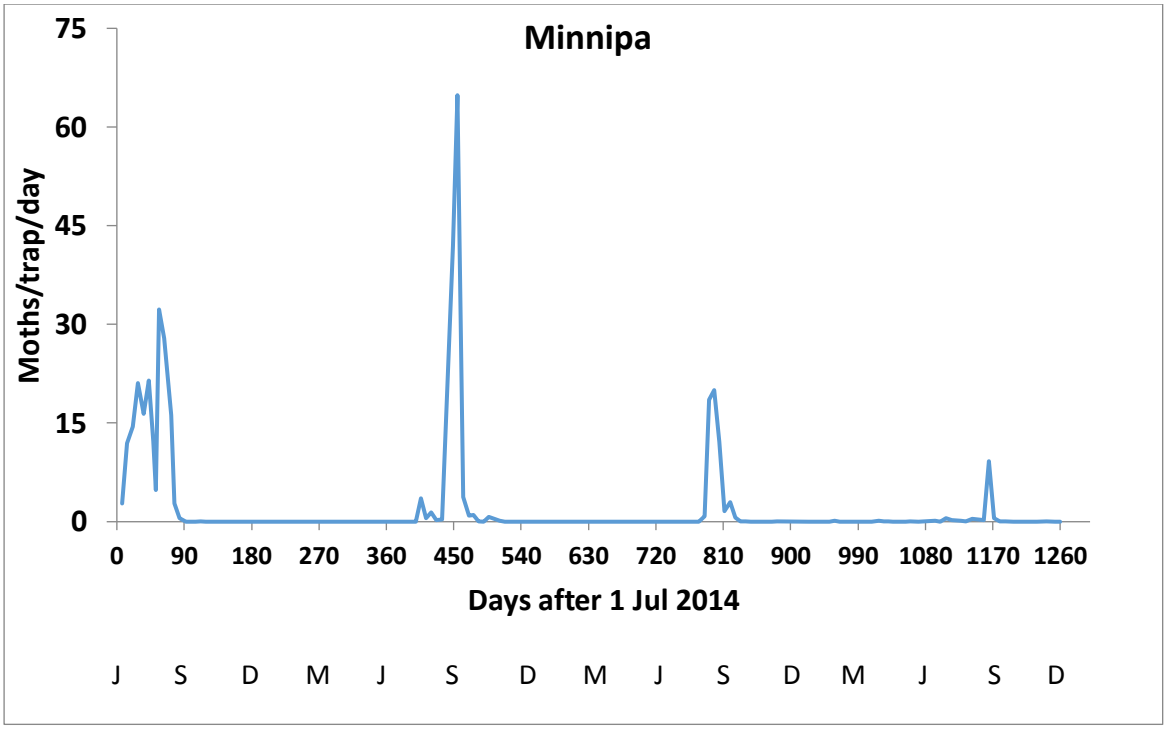


South Australia




Yardea
Moths/trap/day
15
12
9
6
3
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days after 1 Jul 2014
J S D M J S D M J S D M J S D


Roxby Downs
Moths/trap/day
15
12
9
6
3
0
0 90 180 270 360 450 540 630 720 810 900 990 1080 1170 1260
Days from 1 Jul 2014
J S D M J S D M J S D M J S D


Minnipa
Moths/trap/day
75
60
45
30
15
0
0
90
180
270
360
450
540
630
720
810
900
990
1080
1170
1260
Days after 1 Jul 2014
J
S
D
M
J
S
D
M
J
S
D
M
J
S
D

Appendix 2

Reports of field trips 2014 – 2017 for CRDC Project UNE1502, compiled for the Final Report

CRDC Project Inland Trip Report
11-15 June 2014

Peter Gregg and Alice Del Socorro

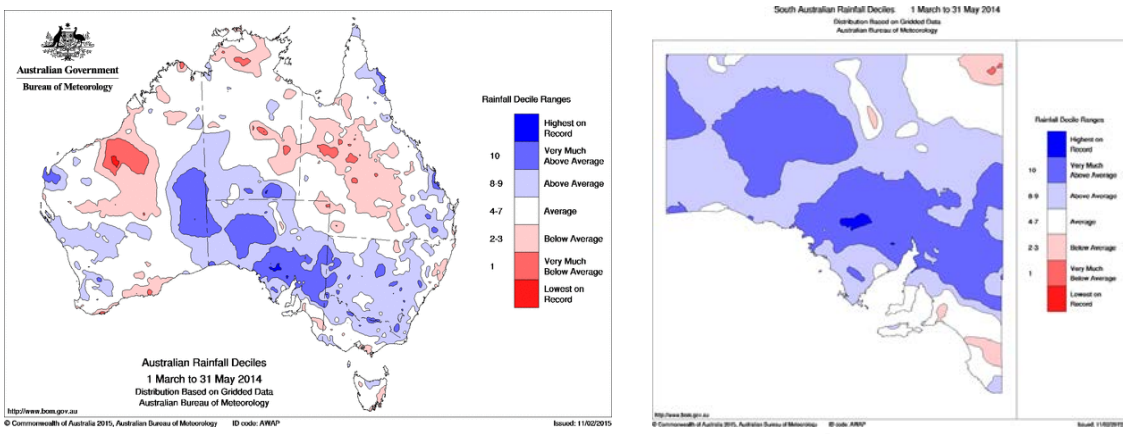
Objectives

The objectives of the trip were: (1) to survey the area north of Ceduna, along Goog's Track and then east through Tarcutta to Kingoonya, for possible *H. punctigera* breeding, (2) to establish some pheromone trapping sites in this region.

Background

Previous work in the HIRG (1991) established the Great Victoria desert of western South Australia as potential breeding habitat for *H. punctigera* following suitable rain. Vegetation and soil maps indicated that this potential area might extend further south, towards Ceduna and the Eyre. We therefore wished to assess the vegetation in this area, and recruit collaborators to run pheromone trapping sites.

Good rain had fallen in the area in April and May, and further out into the Great Victoria desert, whereas most of the rest of inland Australia had been dry during this time (Fig/1)



(a) (b)

Fig. 1. Rainfall deciles for March-May 2014 (a) Australia, (b) South Australia

Methods

Peter Gregg and Alice Del Socorro made the trip during a break from field work on canola at Cummins. Sampling and vegetation assessment methods were as for those used in our previous CRDC project. The route taken is shown in Fig. 2.

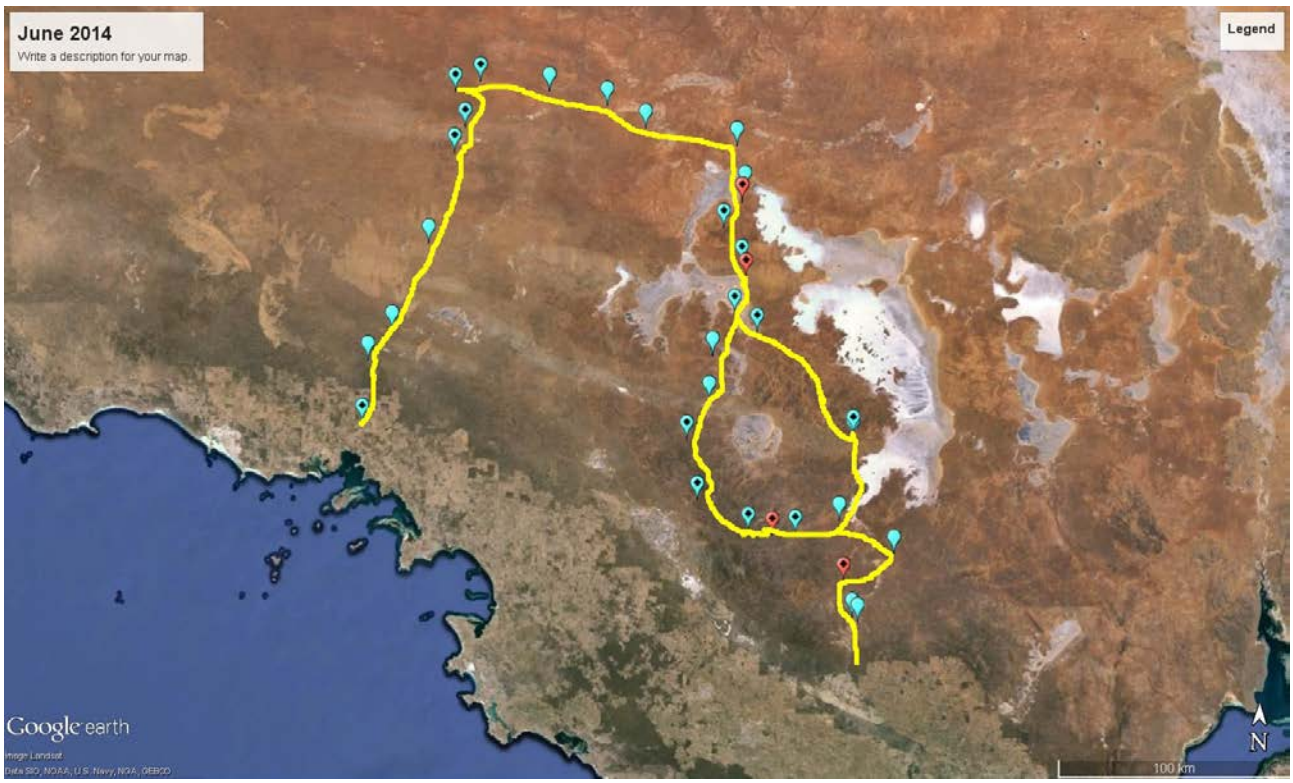


Fig. 2. Route and survey sites. Blue marker with no dot means vegetation survey with no *Helicoverpa* hosts present. Blue marker with dot represents a vegetation survey where hosts were present. Red marker with no dot represents a negative larval survey. Red marker with dot represents a positive larval survey

Trip log

11/6/14 Ceduna to Mt. Finke

Goog's Track is a moderately difficult 4WD track heading north from wheat and canola farming country north of Ceduna, up to the Transcontinental Railway. In farming country around Ceduna there was canola and medic in uncropped areas, but no sampling was undertaken. North of this the vegetation consisted of dense mallee scrub. The southern part of Goog's Track had been burnt by a major bushfire and no hosts were apparent, but it is likely that even without the fire there would have been none, as areas north of the fire zone did not support hosts, probably due to the density of the mallee (Fig. 3). When the mallee thinned out, around the turnoff to Mt. Finke, scattered *Myriocephalus stuartii* became apparent, but no sampling was done. We camped overnight near the Mt. Finke turnoff.

12/6/14 Mt. Finke to Kingoonya

Areas of old dry *Helipterum floribundum* and *Myriocephalus stuartii* from previous seaons were seen, indicating the potential of this area to generate host plants. However, the green vegetation which would have been germinated by the Aporil-May rain was still very low, and though it contained some broadleaf plants as well as grasses, it was not possible to identify any hosts, or do any sweeping

At Kingoonya we established a pheromone trap site, to be looked after by the local publican, Alistair Murray

13/6/14 Kingoonya to Mt. Ive

South of Kingoonya we saw some host plants including a probable Geraniaceae (not identifiable to species because it was not flowering), and *Senecio gregorii* (mostly not flowering but a few early flowering plants and high enough to sweep, Fig. 4). Low to moderate numbers of larvae were recovered from these hosts. Further south, around "Hiltalba and east to 'Yardea" these hosts were replaced by medics, including *M. polymorpha* but probably also other species. These were very green (Fig. 5) but fairly low.

14/6/14 Around Mt. Ive

We first travelled north to "Moonaree", where we sampled on medics and recovered 28 larvae in 100 sweeps, then returned to "Yardea" where we established a pheromone trap, looked after by "Snake" and the manager, Sandy Morris. Additional sampling on medics here produced a few larvae on the "geranium", but not on the medic. We also noted extensive areas of Ward's weed (*Carrichtera anuua*, Brassicaceae) which supported substantial numbers of diamondback moth larvae. This and subsequent sampling for this pest is reported elsewhere.

15/6/14 Mt. Ive to Cummins

After completing further sampling on medics towards "Buckleboo" we returned to Cummins

A summary of larval surveys is given in Table 1.

Table 1. Summary of larval surveys

Date	Location	Host plant	Total larvae collected (per 100 sweeps)
13/6/17	25k S Kingoonya	Unknown "Geranium"	9
13/6/14	67k S Kingoonya	Senecio gregorii	5
14/6/14	"Moonaree"	Medics	28
14/6/14	"Yardea"	unknown "Geranium"	3
"	"	Medics	0
15/6/14	46 k S "Yardea"	Medics	0

Summary and conclusions

The area around the southern part of Goog's Track is unlikely to be a significant source of overwintering *H. punctigera* larvae because of the dense growth of mallee scrub, which appears to crowd out or exclude by allelopathy any host plants. Further north, in more open and sandy country around Tarcoola, Kingoonya and south towards "Moonaree" there are likely to be more hosts, but the growth rate of these hosts appeared to be very slow, since they were either very low, or just beginning to flower, even though it had rained in April. It is likely that these areas are too cold to support true winter breeding, perhaps like the mulga country east of about Eulo in Queensland. However, they might be a significant stepping stone for invasion of the cropping areas of South Australia in August and September, esapecially given the extent of medics in the area, and would be worth surveying again at these times after good winter rain.

Fig. 3. Dense mallee scrub in the southern end of Goog's Track, with no host plants underneath

Fig. 4. Early flowering scattered plants *of Senecio gregorii*, with denser growth of pre-flowering plants underneath, south of Kingoonya

Fig. 5. Dense lush growth of medics and ward's weed near "Moonaree"

CRDC Project Inland Trip Report
21-28 August 2014

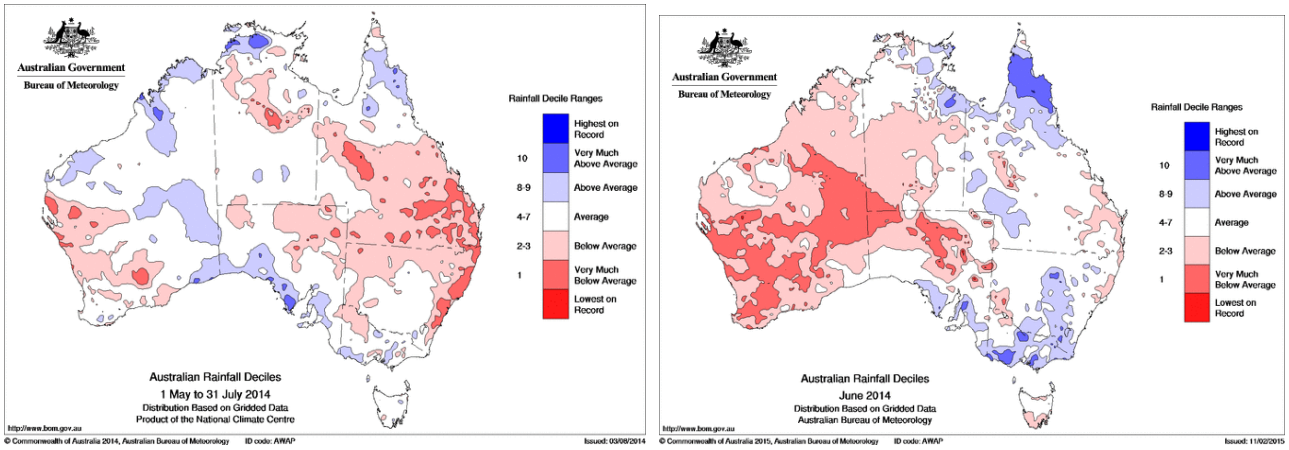
Peter Gregg and Alice Del Socorro

Objectives

The objectives of the trip were: (1) to establish new sites and collaborators at Roxby Downs and Wiudorah, (2) to re-establish contact with collaborators in our previous project, and to explain the new system of payment and complete the paperwork, and to refurbish the traps and leave supplies, and (3) to survey the vegetation and sample for larvae en route.

Background

This trip was undertaken to return the vehicle from field work on canola in the Eyre Peninsula, and was therefore a one-way trip. It had been generally dry through most of the route, except for parts of South Australia immediately north of the Eyre Peninsula (Fig. 1a). However in June there had been a little rain in the area around Eromanga in western Queensland (Fig 1b).



(a) **(b)**

Fig. 1. Rainfall prior to the trip (a) 3 months to July 2014, (b) June 2014 only

Methods

Methods for larval sampling and vegetation rating were as described for earlier trips. Pheromone traps were replaced using the new design of AgriSense traps, secured to the posts by metal strips that were bolted to the side of the traps. The route followed is shown in Fig. 1

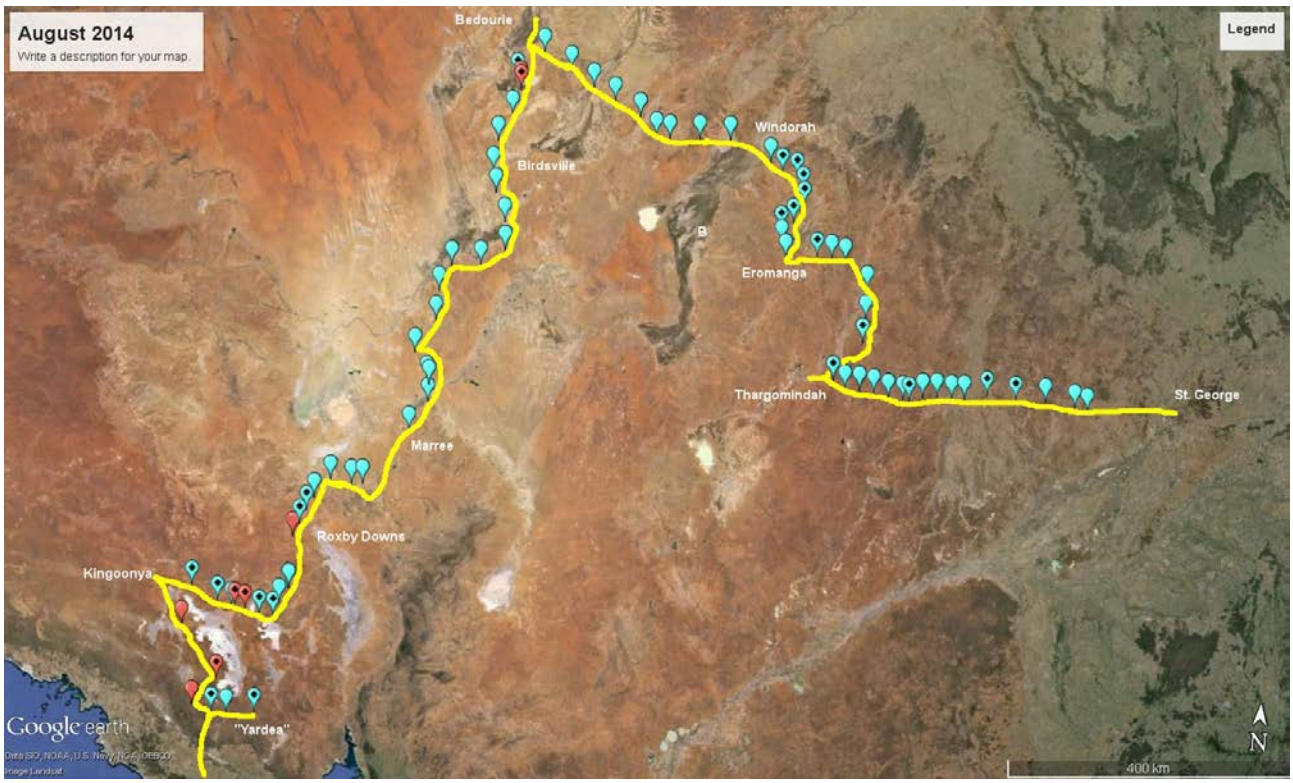


Fig. 2. Route and survey sites. Blue marker with no dot means vegetation survey with no *Helicoverpa* hosts present. Blue marker with dot represents a vegetation survey where hosts were present. Red marker with no dot represents a negative larval survey. Red marker with dot represents a positive larval survey.

21/8/14 Cummins to Kingoonya

Sampling started north of the Gawler Ranges, around "Yardea". From here east to "Mt. Ive" and north to "Moonaree" there were extensive areas of *Medicago polymorpha*, but no daisies Sampling on the medic produced no larvae. The first daisies (*Senecio gregorii* and *Myriocephalus stuartii*) were seen 15 km south of "Moonaree", and sampling produced only 6 larvae in 80 sweeps. Another sample on *Myriocephalus stuartii* 70 km south of Kingoonya (Fig. 3) produced nothing.

We replenished stocks of lures and pest strips at "Yardea" where we had established a pheromone trap site in June, but were not able to contact the collaborator.

22/8/14 Kingoonya to Marree

Daisies including *H. floribundum, S. gregorii* and *M. stuartii* were seen east of Kingoonya and south from Glendambo towards Pimba, on sandy soils. On harder stony downs country only loow button daisies were found. Sampling at two sites in this area produced only low numbers of larvae. North of Pimba conditions became very dry. More daisies (but only scattered) were seen in sandy country around Roxby Downs and north of Olympic Dam, but a sample here did not produce any larvae. The soil and perennial vegetation however suggest this area might produce many host plants given suitable rainfall. North of this area the country reverted to mostly stony downs, with conditions very dry and no hosts present

We established a pheromone trapping site run by the Arid Recovery organisation at their field site north of Olympic Dam (Perri Carter, collaborator).

23/8/14 Marree to Birdsville

Conditions were extremely dry, with no hosts seen on any vegetation type, including the Cooper floodplain and the Diamantina floodplain at Birdsville. In many places, especially in stony downs country, there was no herbaceous vegetation at all.

24/8/15 Birdsville to Bedourie

Conditions continued extremely dry north of Birdsville, with the only hosts being found in the Eyre Creek floodplain around Cuttaburra Crossing (116 km north of Birdsville), where small patches of *Senecio lautus* were found in low-lying areas, along with even smaller patches of *Cullen cinereum* along the roadside (Fig. 4). Sampling on both these hosts produced moderate numbers of larvae, despite the fact that they were only very small patches and no other hosts were found within at least 50 km. Further up the Eyre Creek floodplain, around Bedourie, it was obvious no floodwater had escaped the main channel, it was very dry, and there were no hosts.

At Birdsville we replaced the pheromone traps and established new collaborators: Lyn and Don Rowlands (National Park ranger).

25/8/15 Bedourie to Windorah

We refurbished the traps at Bedourie, including shifting one trap about 50 m north because of road works, made contact with the collaborators and left new supplies. Conditions continued extremely dry, with no host plants seen on the Diamantina floodplain at "Monkira", and no evidence of any flooding.

26/8/14 Windorah to Thargomindah

At Windorah we recruited a new collaborator, Robert Beiby, and shifted the traps from the school yard to a location nearer the floodplain, on the southern edge of town. The Cooper floodplain was very dry, with no host plants. Between Windorah and Eromanga the only host plants seen were recently germinated grasses, and *Sida platycalyx* seedling (Fig 5), with occasional very small *Helipterum floribundum* and *Goodenia* sp. All these hosts were too low and/or too scattered to sweep, and will not make it to flowering without further rain. From Eromanga through Quilipe and down to Thargomindah conditions were even worse, with only scattered *Sida platyclayx* and Goodenia seen in two places. At Thargomindah we made contact with the collaborator and left fresh supplies, but did not replace the traps.

27/8/14 Thargomindah to St, George

Vegetation remained low and dominated by grasses, which in many places appeared heavily grazed down to about 2 cm high. Host plants were seen in only one location, and then it was only low *Sida platycalyx*. Grasses on the Warrego floodplain near Cunnamulla were heavily grazed but there were no broadleaf plants emerging between the tussocks

At Eulo we made contact with the collaborators and left new supplies. We replaced the traps, moving them a few meters to get them further from the trees.

28/8/14 St. George to Armidale

No further sampling or vegetation monitoring was done

A summary of larval surveys is given in Table 1.

Table 1. Summary of larval surveys

Date	Location	Host plant	Total larvae collected (per 100 sweeps)
21/8/14	15k S "Moonaree"	*Senecio gregorii*	6
21/8/14	70k S Kingoonya	*Myriocephalus stuartii*	0
22/8/14	46k S Glendambo	*Helipterum moschatum*	4
22/8/14	60k S Glendambo	*Helipterum moschatum*	3
22/8/14	21k N Olympic Dam	*Myriocephalus stuartii*	0
22/8/14	60k N Olympic Dam	*Cullen cinereum*	0
"	"	Unknown daisy	0
24/8/14	116 k N Birdsville	*Senecio lautus*	9
24/8/14	121k N Birdsville	*Cullen cinereum*	22
"	"	*Senecio lautus*	8

Summary and conclusions

This survey revealed very little potential for winter breeding and spring migration in inland Australia. Conditions were very dry except in the southern part of South Australia, where host plants were found but they supported very few larvae. Further north in South Australia, and in western Queensland, there were virtually no hosts. None of the inland floodplains had any significant flooding, except for a very small area of the Eyre Creek floodplain around "Glengyle", where some larvae were found on small patches of *Cullen cinereum* and *Senecio lautus*. This provides further evidence of the remarkable capacity of *H. punctigera* to locate isolated areas of host plants in large areas of surrounding dry habitat, but these populations will not contribute significantly to spring migrations.

The survey located areas that are likely to support good host growth north of the Gawler Ranges, and around Roxby Downs, and although hosts were only scattered and did not support many larvae on this occasion, they will be worth watching following suitable rainfall in autumn and winter in future years.

Around Eromanga where some limited rain had fallen in June there was evidence of some host plants, but these were mostly *Sida platycalyx* (not a particularly good host), along with scattered very small *Helipterum floribundum* and *Goodenia* spp., which were already flowering even though they were only a few cm high. These plants, although they are better hosts, will also not support many larvae due to their size, and they are now rapidly senescing.

The vegetation in western Queensland is losing the extensive growth of grasses that followed the heavy summer rains of 2010-2012, with most of it now being grazed off. There are now large areas of bare ground in many regions. This means that if there is good autumn or winter rain in the next season, we might expect less competition from grasses and more broadleaf hosts, if the seed banks of these hosts are still functional

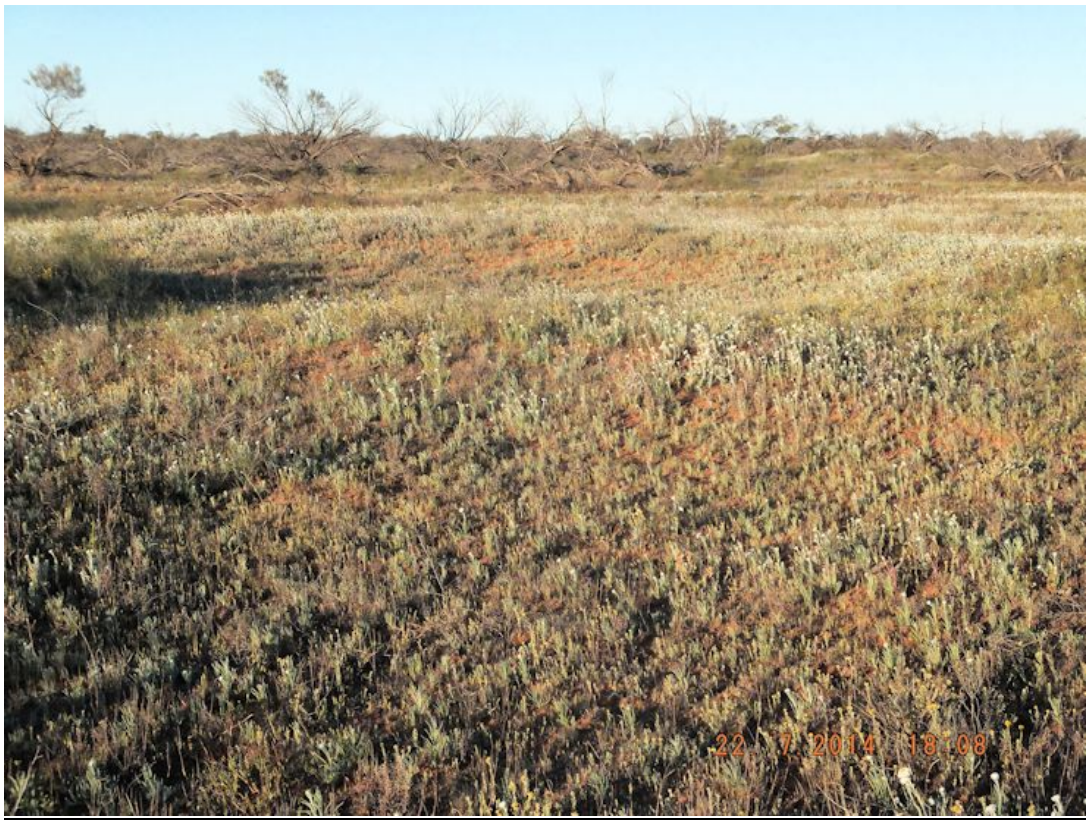


Fig. 3 *Myriocephalus stuartii* in sandy areas south of Kingoonya

Fig. 4. Isolated stand of *Cullen cinereum* and *Senecio lautus*, roadside only, Eyre Creek floodplain near "Glengyle".

Fig. 5 Low vegetation near Eromanga, showing seedlings of *Sida platycalyx*, probably germinated from rain in June

CRDC Project Inland Trip Report
14-21 July 2015

Peter Gregg and Alice Del Socorro

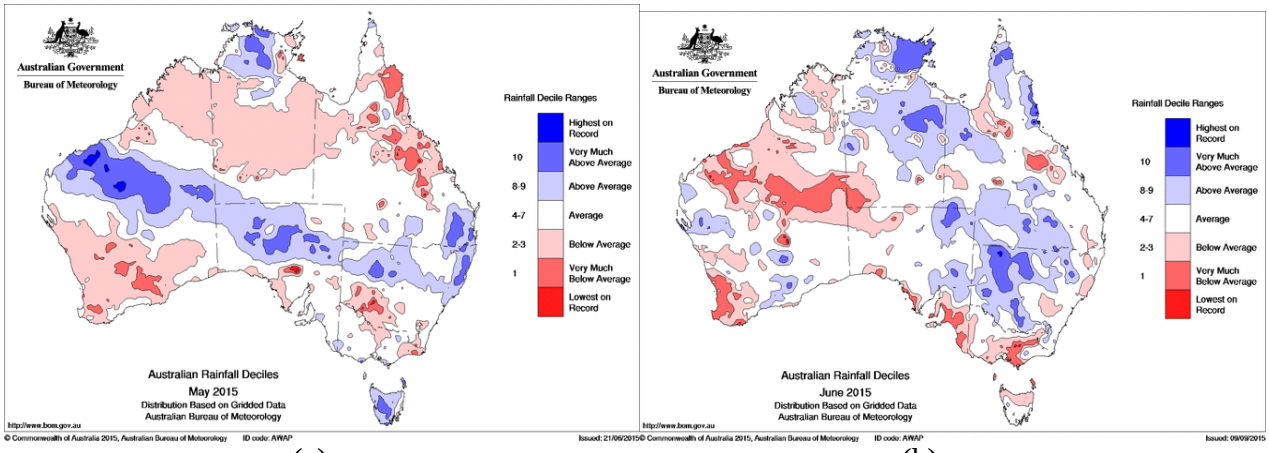
Objectives

The objectives of this trip were to survey areas of western Queensland and northern SA following rain in May and June which had been patchy, but moderately heavy in some locations. We also planned to contact collaborators and refurbish some traps and permanent vegetation markers along the way..

Background

The trip was planned as a one-way survey of western Queensland and South Australia, as it was intended to leave the vehicle at Cummins for winter work on canola, and fly back to Armidale. As well as vegetation surveys and sweep netting for larvae, it was intended to refurbish some of the permanent marker sites with new posts, to contact current collaborators and establish a new site at Roxby Downs.

After a prolonged dry spell from February to April, moderate rain had fallen in the arid zone of South Australia in May (Fig 1a) and in the Warrego area of Queensland and around Birdsville in June (Fig. 1b). These conditions might have been expected to germinate some host plants.



(a) (b)

Fig. 1. Rainfall deciles for May (a) and June (b) in 2015

Methods

The trip was conducted between 14/7 and 21/7/15 by Peter Gregg and Alice Del Socorro. Methods for sampling and vegetation scoring were as for previous trips, except that visual samp0les were necessary in some areas because the host plants were too low to sweep. Timed samples, in which the operator searched as many host plants as possible during a 15 min interval, were used as hosts were often too patchy to do visual sampling by fixed areas. The route taken is shown in Fig. 1.

Fig. 2. Route and survey sites. Blue marker with no dot means vegetation survey with no *Helicoverpa* hosts present. Blue marker with dot represents a vegetation survey where hosts were present. Red marker with no dot represents a negative larval survey. Red marker with dot represents a positive larval survey.

Trip log

NB the dates shown in the Fuji images for this trip are wrong because the camera date was set incorrectly. Use the information in this log instead

14/7/15 Armidale to St. George

No sampling was done, but the vegetation was quite green in places, with medics and milk thistle present.

15/7/15 St. George to Eulo

The vegetation was greener than we have seen in recent years, but still not abundant. In places there were extensive areas of low green broadleaf plants, just germinating and < 2cm high, but it was not possible to determine whether these included hosts. Some *Helichrysum apiculatum* was seen west of Bollon, and some native geranium (*Erodium crinitum*) both in the mulga and on the Warrego floodplain. The floodplain had the grass eaten down, and a sub-story of quite green broadleaf plants (Fig. 3) including *Medicago polymorpha* and *Helipterum strictum*, as well as potential host plants that were just germinating but could not be identified. Sampling on *H. strictum* 5 km east of Cunnamulla yielded 25 larvae in 100 sweeps. However, many of these larvae turned out to be parasitised by Tachinid flies and wasps.

West of Cunnamulla conditions dried out rapidly and few host plants were seen. Contact was made with collaborators at Eulo, and lures and pest strips were restocked. No moths had been caught for many months.

16/7/15 Eulo to Windorah

Between Eulo and Toompine conditions were very dry. In many places there was no herbaceous vegetation at all (Fig 4), and much of the mulga was either dead or knocked down for drought fodder.
North of Toompine, on the Bulloo floodplain, there were some areas of low green vegetation, mostly grasses but with a few broadleaf plants that were too small to identify. Further rain will be required for any hosts here to grow to flowering. From Quilpie to Eromanga similar conditions prevailed, with some patches of low green broadleaf herbage which included *Sida platycalyx* (of which there were many seed capsules scattered on the ground), but < 5 cm high. Again, more rain would be required for these hosts to amount to anything. There was a slightly greener area 20k north of Eromanga, but from there through Thylungra to Windorah it was extremely dry, with no hosts and often no herbaceous vegetation at all.

No sampling was done on this day because when hosts were present, they were pre-flowering and either too young to identify or too small to sweep net.

At Eromanga we intended to recruit a new collaborator to replace the school, but on hearing that Craig Brin would be returning to the school at the end of the month, we decided to refurbish the traps in their current position and discuss his continued collaboration on his return.

17/7/15 Windorah to Birdsville

Conditions remained extremely dry west of Windorah, with only very occasional patches of *Cullen australasicum* in creeklines. However, beginning about 35 km east of Birdsville increasing quantioties of hosat plants became apparent, especially in sandy country. They included *Calotis multicaulis, Senecio gregorii* and *Myriocephalus stuartii* , and in claypans, *Cullen cinereum*. However in many places the hosts were quite small, and pre-flowering, having apparently germinated only after the rains in June (Fig 1b). One sample 25 k E of Birdsville on slightly larger S. gregorii yielded 22 larvae per 100 sweeps, but elsewhere the plants were too low to sweep.

We replaced the posts at permanent marker sites 1, 2, 3 and 4.

18/7/15 Around Birdsville

We travelled out into the Simpson Desert as far as the Eyre Creek bypass. On sandy country there were many small seedlings of *Senecio gregorii* and *Myriocephalus stuartii*, with some low *Calotis multicaulis* in depressions. All of this was too low to sweep. We attempted visual sampling on two sites, but found nothing in 2 x 15 minute samples. The hosts would have germinated in response to the June rain and are likely to be flowering abundantly in a few weeks.

There was no activity in the floodplains of either Eyre Creek or the Diuamantina, other than a few patches of *Calotis multicaulis*. There was no *Cullen cinereum*, and it was apparent that no water had broken out of the main channels. We travelled 80 km down the Birdsville track towards Marree, and found similar results.

We replaced the posts on Markers 13, 14 and 15, and made contact with the collaborator, replenishing trapping supplies. There were substantial numbers of moths in the traps, but they had not been checked since the end of May so it is difficult to assess when they arrived.

19/7/15 Birdsville – Marree

We travelled down the Birdsville Track to Mungerannie. Much of this area is stony downs country which supports few hosts, but from about 120 km south of Birdsville, there were areas of surface water, and in a few drainage lines with softer soil there were patches of *Cullen australasicum*. A sample on this 151 km south of Birdsville yielded 11 larvae in 100 sweeps.

The Cooper floodplain where it crosses the Birdsville Track showed no evidence of flooding during the summer or since, and no *Cullen cinereum* was present.

Between Mungerannie and Marree it became increasingly green, with *Cullen australasicum* and *Calotis multicaulisin* low-lying areas (Fig. 5), and scattered *Senecio gregorii* and *Myriocephalus stuartii* in sandy areas. The latter were too scattered to sweep net but sampling on the *C. australasicum* 40 km south of Mungerannie yielded only one laerva in 100 sweeps, and visual sampling on very low plants, possibly *Erodium crinitum*, 23 k north of Marree produced nothing

20/7/15 Marree – Kingoonya

Southwest of Marree the country is mostly stony downs with few hosts, although there were patches of *Cullen australasicum* in some drainage lines, and in one location 68 km south of Marree, one larva was recovered in 100 sweeps. After the turnoff to Roxby Downs, from the Oodnadatta Track, the country became increasingly sandy and *Senecio gregorii* and *Myriocephalus stuartii* (more advanced than further north) became more common, though they were scattered, and therefore difficult to sweep. This vegetation type persisted through Roxby Downs and Pimba, towards Glendambo, but several samples in these areas produced only very low numbers of larvae. It is likely that there will be more hosts here in a few weeks.

At Roxby Downs we met the collaborator and examined the re-established trap site at the Arid Rescue field base.

21/7/15 Kingoonya-Cummins

From Kingoonya down though the Gawler Ranges to Minnipa there were no host plants, though the vegetation became quite green with non-hosts, especially Ward's weed, in the south of this region. No further sampling was done. Contact was made with collaborators at Kingoonya and Yardea, where pheromone catches had been very low.

A summary of larval surveys is given in Table 1.

Table 1. Summary of larval surveys

Date	Location	Host plant	Total larvae collected (per 100 sweeps)
15/7/15	5k E Cunnamulla	*Helipterum strictum*	25
"	"	Unidentified daisy (PGN1)	0
17/5/15	25k E Birdsville	*Senecio gregorii*	22
18/7/15	2k N Eyre Creek Bypass	*Senecio gregorii*	0 (in 2 x 15 min visual)
18/7/15	6k E Eyre Creek	*Senecio gregorii*	0 (in 2 x 15 min visual)
19/7/15	151k S Birdsville	*Cullen australasicum*	11
19/7/15	40k S Mungerannie	*Cullen australasicum*	3
19/7/15	23k N Marree	PGN3 (E. crinitum?)	0 (in 2 x 15 min visual)
20/7/15	68k W Marree	*Cullen australasicum*	1
20/7/15	17k S Roxby Downs	*Senecio gregorii*	0
20/7/15	1k E Olympic Dam	*Senecio gregorii*	0
20/7/15	29k N Pimba	*Myriocephalus stuartii*	2
20/7/15	33k N Pimba	*Senecio gregorii*	1

Summary and conclusions

Numbers of *Helicoverpa* larvae found during the trip were relatively low, even though there were places where host plants were abundant. However, in most of these places the plants were very low, and often difficult to sample. They appeared to have germinated from the rain in June rather than May, as their distribution followed the patterns of Fig 1b more than Fig 1a, with the exception of the area around Roxby Downs where the hosts were more advanced. Some of these areas might support more hosts in a few weeks and could be colonised by larger numbers of *H. punctigera in* late winter. Areas worth watching include the Warrego floodplain around Cunnamulla, the sandy desert around Birdsville, and sandy areas around Roxby Downs. Elsewhere it continues to be dry and unlikely to be the source of significant migrations in spring. In particular it is obvious there has been no activity in any inland floodplains this winter.

Fig. 3. Green vegetation including *Helipterum strictum, Erodium crinitum, Calotis multicaulis* and *Medicago polymorpha* on the Warrego floodplain, 5km east of Cunnamulla

Fig. 4. Permanent vegetation marker at Site 8, near Toompine in the mulga, showing almost complete absence of herbaceous vegetation, with many mulga trees either knocked down for grazing, or dying.

Fig. 5. Cullen australsicum in a drainage line, 40 km south of Mungerannie

CRDC Project Inland Trip Report
30 August – 4 September 2015

Peter Gregg and Alice Del Socorro

Objectives

The field trip undertaken between 14-21 July had indicated that there were several areas where rain had fallen in June, and host plants germinated, but were either too small to sweep or had no larvae on them. These areas included the Warrego floodplain near Cunnamulla, some areas of mulga around Eromanga, and sandy country around Birdsville. Insufficient time was available for a trip to Birdsville, but it was decided that a quick trip as far as Eromanga would be worthwhile, to determine whether the host plants had continued to grow, and whether they had been colonised by *H. punctigera*.

The objectives also included refurbishing the permanent vegetation markers that had not been replaced in the July trip, and making contact with the principal at Eromanga State School, Craig Brin, to re-establish the pheromone traps there.

Background

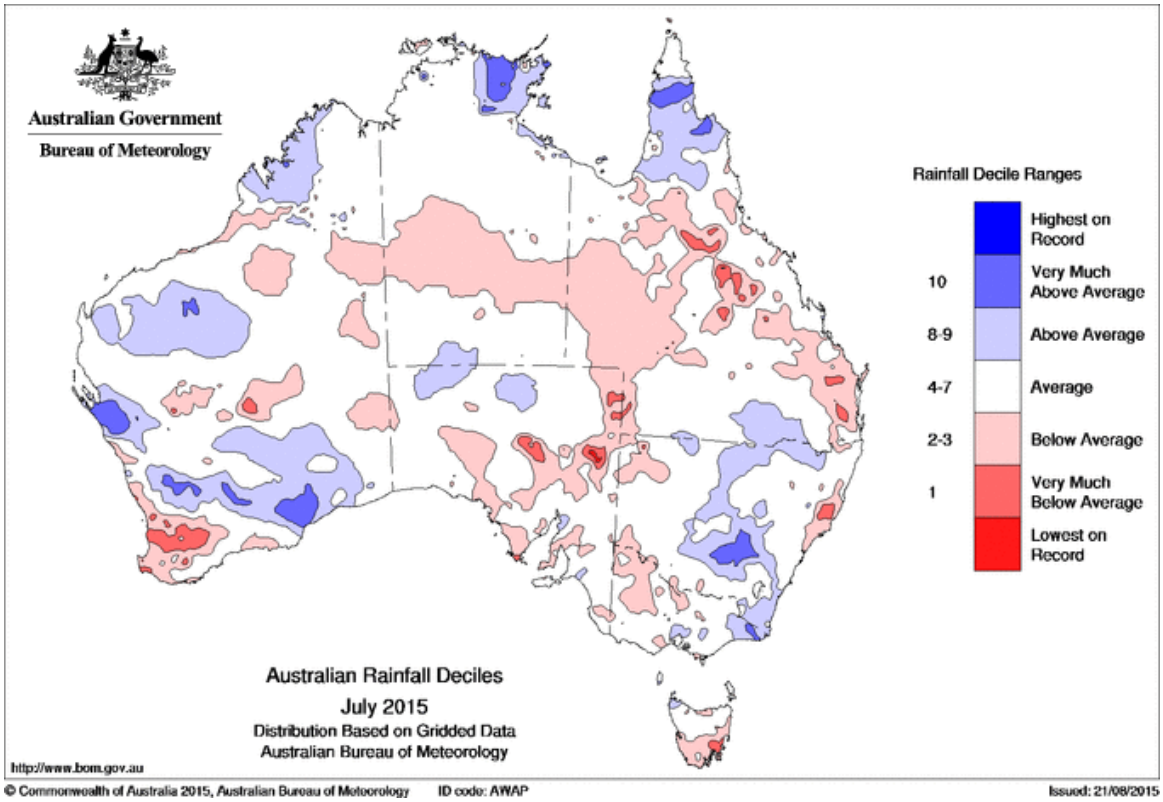


Fig. 1. Rainfall deciles for July 2015

Methods

Methods for sampling and vegetation ratings were as for previous trips. The trip was conducted between 130/8 2015 and 4/9/2015 by Peter Gregg and Alice Del Socorro.
The route taken is shown in Fig. 1.

Fig. 2. Route and survey sites. Blue marker with no dot means vegetation survey with no *Helicoverpa* hosts present. Blue marker with dot represents a vegetation survey where hosts were present. Red marker with no dot represents a negative larval survey. Red marker with dot represents a positive larval survey.

Trip log

30/8/15 Armidale to St. George

No sampling done. Conditions were quite green with abundant medic especially along the roadside

31/8/15 St. George to Thargomindah

Between St. George and Bollon conditions were better than we had seen in recent years, but there were only patchy hosts, including wild geranium (*Erodium crinitum*) *Velleia glabrata, Calotis multicaulis* and *Helipterum strict*um. One sample 14km west of Bollon yielded 7 larvae in 100 sweeps. However, after that almost no hosts were seen, and there was very little herbaceous vegetation until the Warrego floodplain. On the floodplain we noted some *Medicago polymorpha, Helipterum strictum* and *Helipterum floribundum* but decided to leave sampling until we returned.

West of the Warrego, the herbaceous vegetation deteriorated rapidly, and from there through Eulo to Thargomindah there was virtually none, with much of the mulga knocked down for drought fodder (Fig. 3a)

We refurbished the markers at permanent vegetation sites 11, 10 and 9.

1/9/15 Thargomindah – Quilpie – Thylungra - Eromanga

Conditions remained very dry with almost no herbaceous vegetation, and no host plants, until north of Toompine, where small areas of *Calotis multicaulis, Velleia glabrata* and *Helipterum floribundum* were found on the Bulloo floodplain in drainage areas, but they were very low (< 3cm) so we did not sample them, although in some areas they were flowering. After then, through Quilpie and up to Thylungra there were virtually no host plants (Fig. 3b), although in many places there were many seed capsules *of Sida platycalyx* scattered on the ground. From about 40 km north of Eromanga into the town, in the area where green seedlings were noted in July, there were only a few patches of an unidentified daisy, collected as PG999, and *Calotis multicaulis*. Sampling on the unknown daisy did not produce any larvae.

We refurbished the marker posts at Sites 8 and 6, and made contact with Craig Brin at Eromanga State School, who agreed to take over running the pheromone traps, which we replaced with new traps.

2/9/15 Eromanga to Cunnamulla

Small areas of the same hosts as found the previous day, the unknown daisy PG999 and *Calotis multicaulis*, were found up to about 20 km east of Eromanaga, but only in drainage areas and along the roadside. East of there, through Quilpie and all the way to Charleville, there was virtually no herbaceous vegetation. From about 40 km south of Charleville increasing areas of diverse host plants including the daisies *Verbesina encelioides, Helipterum moschatum, Helipterum strictum, Helipterum floribundum* and an unknown daisy collected as PG998 were found on the Warrego floodplain. There were also hosts in other families, including *Velleia glabrata*, (Goodeniaceae), *Erodium crinitum* (Geraniaceae) and *Medicago polymorpha* (Fabaceae). Three samples were done which yielded only low numbers of larvae. More hosts were noted but as darkness was approaching we decided to return to the area the next day.

We refurbished the marker for the permanent vegetation Site 7.

3/9/15 Cunnamulla to St. George

Heading back up the Warrego floodplain, substantial areas of host plants, especially *Helipterum floribundu*m, were seen in the first 60 km north of Cunnamulla. Samples on this produced large numbers of larvae, up to 156 per 100 sweeps, with smaller numbers being found on other hosts including *Helipterum moschatum, Helipterum strictum* and the an unknown daisy collected as PG998. However, there were few hosts in higher mulga parts of this area. Returning to Cunnamulla and heading east, hosts including *Medicago polymorpha*, the daisy PG998 and wild tobacco, *Nicotiana velutina*, supported moderate numbers of larvae. Substantial areas of *Erodium crinitum* were seen near St. George, but a sample yielded only one larva in 100 sweeps

4/9/15 St. George to Armidale

No further sampling or vegetation monitoring was undertaken

A summary of larval surveys is given in Table 1.

Table 1. Summary of larval surveys

Date	Location	Host plant	Total larvae collected (per 100 sweeps)
31/8/15	14k W Bollon	*Helipterum strictum*	7
1/9/15	60 k S Thylungra turnoff	PG999 daisy	0
2/9/15	2k E Eromanga	PG999 daisy	0
2/9/15	166k E Quilpie	*Calotis multicaulis*	0
2/9/15	30 k S Charleville	*Verbesina enceliodes*	2
2/9/15	43k S Charleville	*Helipterum moschatum*	0
2/9/15	46k S Charleville	*Helipterum strictum*	3
3/9/15	9k N Cunnamulla	PG998 daisy	5
3/9/15	16k N Cunnamulla	*Helipterum moschatum*	12
3/9/15	40k N Cunnamulla	*Helipterum floribundum*	70
3/9/15	49k N Cunnamulla	*Helipterum floribundum*	156
3/9/15	62k N Cunnamulla	*Helipterum strictum*	*25*
3/9/15	*14k E Cunnamulla*	*Erodium crinitum*	12
"	"	*Medicago polymorpha*	11
3/9/15	24k E Cunnamulla	*Nicotiana velutina*	11
3/9/15	48 k E Cunnamulla	PG998 daisy	1
3/9/15	137 k E Cunnamulla	PG998 daisy	15
3/9/15	99 k E Bollon	*Erodium crinitum*	1

Summary and conclusions

The major finding for this trip was the relative abundance of hosts on the Warrego floodplain, which supported many larvae. These hosts had been germinated by the rain in June, with follow-up rain in July. No flooding was involved. This observation indicates that the seed banks for host plants in the Warrego are still present. We should consider the easternmost floodplains (Warrego, Bulloo and Paroo) as potential components of a "green bridge" for spring migration, if suitable rain has fallen, even though the larger areas of mulga may not be functional in this regard.

In contrast, in nearby areas of mulga which received the same rain there were few hosts, suggesting that the seed banks may be depleted in these areas following the Millenium drought. In both mulga and floodplain areas which received the June rain but not the follow-up July rain (on the Bulloo floodplain around Toompine, and north of Eromanga) host growth was limited to low-lying patches where the rain had pooled. These hosts were very low, and had flowered without much vegetative growth. They were unsuitable for sweeping, except in two sites where no larvae were recovered.

Elsewhere in the mulga conditions were worse than we have ever seen them, with herbaceous vegetation scores of zero in most sites, and a complete absence of host plants, although there are clearly many seeds of *Sida platycalyx* on the surface which will germinate following any spring or summer rain.

(a) (b)

Fig. 3. Dry conditions with almost no herbaceous vegetation in mulga areas. (a) 60 km west of Eulo, (b) 2 km south of the Thylungra turnoff, the site where previous long-term comparative photos showing extensive host growth were taken

(a) **(b)**

Fig. 4. Host plant growth on the Warrego floodplain, from Cunnamulla to about 50 km north. (a) *Helipterum moschatum*, (b) *Helipterum floribundum* and *Helipterum strictum*

CRDC Project Inland Trip Report
5-10 October 2015

Peter Gregg and Alice Del Socorro

Objectives

The objectives were to survey grain legume and canola crops in South Australia, northern Victoria and southern NSW for larvae of *H. punctigera*, to assess both likely pest problems on these crops in the near future, and the potential for immigration to cotton areas later in the season.

Background

Following completion of field work in the Eyre Peninsula for our GRDC project on canola, it was necessary to return the vehicle to Armidale. The intention had been to do this via northern SA and western Queensland, but after our surveys there in July and August, conditions had remained very dry (Fig. 1) and our pheromone catches from this region indicated little activity. On the other hand, pheromone trap catches from South Australia (including our own from the Eyre Peninsula) had indicated a major influx of *H. punctigera* moths into cropping regions of South Australia.in mid- to late- September.

There is now a substantial network of pheromone traps in southern Australia, (~35 sites) which are coordinated by the CESAR centre at the University of Melbourne, with funding from GRDC. See:

http://cesaraustralia.com/sustainable-agriculture/pestfacts-south-eastern/

We are collaborating with this group, including sharing our data on pheromone trap catches and inland surveys and in turn receiving broad information on *H. punctigera* population dynamics in winter/spring crops in southern Australia, which might help us understand whether these regions can be sources of *H. punctigera* immigration to cotton areas later in the season.

Given the likely lack of activity in western Queensland, we decided it would be more useful to return from South Australia via northern Victoria and southern NSW, surveying grain legume and canola crops as we went.

Methods

Methods for sampling were the same as for inland surveys. At each site 5 x 20 sweep samples were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to check identify and determine parasitism levels. Since crops rather than non-crop vegetation were being samples (with only a few exceptions) no vegetation assessment of the type used in inland surveys was undertaken, but the growth stage of the crop was recorded

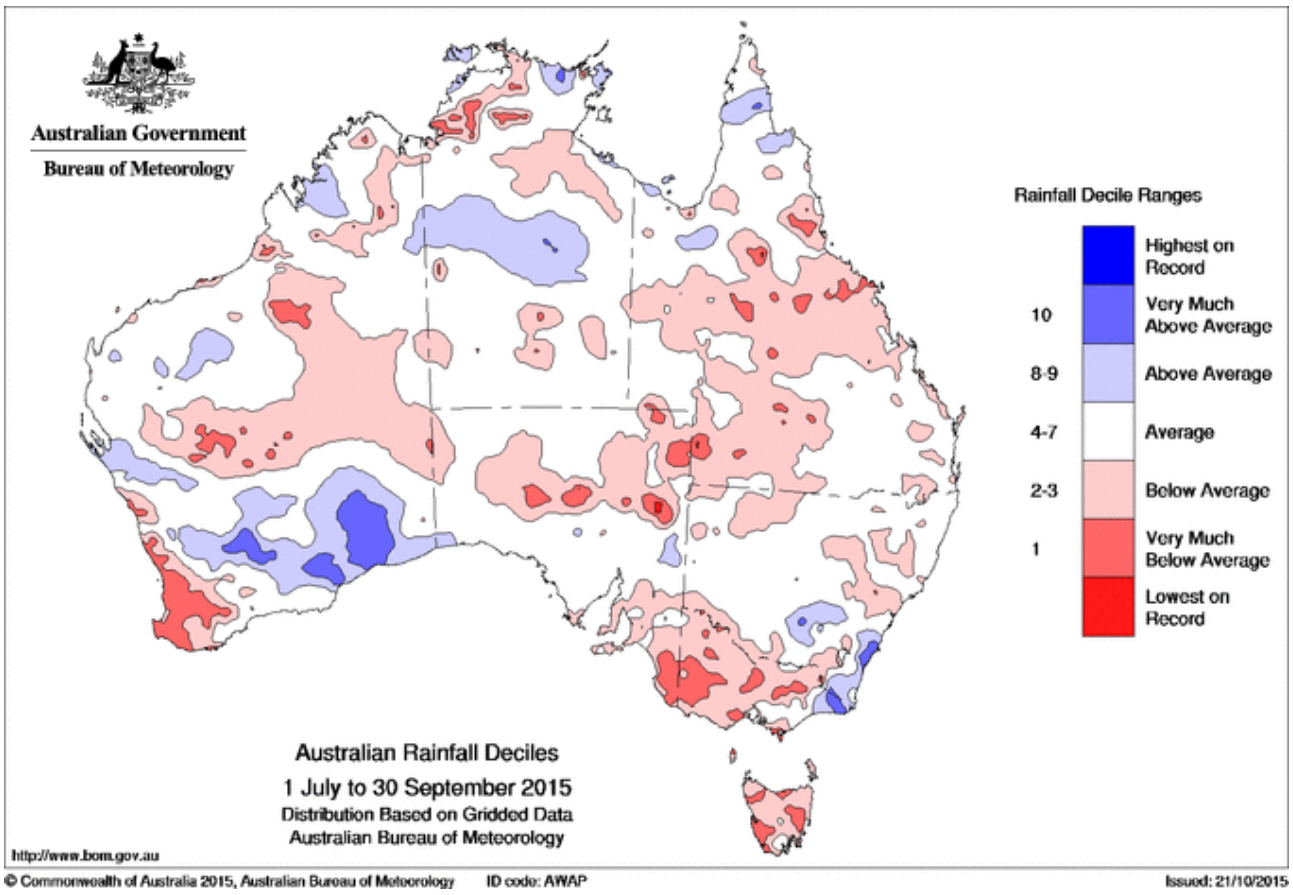


Fig. 1. Rainfall deciles for July - September 2015

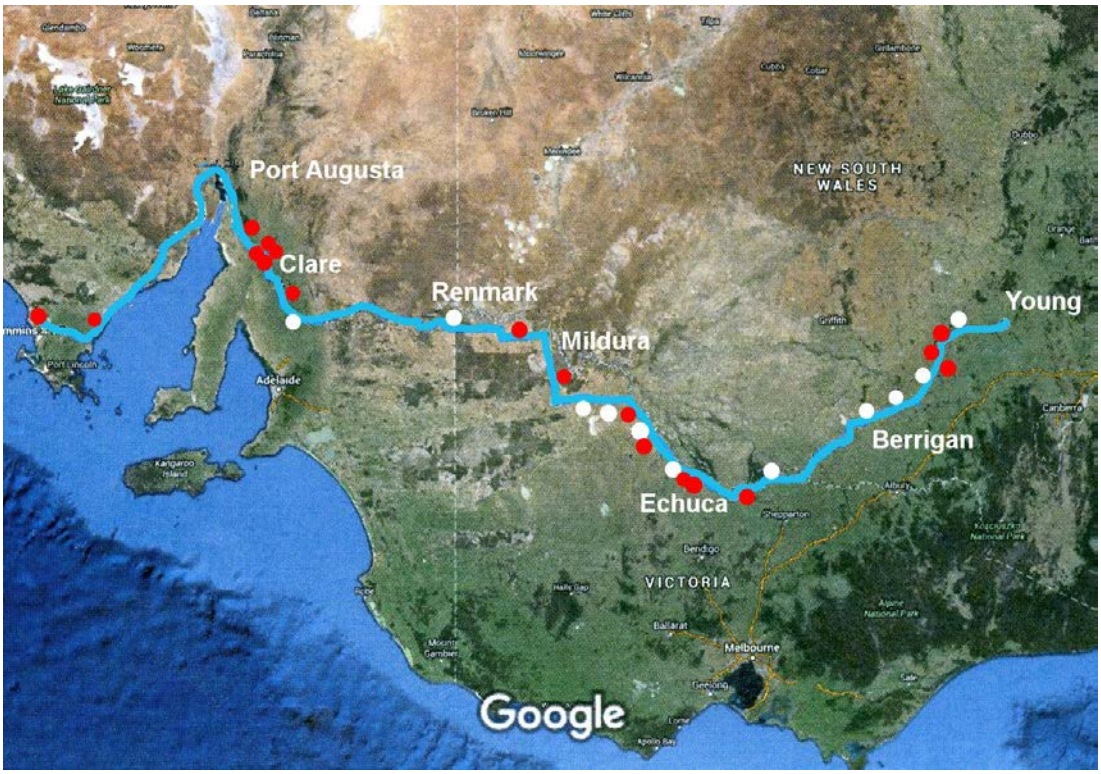


Fig. 2. Route and survey sites. White circles represent a site with no *Helicoverpa* larvae found. Red circles represent sites where larvae were found.

Trip log

5/10/15 Cummins to Port Augusta

Crops in this region were mostly canola or faba beans, both post-flowering. only two sites were sampled, both with moderate numbers of larvae

6/10/15 Port Augusta to Waikerie

Crops here consisted of mostly of field peas and faba beans. In the north they were mostly post-flowering, but further south there were many crops that were at peak flowering or early flowering stages. Some very high numbers of larvae were found between Crystal Brook and Clare, but numbers were patchy elsewhere. It is possible that some crops had been sprayed for *Helicoverpa*.

7/10/15 Waikerie to Mildura

There were very few grain legume crops in this area, since most irrigated areas supported horticultural crops, and non-irrigated areas were very dry. One sample on field peass south of Renmark produced very large numbers of larvae. Another sample, the only one of the trip on native vegetation (an unknown daisy), produced no larvae

8/10/15 Mildura to Echuca

In the western Mallee region most of the field pea crops were post-flowering and drying off very rapidly following a burst of hot dry weather in the first week of October. Many crops were subsequently cut for hay. In some places no larvae were found, in others moderate numbers were recovered. As in South Australia, it is possible that some crops had been sprayed for *Helicoverpa* in the previous 2-3 weeks. Very few larvae were found in crops to the south and east of Sea Lake, through to Echuca

9/10/15 Echuca to Cootamundra

In this region the dominant grain legume crop was lupins, with a few faba bean crops, as well as canola. Most crops were post-flowering. Very few *Helicoverpa* larvae were found, except for one faba bean crop near Lockhart.

10/10/15 Cootamundra to Armidale

No further sampling was undertaken. Survey data were forwarded to the CESAR PestFacts team on our return to Armidale.

A summary of larval surveys is given in Table 1.

Table 1. Summary of larval surveys

Date	Location	Host plant	Total larvae collected (per 100 sweeps)
4/10/15	Mt. Hope	Canola	9
5/10/15	20k S Tumby Bay	Faba beans	2
6/10/15	7 k S Port Pirie turnoff	Chickpeas	8
6/10/15	3k S Crystal Brook	Faba beans	1
6/10/15	7k S Crystal Brook	Field peas	0
6/10/15	23k S Crystal Brook	Field peas	270
6/10/15	15k S Yacka	Faba beans	32
6/10/15	3k E Saddleworth	Field peas	2
6/10/15	2k E Marrabel	Faba beans	0
7/10/15	5k E Renmark	Unknown daisy	0
7/10/15	61k E Renmark	Field peas	124
8/10/15	61k S Red Cliffs	Field peas	9
8/10/15	21k SE Ouyen	Field peas	0
8/10/15	41k SE Ouyen	Lentils	0
8/10/15	67k SE Ouyen	Field peas	11
8/10/15	7k SE Sea Lake	Lentils	0
8/10/15	21k SE Sea Lake	Field peas	7
8/10/15	41k E Culgoa	Field peas	0
8/10/15	71k E Culgoa	Field peas	5
8/10/15	72k E Kerang	Vetch	5
9/10/15	40k E Echuca	Canola	2
9/10/15	10k NE Cobram	Lupins	0
9/10/15	4k N Berrigan	Canola	0
“	“	Lupins	0
9/10/15	26k N Berrigan	Faba beans	0
9/10/15	32k N Urana	Lucerne	6
9/10/15	5k N Lockhart	Faba beans	14
9/10/15	37k N Lockhart	Lucerne	3
9/10/15	16k S Coolamon	Lucerne	0
“	“	Canola	0
9/10/15	22k E Coolamon	Lucerne	0

Summary and conclusions

The results from this trip supported the hypothesis that there had been a major influx of *H. punctigera* to the Eyre Peninsula and mid-north region of South Australia 2-3 weeks before our trip. These moths were probably immigrants from the north-west (eg Great Victoria desert) or the north (Simpson Desert and areas around Roxby Downs where we had found larvae in July). The potential for local overwintering on the Eyre Peninsula, especially northern areas around Minnipa, to contribute to these populations cannot be excluded and warrants further study, but it is difficult to imagine an autumn source population in these regions due to lack of hosts (either crop or non-crop).

Our results suggested that some immigrants had reached as far as the western Mallee of Victoria, and there were reports of widespread spraying before our trip there, so in some cases we may have been sampling sprayed fields. This would account for the patchy nature of larval populations.

In the two weeks subsequent to our trip there were also substantial moth catches recorded from southwest Victoria, suggesting immigration from the earlier component of the Eyre Peninsula and mid-north SA populations. These immigrants would have laid eggs, but most crops had reached maturity and little damage resulted.

It is becoming clear that immigration from the southwest, as well as the northwest, might contribute substantially to gene flow in *H. punctigera* populations in cotton areas. The extremely large areas of winter crops which are hosts for H. punctigera in South Australia and Victoria (over 1.2 million ha; Table 2) can clearly support the breeding of large *H. punctigera* populations, even though economic thresholds for these crops are quite low and they are regularly sprayed. Thresholds are generally less than 10 larvae per 100 sweeps; about 40% of our samples showed numbers above the economic thresholds for their respective crops (G. McDonald pers. comm.), even though some of them may have already been sprayed. There were many reports of at least partial spray failures, most likely due to the larvae boring into pods prematurely, in hot weather. Also, many of the larvae may have already pupated in the more northerly sites we sampled. All these considerations suggest that there may be significant numbers of moths emerging from South Australia and Victoria in November, and it is possible that they might migrate northwards, towards cotton areas. Post-frontal south-westerly winds are probably warm enough to support high altitude nocturnal flight at this time of year, unlike in early spring. It will be interesting to note pheromone trap catches in cotton areas in late spring-early summer.

Crop	South Australia	Victoria
Chickpeas	15	19
Faba beans	86	75
Field peas	90	47
Lentils	110	116
Canola	225	443
TOTAL	526	700

Table 2. Winter/spring host crops for H. punctigera in South Australia and Victoria. Source: ABARES Crop Report June 2015.
http://www.agriculture.gov.au/abares/display?url=http://143.188.17.20/anrdl/DAFFService/display.php?fid=pb_aucrpd9aba_20150610_11a.xml

CRDC Project Inland Trip Report
12-18 May 2016

Peter Gregg, Alice Del Socorro and Kris Le Mottee

Objectives

The main objective was to assess the vegetation and survey any *Helicoverpa* hosts that may have grown following rain in mid-March in the Great Victoria Desert region and adjacent parts of South Australia. A secondary objective was to sample chenopods for *Helicoverpa* larvae.

Background

We have not investigated the Great Victoria Desert region of South Australia and Western Australia since a trip in September 1991, as part of the Heliothis Inland Research group project. In that trip we established that good winter rains could produce extensive growth of daisies in the region that supported *H. punctigera* larvae. Since then, we have suspected in some years that the region has produced moths that emigrated to cropping regions of SA and Vic, and possibly further afield, but there have been no surveys.

There had been good rain in part of the region in mid-March (Fig. 1a). We know that in western Queensland rain at this time does not germinate daisies, but favours the growth of grasses and perennials that are not *Helicoverpa* hosts. However we speculated that since it was further south, March rain might produce germination of hosts in the Great Victoria Desert. After a very dry April, there had been some further rain in May, about a week before the trip (Fig 1b). We did not expect to find hosts originating from this rain as it was too recent, but it might have promoted the growth of hosts germinating from the March rain.

A secondary objective was to sample chenopods (saltbushes, copperburrs etc) for *H. punctigera* larvae. This originated from reports of substantial numbers of *H. punctigera i*n cropping areas which have carbon isotope profiles consistent with origins on C4 hosts (G. Baker and C. Tann, pers. comm.). This has led to suggestions that chenopods may be important hosts for *H. punctigera,* and though we have never found them on these plants in the inland, we decided that further sampling was required.

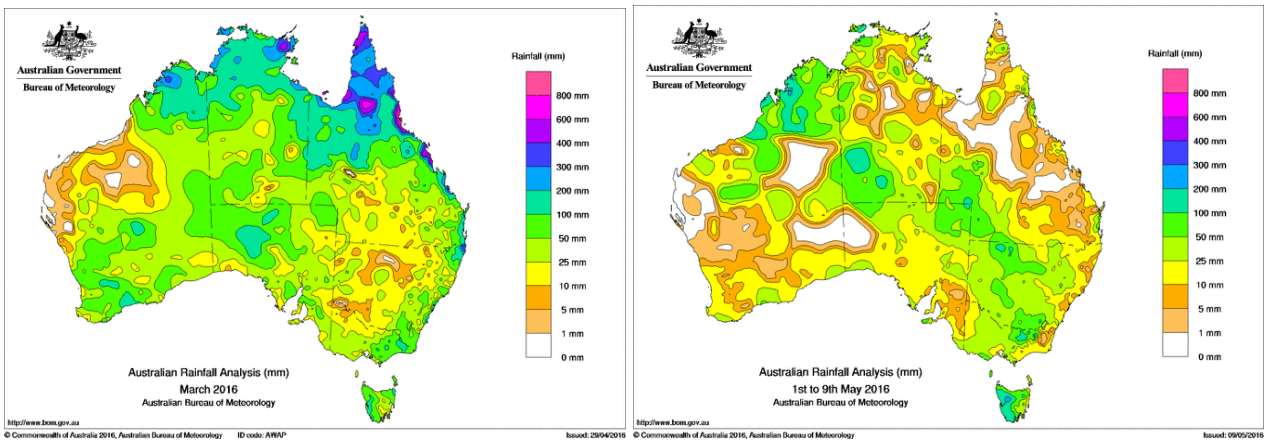


Fig. 1. Rainfall deciles for (a) March and (b) May 2016

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to check identify and determine parasitism levels.

Vegetation scores and the presence of host plants were recorded every 20 km using methods described in previous trip reports. A novel feature was the use of a GPS-enabled tablet computer (Motion Computing Model F5v) running ArcMap and loaded with digital vegetation maps for South Australia and NSW. This system has been developed by Kris Le Mottee and now allows us to relate survey data top precise vegetation associations for each site.

The survey route taken and sample sites are shown in Fig. 2.

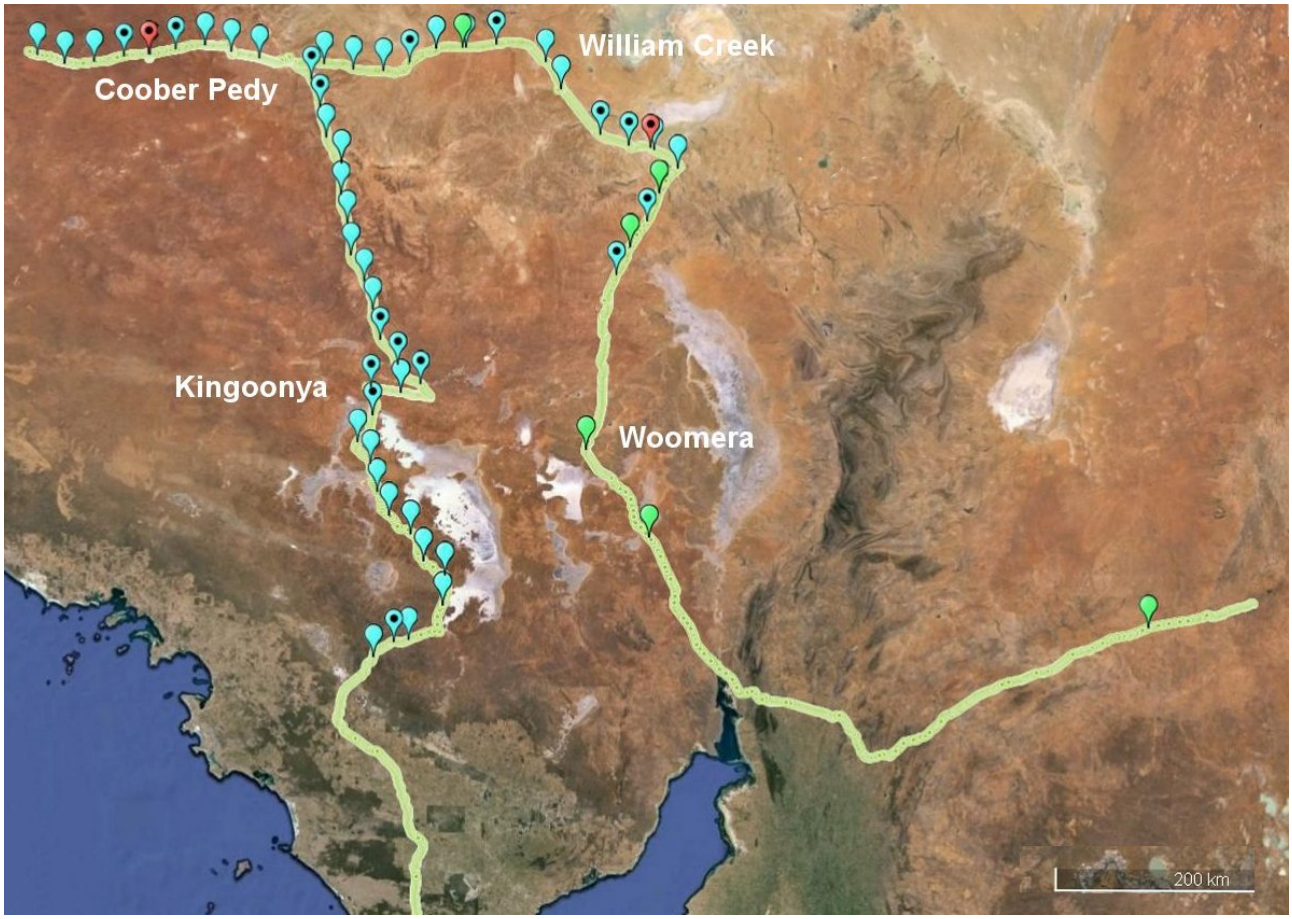


Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants, green markers represent sample sites on chenopods, not known to be hosts.

Trip log

10/5/16 Armidale to Nyngan

No sampling

11/5/16 Nyngan to Broken Hill

No sampling done. Few host plants observed.

12/5/16 Broken Hill to Woomera

No host plants were seen, but sampling was undertaken on chenopods at three locations, 25 k west of Cockburn, 96 k north of Port Augusta and 1 k west of Pimba (near Woomera). No larvae were found. The vegetation was reasonably green in many areas, but consisted mostly of saltbush and grasses.

13/5/16 Woomera-Roxby Downs-William Creek

We visited the Arid Zone Recovery site at Roxby Downs, met collaborator Kimberley Sully and checked the traps. A few moths had recently been caught in the traps. The vegetation was quite green but there were few hosts. However there were large numbers of germinating dicot plants which included some *Rhodanthe floribunda* in the claypans and some poached egg daisies (*Myriocephalus stu*artii) on the dunes. Some of these were just emerging, but others were 2-4 cm high. It appeared that the seedlings just emerging had germinated from the recent May rain, while the larger plants had germinated earlier. While it was probably the March rain that was responsible for the germination of these larger seedlings, they should have been more advanced if they had germinated immediately. We formed the impression that they had remained inactive until sometime after March, when autumnal conditions (probably falling temperatures) had developed enough to stimulate germination. Such vegetation was seen in patches through to William Creek.

In places a few post-flowering *Cullen cinereum* plants were seen in creeklines, and at one site, 25 k north of the turnoff on the Oodnadatta Track, near Lake Eyre South, we sampled on this but found nothing. At the same site there were scattered plants of wild tobacco, *Nicotiana velutinum*. While mostly too scattered to sweep net, we found one patch that could be swept, and recovered 7 larvae per 100 sweeps. We also conducted a further two samples on chenopods, with negative results.

14/5/16 William Creek – Coober Pedy – Great Victoria Desert

West of William Creek, around Anna Creek station, we found an area of sand dunes and claypans which had very green vegetation, including *Rhodanthe floribunda*, poached egg daisies and *Calotis cuneifolia* and scattered wild tobacco (Fig 3a). Again these were very small, except for the wild tobacco, and could not be swept. They appeared, like the vegetation around Roxby Downs, to have resulted from the March rain but not immediately – it appeared that germination had been delayed until suitable temperatures and/or photoperiods developed, at some time in April. There were also very green saltbushes and other chenopods, on which we made two samples but found nothing.

From Anna Creek to Coober Pedy was mostly stony downs country, quite dry with few host plants. The same type of vegetation was found west of Coober Pedy, as far as Mabel Creek station, but west of here in sandy country there was evidence of recent rain, and quite green vegetation that contained similar low seedlings to those seen around Anna Creek. There was also a *Goodenia* species and some Malvaceae, including possibly *Sida platycalyx*. The only host plant tall enough to sweep was wild tobacco (Fig 3b), and on two samples on this we recovered 7 and 13 larvae per 100

sweeps. On rearing the majority of these larvae turned out to be lesser budworm, *Heliothis (=-Neocleptria) punctifera*, not *Helicoverpa punctigera*. We camped in such vegetation about 30 km west of the border of the Tallaringa Conservation Park and ran a light trap. At least three *H. punctigera* were caught, and there may have been more because it was difficult to sort the catch due to large numbers of other moths, that included some *Agrotis munda*.

15/5/16 Great Victoria Desert – Coober Pedy

We travelled another 50 km to the west along the Anne Beadell Highway, to the western boundary of the Tallaringa Conservation Park. The area west of here was inaccessible due to the temporary closure of the Woomera Prohibited Area by the Defence Dept., so we returned to Coober Pedy. The western parts of this route quickly became very dry with no host plants. It was apparent that the area of high rainfall in March had not been as extensive to the west as indicated in Fig. 1a.

16/5/16 Coober Pedy – Glendambo

Kris flew back to Armidale from Coober Pedy while Peter and Alice proceeded south in order to set up diamondback moth trials near Cummins, and leave the vehicle there. From Coober Pedy to Glendambo there were similar patches of low green vegetation, including host plants, that probably resulted from the March rain, but not immediately. No sampling was undertaken. At the Kingoonya trap site we made contact with the collaborators and checked the traps. There had been an error in the supply of lures, and for the previous week *H. armigera* lures had been placed in the traps. Interestingly one moth had been caught, and examination of the wing markings indicated that it was *H. armigera*. This species is known to occur in South Australia, but is much less common than H. *punctigera*. We replaced the lures with the correct ones. Around the trap sites the saltbush was very green and there were many small dicot seedlings emerging, which included some host plants (Fig 3c). The only ones of these that were flowering appeared to be a *Calotis* or *Brachycome* species, and they were too scattered to sweep. A few *H. punctigera* moths were seen around the lights at Glendambo.

17/5/16 Glendambo – Kingoonya - Cummins

South of Kingoonya the host plants disappeared and the saltbush became much drier. We checked the Yardea trap site and left supplies for the collaborator, who was absent. In the southern part of this region there was abundant Ward's weed (*Carrichtera annua*) which is not a *Helicoverpa* host but supports diamondback moth larvae. However, it was too small to sweep, as were the medics that were common from around Yardea south (Fig 3d).

No further sampling was undertaken until we reached the cropping areas around Cummins, where we sampled on canola and lucerne, but no larvae were found.

A summary of larval surveys is given in Table 1.

Date	Location	Host	Larvae/100 sweeps	% *H. punctigera*
12.5.16	25k W Coburn	*Atriplex vesicaria**	0	
12.5.16	96k N Pt Augusta	*A. vesicaria**	0	
"	"	*Maireana* spp.*	0	
12.5.16	1k E Pimba	*A. vesicaria**	0	
"	"	*Tecticornia* spp.*	0	
13.5.16	60k N Roxby Downs	*A. vesicaria**	0	
13.5.16	100k N Roxby Downs	Copperburr (*Sclerolaena articulata*)*	0	
13.5.16	25k N Oodnadatta T/O	*Cullen cinereum*	0	
"	"	*Nicotiana velutinum*	7	100
14.5.16	43k N William Creek	Chenopod 1*	0	
"	"	Chenopod 2*	0	
14.5.16	97k W Coober Pedy	*N. velutinum*	7	0
14.5.16	3k W Tallaringa Conservation Park border	*N. velutinum*	13	33

*plants not known to be hosts

Table 1. Summary of larval surveys

Summary and conclusions

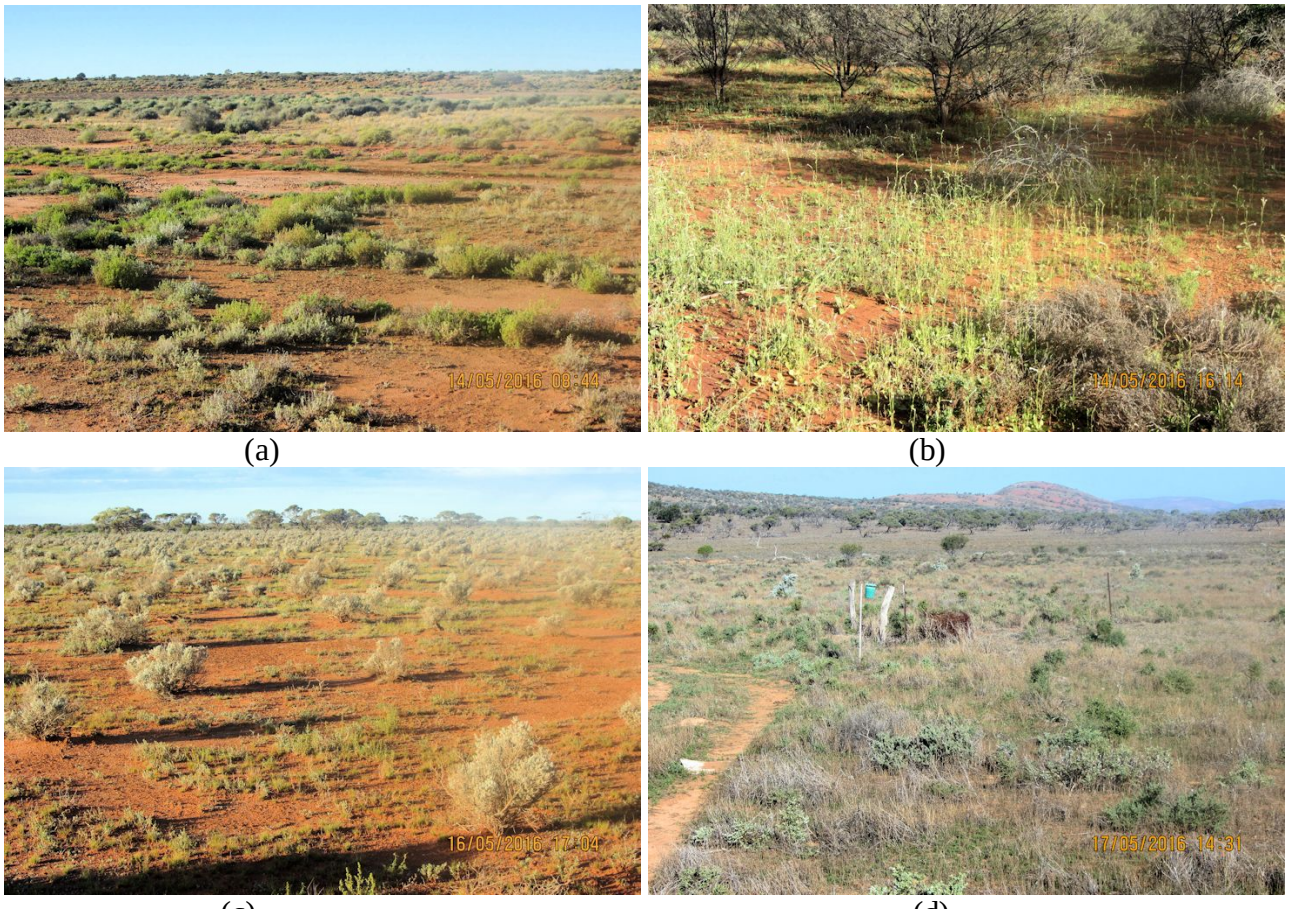
The main conclusion from this trip is that March rain will not directly germinate *H. punctigera* hosts in South Australia, even though the areas that can produce hosts are further south than in Queensland. It appears that the mechanisms that restrict germination of daisies in summer (presumably temperature and/or photoperiod) are effective at latitudes as far south as the middle of SA. However, if there is enough rain to keep the soil wet, hosts will germinate when those inhibitory mechanisms are relaxed, which seems to occur at some time in April.

In the sandy areas of SA there are now many host plants in the early stages of growth, and though we were unable to sample them because they were too low, it is likely that they are already being colonised by *H. punctigera*. Low numbers of moths were seen in many areas (recent pheromone catches from Roxby Downs and Kingoonya, the light trap in the Great Victoria Desert, town lights in Glendambo). The only host we were able to sample was wild tobacco (which does seem to germinate in response to March rain), but whenever we found enough to sample, there were larvae on it. With recent rain these hosts will continue to grow, so conditions are set for substantial *H. punctigera* populations to develop later in the winter. The same is probably true of central Australia and western Qld, where the May rains have been much heavier (Fig 1b), though we have not been to these areas yet.

Of interest was the collection of lesser budworm in the northern parts of the trip, around Coober Pedy and the Tallaringa Conservation Park. This species used to be quite common in western Queensland in the 1980's and 90's, but we have not seen it since the drought until now. It is

possible that more consistent rain in South Australia compared to western Queensland has allowed populations to persist there.

This trip provided no evidence to support the hypothesis that C4 *punctigera* come from chenopods, as all our samples were negative. This is consistent with past experience in inland surveys, but the hypothesis should not be dismissed yet. There are many species of chenopods which are C4, and we have only sampled a few of the most common ones. The leaves on the ones we have sampled are very fleshy, and (like the leaves of poached egg daisies) probably do not provide enough nutrition to support larval development. However there might be some species of chenopods which are not like this, and we should continue to sample such plants.



(a) (b)

(c) (d)

Fig. 3. Vegetation types: (a) near Anna Creek station, west of William Creek. Green shrubs are chenopods, but many of the low seedlings are host plants including *R. floribundum* on the flats and *M. stuartii* on the dunes, (b) wild tobacco (*N. velutinum*) under mulga trees in the Great Victoria Desert, (c) chenopods with low seedlings including *Calotis*, *Brachycome* and *R. floribundum* at the Kingoonya trap site, (d) Ward's weed and medics under saltbushes at the Yardea trap site.spp.

CRDC Project Inland Trip Report
22-26 June 2016

Peter Gregg, Alice Del Socorro, Colin Tann and Kris Le Mottee

Objectives

This report covers two separate trips, one by Peter Gregg and Alice Del Socorro in South Australia (travelling northg from the Eyre Peninsula where bthey were based for GRDC work on canola), and the other by Colin Tann and Kris Le Mottee in western Queensland, travelling west from Narrabri. The objectives were to survey western Queensland and adjacent areas of northern South Australia for host plant growth and larval numbers, and to collect specimens for genetic studies by CSIRO researchers. It was intended that the two teams would meet in Birdsville and be joined by Geoff Baker, who would fly in. The two teams would then jointly survey that area and adjoining parts of the Simpson Desert, where rainfall patterns suggested that the largest numbers of *H. punctigera* would be found. Unfortunately rain during the trips closed many roads in northern South Australia, which meant that the southern team had to turn back, and the western team had to modify their routes and abandon plans to sample in the Simpson. Colin Tann and Geoff Baker then travelled north and west to collect moths for genetic studies, using funds from their CSD Researcher of the Year award. That part of the trip is not included in this report. Kris then flew back to Armidale.

Background

It had rained in mid-March, especially in western SA, but the field trip we did in May indicated that few hosts had germinated in response to this rain, probably because it was too early (see *Inland Field Trip Report, 12-18 May 2016*). This is likely to have been the case in western Queensland as well as South Australia. Following the March rain, April had been generally dry throughout the inland (Fig 1a), but there had been good rain in western Queensland and northern SA in mid-May (Fig 1b). While it was expected that a field trip in June might be too early for much host growth and larval development following the May rain, this timing fitted with the CSIRO project of Geoff Baker and Colin Tann, and it was envisaged that there might be further trips during the season by the UNE team.

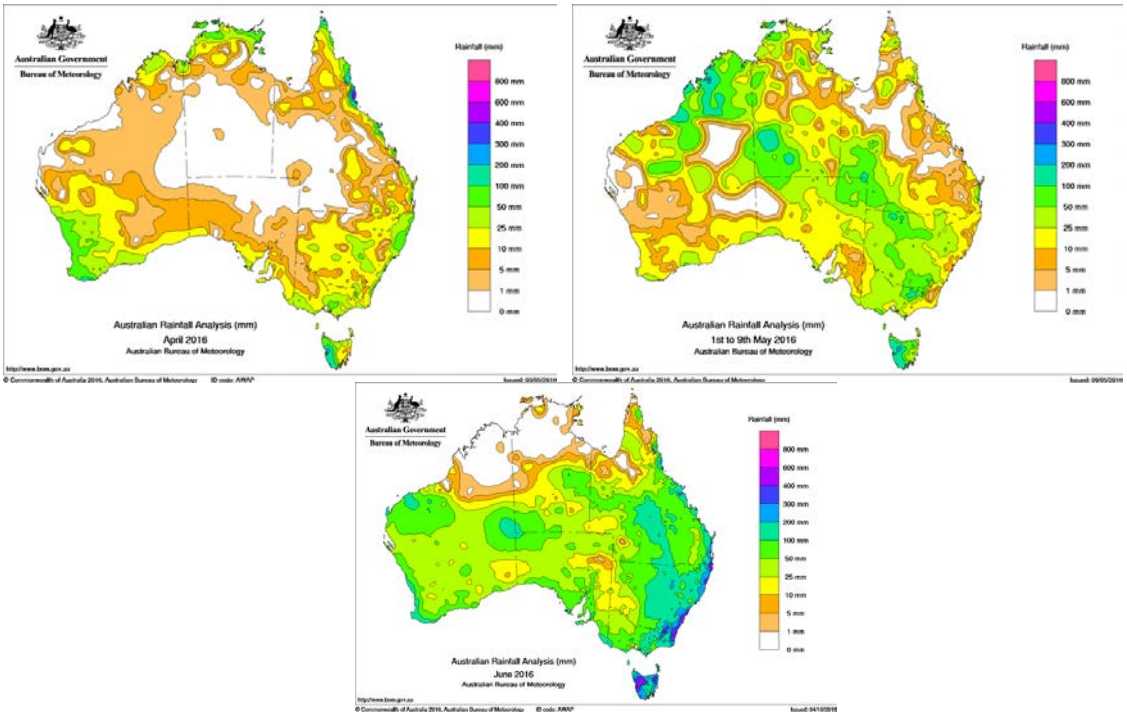


Fig. 1. Rainfall totals for (a) April (b) May 2016 (c) June 2016

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples (4 x 25 sweeps for the northern team) were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to check identify and determine parasitism levels. Larvae from the northern team were not reared but kept in alcohol for genetic analysis.

Vegetation scores and the presence of host plants were recorded about every 20 km using methods described in previous trip reports. The GPS-enabled tablet computer running ArcMap and loaded with digital vegetation maps for South Australia and NSW (see May field trip report) was updated with Queensland vegetation maps by Kris le Mottee and used for the Queensland component of the trip, but not the southern trip because only one computer was available.

The survey route taken and sample sites are shown in Fig. 2.

Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants. Where these have no dots there were no larvae found. Red markers with dots represent sample sites where larvae were found.

Trip log

Southern trip

24-6-16 Port Augusta to Roxby Downs

Medics, mostly *M. polymorpha*, were abundant in the first 60 km north of Port Augusta. Two samples on this yielded only 1 and 2 small larvae per 100 sweeps. After this the only potential hosts were very small seedlings, too low to sweep and not identifiable, but they may have included some daisies. There was also a geranium-like plant (but probably not *Erodium crinitum*) on which two samples were done, with only low numbers of very small larvae, which did not survive rearing so we cannot be sure they were *H. punctigera*. It was mostly restricted to roadsides. Stony downs and saltbush country between Pimba and Roxby Downs supported very few hosts.

25-6-16 Roxby Downs to Marree

In sandy country north of Roxby Downs, along the Borefields Track, several host species were common though usually scattered. They included *Myriocephalus stuartii*, *Senecio gregorii, Nicotiana velutinum, Goodenia* spp., *Erodium crinitum* and various Brassicas. The area was very green and there were also many low green seedlings .Sampling on the scattered hosts yielded no or very few larvae, with the exception of one site 62 km north of Roxby Downs, where 99 larvae per 100 sweeps were collected on *S. gregorii*. However, on rearing about half off these proved to be *Heliothis punctifera*, not *Helicoverpa punctigera*. When the sandy country ended about 100 km north of Roxby Downs, the hosts disappeared and the only host present was Cullen cinereum, mostly post-flowering and restricted to drainage lines. A sample on this yielded only one larva in 100 sweeps.

26-6-16 Marree-Birdsville Track-Marree

We drove about 120 km up the Birdsville Track, but rain and the deteriorating condition of the road forced us back to Marree. For the first 70 km hosts were restricted to *Cullen cinereum* in the drainage lines. Two samples on this yielded no larvae. Further up the track low *Helipterum floribundum* was common, and in one sandy patch *Senecio gregorii* was noted. However rain prevented sampling on these hosts.

27-6-16 Marree – Port Augusta

Following further overnight rain we decided to abandon attempts to get to Birdsville and return to port Augusta, via the Flinders Ranges. For the first 100 km south of Marree the vegetation was dominated by saltbush, with no obvious hosts although there were patchy low green plants germinating which might have included some host species. In the Flinders Ranges Ward's weed (*Carrichtera annua*) was abundant. It is a host for diamondback moth but not *Helicoverpa*. There were also patches of medic, increasing towards the south, and the geranium-like plant that was also noted between Port Augusta and Roxby Downs. A sample on the geranium-like plant 2 km south of Hawker yielded 2 larvae per 100 sweeps, but again they did not survive rearing.

Northern trip

The following information was compiled by Colin Tann, and refers only for the part of the trip when he was accompanied by Kris Le Mottee

21st June-
Narrabri to Cunnamulla

No sampling carried out. (O/N @ Cunnamulla)
The country was green and the vegetation generally fresh and young.

22nd & 23rd June-
Cunnamulla, Eulo, Thargomindah, Innamincka

First night camped east of Thargomindah and successfully caught moths in pheromone and light traps.
The Mulga country we passed through initially was generally devoid of fresh green vegetation : this area appeared to have missed the recent rains in May and before. However Eulo reported 44mm of rain a couple of days before, and we would expect some vegetation response over coming weeks. As we passed Thargomindah and progressed along the Innamincka road, the country-side improved markedly with large expanses of fresh green herbage, but very few hosts, until we got closer to Innamincka and into some of the sandy country. Here we observed *Senecio gregori* starting to flower and on sampling found some larvae developing. Also *Ptilotus sp.* and various *Brassicaceae spp.* but sampling resulted in nil catch. *Myrocephalus stuartii* was found with flower buds developing. There is certainly some potential for reasonable expanses of hosts to develop further in this area
The weather was problematic with rain periods and wet and 'sloshy' roads. It also made sampling difficult as vegetation was generally very wet.
Sliding into Innaminka we decided to stay the night at the roadhouse cabins as rain was persisting and all roads had been closed.
Able to dry off gear in the camp kitchen
The chance of progressing on up to Birdsville via Arrabury and Cordillo Downs was now out of the question. We were forced to backtrack!
Light traps were set on the veranda and a few moths collected the following morning.

24th June-
Innamincka, Eromanga, Windorah

All roads remained closed out of Innamincka, but the Thargomindah road was only about 30kms of dirt/mud, so we headed back on that.
We continued vegetation assessments once we turned off to Eromanga.
Travelling through Mulga and Gidgee country there were very few hosts. Once on the Quilpie – Windorah road young, green immature herbage could be observed in the rather wet conditions, but very few hosts were sighted.
Camped in Mulga country and set up light and pheromone traps resulting in only one moth being caught after a very cold night resulting in early morning frost.

25th June
Windorah area

Travelling westward we observed a lot of water lying about, and some fresh green immature herbage developing in sections. The creek crossings were mostly flooded, and in the main Cooper creek channels we found ourselves driving through large stretches of 0.5+ M water.
Arriving in Windorah we discovered that the road we had just driven on was now closed, expecting further rises over the next couple of days. We were lucky to get through!.
Whilst in Windorah we caught up with Peter Gregg's collaborator who is running pheromone traps.

Continuing on towards Birdsville the country-side was generally grassy with a distinctive fresh green base and various Chenopods being refreshed by recent rains. We sampled both *Cullen sp.* and *Malvastrum americanum* but found no larvae.
Camped along a dune system about 70 km east of Birdsville where we had noticed some *Senecio gregorii* flowering and supporting larvae.
Set light and pheromone traps that night resulting in three moths the following morning. A wet night…..again!

26th June
Birdsville

Arrived in Birdsville quite wet and cold and managed to secure a room at the Birdsville hotel. Very lucky to get the room considering there were quite a few travellers now stranded here.
The road out into the desert was a muddy mess, the Birdsville track was closed which meant that Peter Gregg and Alice Del Soccoro were unable to meet us in Birdsville as planned. The road to Windorah and Quilpie had remained closed with water levels reaching above the 1M mark where we had travelled earlier. Light rain persisted. Time to dry off wet camping gear once more in a nearby shed.

Sampling Results-

Moth collections-

23rd June
Camp site=Thargominda Road, Noccundra (S 27 42.52, E 142 59.03)
9 male, 2 female *H.punctigera*

24th June
Innamincka Roadhouse
I Male & 1 female Hp

25th June
Qulpie- Windorah road (S26 27.076, 145 45.324)
1 male Hp

26th June
70 km east of Birdsville (S25 43.896, E 139 53.669)
3 male Hp

A summary of larval surveys is given in Table 1.

Date	Location	Host	Larvae/100 sweeps	% *H. punctigera*
	Southern trip			
24/6/16	20 k N Port Augusta	"wild geranium"*	1	NA
	60 k N Port Augusta	"wild geranium"*	2	NA
25/6/16	38.6 k N Roxby Downs	*Myriocephalus stuartii*	0	
	"	*Nicotiana velutinum*	0	
	"	*Sclerolaena sp.**	0	
	53 k N Roxby Downs	*Myriocephalus stuartii*	0	
	67 k N Roxby Downs	*Myriocephalus stuartii*	99	47
	80 k N Roxby Downs	*Myriocephalus stuartii*	2	NA
	"	*Nicotiana velutinum*	6	100
	183 k N Roxby Downs	*Cullen cinereum*	0	
26/6/16	2 k SE Marree	*Cullen cinereum*	0	
	71 k N Marree	*Cullen cinereum*	0	
27/6/16	2 k S Hawker	"wild geranium"*	2	NA
	Northern trip			
23/6/16	Innaminka area S27 30.228, E141 12.635	*Senecio gregorii*	36	NA+
24/6/16	Innaminka area S27 44.531, E140 48.352	*Senecio gregorii*	16	NA+
	Eromanga area S26 46.369 E143 03.686	*Abutilon* sp.	0	
25/6/16	Windorah area S25 42.034 E141 09.819	*Malvastrum americanum*	0	
26/6/16	Birdsville area S25 40.28 E140 03.823	*Cullen cinereum*	6	NA+
	Birdsville area S25 43.638 E139 53.561	*Senecio gregorii*	14	NA+

*plants not known to be hosts + larval rearing data unavailable because larvae were preserved in alcohol for genetic analysis, but identification will be available eventually.

Table 1. Summary of larval surveys

Summary and conclusions

While this trip was hampered by wet weather, some substantial populations of *Helicoverpa* larvae were located, in sandy country north of Roxby Downs and around Innamincka. Many of these larvae were quite small, and the host plants were either pre-flowering or in the early flowering stages. This indicates that colonisation of many areas was just beginning. The rain that hampered

the trip will ensure that there is widespread growth of hosts in late winter and spring, and there are likely to be substantial populations of *H. punctigera,* especially in sandy areas. It will be particularly interesting to see how the mulga country of SW Queensland responds to the winter rain, as this is the first year we have seen good rain during the winter in this area.

The large numbers of larvae that we found on one site, north of Roxby Downs, consisted of about equal proportions of *H. punctigera* and lesser budworm *Heliothis (= Neopcleptria) punctifera*. This follows detection of this species in our May 2016 trip, the first time we have seen this species for many years. It indicates that the potential large populations later in winter and spring might have substantial numbers of this species as well as *H. punctigera*.

CRDC Project Inland Trip Report
5-13 August 2016

Peter Gregg, Alice Del Socorro and Kris Le Mottee

Objectives

The objectives of the trip were to survey the host plants and larval numbers in South Australia and western Queensland, and to make contact with new trap operators at Birdsville and renew contact with previous trap operator at Eromanga. We also wanted to sample some chenopods, and collect some inland plants for stable carbon isotopes to determine their photosynthetic pathways, in order to investigate the origins of C4 *H. punctigera* detected by Geoff Baker and Colin Tann in cotton regions. Peter and Alice travelled from South Australia after field work for our GRDC project, and Kris joined us after flying into Birdsville.

Background

In June we had attempted to survey this area but were restricted by rain (see June 2016 trip report). Nevertheless it was clear that above average rainfall, including the rain that was in progress, would result in extensive host growth, especially in the Birdsville and Innamincka areas. It also provided the opportunity to determine whether there was finally a recovery in host plant growth in the mulga, after successive surveys following the Millennium drought had failed to find many hosts and larvae.
After the June rain it had remained fairly dry in July (Fig. 1), but summed over the preceding three months rain had been well above average, especially in the Birdsville-Innamincka areas and in a band towards Thargomindah.

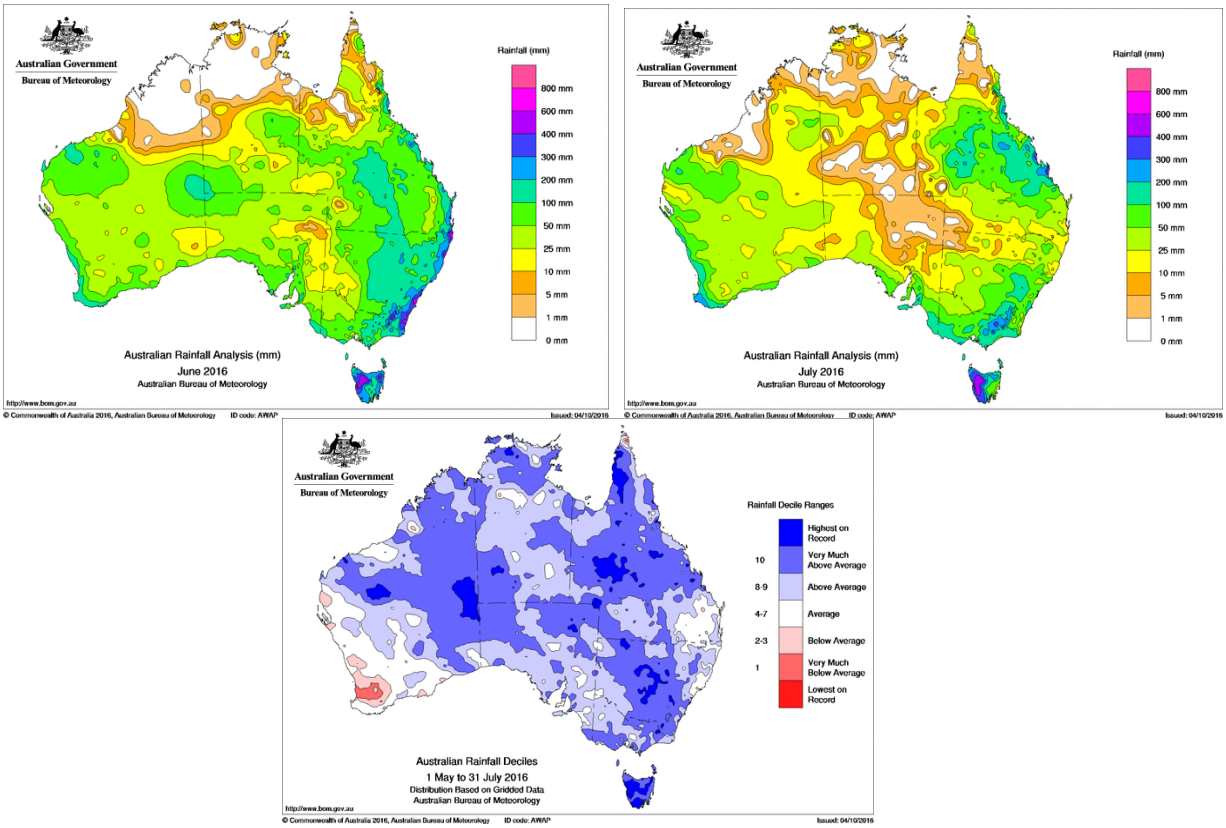


Fig. 1. Rainfall totals for (a) June (b) July 2016 (c) deciles for May-July 2016

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples (4 x 25 sweeps for the northern team) were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to identify species and determine parasitism levels.

Vegetation scores and the presence of host plants were recorded about every 20 km using methods described in previous trip reports. The GPS-enabled tablet computer running ArcMap and loaded with digital vegetation maps for South Australia and NSW (see May field trip report) was updated with Queensland vegetation maps by Kris le Mottee and used for this trip. The survey route taken and sample sites are shown in Fig. 2.

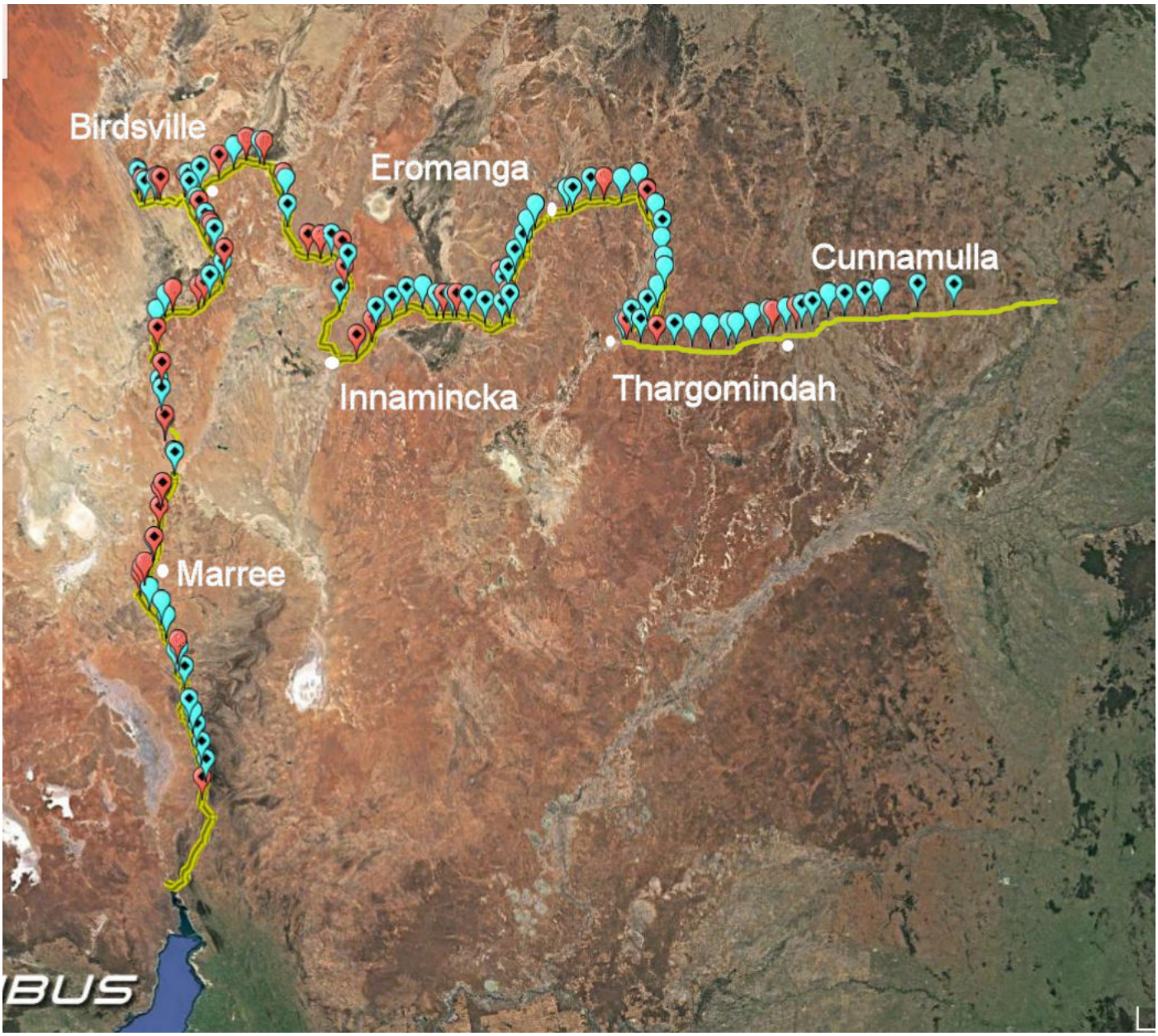


Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants. Where these have no dots there were no larvae found. Red markers with dots represent sample sites where larvae were found.

5-8-16 Port Augusta to Marree
Since the Borefield Track north of Roxby Downs was still closed due to rain, we travelled to Marree via Leigh Creek, up the western side of the Flinders Ranges.

From Hawker north, *Medicago polymorpha*, an unidentified geranium-like plant that we have previously found larvae on (see June 2016 report), occasional *Swainsona* sp. and the daisy *Rhodanthe pygmaea* (= *Helipterum pygmaeum* were seen. A sample on the latter 20 km north of Hawker yielded 5 larvae, making this a new host record. At the northern end of the Flinders, these hosts disappeared, leaving only roadside *Rhodanthe stricta* and, closer to Marree, *Cullen cinereum* in the drainage lines. No larvae were found in two samples on these hosts. The area between Lyndhurst and Marree was dominated by saltbush with no hosts. At Marree we ran a light trap overnight and caught one *H. punctigera* moth. No moths were caught in four Megaview pheromone traps.

6-8-16 Marree – Birdsville Track
Heading north on the Birdsville Track all the drainage lines contained *Cullen cinereum*, mostly post-flowering. There was also some orange Darling pea, *Swainsona stipularis*, in drainage lines, but sampling on these hosts produced no larvae.

After about 20 km *Rhodanthe floribunda* appeared, at first quite low but increasing in size as we went north. From about 443 km north of Marree larvae were found on this host, but the numbers were low. There were also patches of *Senecio gregorii* in sandy areas, and the *Cullen cinereum* and *Swainsona stipularis* became fresher and more abundant. Low numbers of larvae were found on all these hosts. This is an area where in the past we have seen very few hosts, mostly *C. cinereum* confined to drainage lines, and on this trip, although the larval density was low, hosts were common if scattered, indicating that many *H. punctigera* might be produced in this large region in suitable seasons.

We camped at Poongalassie Creek, about 70 k north of Mungerannie, and ran a light trap and four Megaview pheromone traps. No moths were caught in the pheromone trap, and the light trap caught 2 *H. punctigera* and about 10 *N. punctifera*.

7-8-16 Birdsville Track – Birdsville
Heading north from the campsite conditions continued to be similar for about 20 km, with occasional *R. floribunda* and *S. gregorii*, and *C. cinereum* and *S. stipularis* in the creeks. For the next 80 km or so, the road covered stony downs country with very few hosts except in creeklines, and very few larvae found.

However, from about 248 km north of Mungerannie (about 70 km south of Birdsville) sandhills with extensive coverage of flowering *S. gregorii, Rhodanthe moschata* and *Myriocephalus stuartii* were found. One sample here yielded very large numbers of *H. punctigera* larvae. As it was getting dark we decided to continue to Birdsville and return to this area the next day.

8-8-16 Around Birdsville

We checked and repaired the pheromone traps at Birdsville. One trap contained just one *H. punctigera* moth. On making contact with new collaborator Kylie Bailey-Hill we discovered that although she had been checking the traps regularly, she had not changed the lures, which had been in place since they were first installed (15 June), making data from about the end of July unreliable. We immediately replaced the lures. We then headed south down the Birdsville Track to survey areas we had missed the previous day due to darkness. We found two more sites where very high numbers of *H. punctigera* larvae were found on *S. gregorii* and *M. stuartii*.

Kris arrived on the afternoon flight.

9-8-16 Simpson Desert

We checked the pheromone traps at Birdsville after having replaced the lures. No moths were caught. We then headed out into the Simpson Desert as far as the monitoring site on the Eyre Creek bypass, finding three more sites where very large numbers of larvae were present on *S. gregorii*, *M. stuartii*, *R. moschata* and *R. floribunda*. We also discovered a few larvae on *Blennodia canescens*, a brassicaceous plant that was abundant but is not known as a good host. These larvae were found while searching for diamondback moth larvae, of which a few were recovered. This marks the first finding of these insects in central Australia, and a new host record for them. The status of *B. canescens* as a *punctigera* host is uncertain, because when high populations of larvae are found on good hosts, a few may incidentally crawl onto other plants. We also saw many larvae crawling on the ground in some areas, and on large pigweed (*Portulaca intraterraneae*). Similarly the host status of this plant is uncertain, but it is of interest because it has the C4 photosynthetic pathway. On return to Birdsville we set up a light trap, but it failed due to a poor connection. No moths were found in the pheromone traps the next day.

10-8-16 Birdsville –Innamincka

Heading east on the Windorah road, the country was mainly stony downs with a few sandy patches. *C. cinereum*, *Swainsona* sp. and *Malvastrum americanum* were found in creeklines, but there were very few larvae on these hosts. This pattern continued down the "Cordillo Downs" road, but near "Cordillo Downs" station we encountered sandy country with extensive stands of *S. gregorii* and *M. stuartii*. More larvae were found here but the numbers were not as high as around Birdsville. Between "Cordillo Downs:" and Innamincka the country was often sandy, but undulating rather than high sandhills. *M. stuartii* and *S. gregorii* were seen, but more scattered than further north. *R. floribunda* was common in lower areas, as were brassicas. However darkness prevented sampling for the last 60 k into Innamincka.

11-8-16 Innamincka-Eromanga

Heading east from Innamincka we found a sandhill site where high numbers of larvae were present on *M. stuartii*, but the sandy country soon gave way to stony downs where the main host was *Rhodanthe stricta*, and lower numbers of larvae were present. On the Cooper floodplain near Jackson moderate numbers of larvae were found on *Senecio lautus*, and on sandy country on the edge of the floodplain on *S. gregorii*. Going northeast towards Eromanga the country was a mixture of stony downs and mulga, with patches of *R. floribunda* and *Calotis multicaulis* in the latter, but few larvae were found. We ran a light trap and two Megaview pheromone traps 10 k west of Eromanga but no moths were caught.

12-8-16 Eromanga-Thargomindah

We visited Craig Brimms at Eromanga State School, who is again running the traps. Two moths were found in one of the traps, and three in the other. The mulga country between Eromanga and Quilpie had very few host plants despite the rain. There were scattered *Calotis multicaulis* and *Velleia glabrata*, with moderate numbers of larvae on one sample on *C. multicaulis*. We sampled on two species of chenopods, with negative results. South of Quilpie the Bulloo floodplain had patches of very low, but flowering, *R. floribunda*, and moderate numbers of larvae were found at one site. However the mulga country had very few hosts until we got close to Thargomindah, where there had been more rain and larger patches of *Calotis multicaulis* and *Velleia glabrata* were seen. We ran a light trap near the pheromone traps at Thargomindah and checked the pheromone traps. No *H. punctigera* moths were caught but there were many *N. punctifera*. No moths were found in the pheromone traps.

13-8-16 Thargomindah – St George

East of Thargomindah there were substantial patches of *Velleia glabrata* and *Calotis multicaulis*, with more scattered *R. floribunda* in some places. These hosts in the mulga appeared to be finally recovering from the drought, but have a long way to go. Hosts became less common after about 40 km from Thargomindah, and there were very few through Eulo to the Warrego floodplain. Few larvae were found except for one site where large numbers were recovered from wild tobacco, *Nicotiana velutinum*, which is known to be a good host. On the floodplain medics, *R. stricta* and *Helipterum sturtianum* were present, but few larvae were found. The mulga between Cunnamulla and St. George contained few hosts, but in places there were large

areas of low green vegetation which might have included some daisies and *Erodium crinitum*, and the marker post at Bollon had more of these hosts than we have previously seen at this site.

Stable carbon isotope analysis

Two samples were collected and analysed from each of the following plants:

Asteraceae

Calotis cuneifolia – purple burr daisy
Calotis multicaulis – woolly-headed burr daisy
Myriocephalus stuartii – poached egg daisy
Rhodanthe floribunda – large white sunray
Rhodanthe moschata – musk sunray
Senecio lautus – groundsel
Senecio gregorii – fleshy groundsel

Fabaceae

Cullen cinereum – annual verbine
Cullen pallidum – woolly scurf pea
Swainsona sp. – Darling pea

Goodeniaceae

Velleia glabrata – pee-the-bed
Goodenia fascicularis – silky goodenia

Solanaceae

Nicotiana velutinum – wild tobacco

Geraniaceae

Erodium crintum – wild geranium

All these proved to have the C3 photosynthetic pathway. One plant, large pigweed, *Portulacca intraterranea* (Portulaceae) was identified as C4, but as discussed (daily log, 9/8/16) its host plant status is uncertain.

Date	**Location**	**Host**	**Larvae/100 sweeps**	**% *H. punctigera***
	Southern trip			
5/8/16	35 k N Hawker	*Rhodamthe pygmaea*	5	100
	28 k N Leigh Creek	*Rhodanthe stricta*	0	
	2 k E Marree	*Cullen cinereum*	0	
6/8/16	7 k N Marree	*Swainsona stipularis*	0	
	14 k N Marree	*Cullen cinereum*	0	
	43 k N Marree	*Rhodanthe floribunba*	3	NA
	78 k N Marree	*Rhodanthe floribunda*	9	100
		Cullen cinereum	0	
	98 k N Marree	*Senecio gregorii*	4	100
	103 k N Marree	*Cullen cinereum*	3	100
		Swainsona stipularis	1	100
	142 k N Marree	*Senecio gregorii*	4	100
	183 k N Marree	*Rhodanthe floribunda*	1	NA
	39 k N Mungerannie	*Cullen cinereum*	1	NA
		Rhodanthe floribunda	1	NA
7/8/16	80 k N Mungerannie	*Rhodanthe floribunda*	1 (4)	NA
	131 k N Mungerannie	*Cullen cinereum*	0	
		Swainsona stipularis	0	
	170 k N Mungerannie	*Cullen cinereum*	5	100

		Senecio gregorii	6 (2)	NA
		Nicotiana velutinum	32	100
	216 k N Mungerannie	*Rhodanthe floribunda*	2 (3)	100
		Cullen cinereum	2 (3)	NA
	248 k N Mungerannie	*Senecio greorii*	124	100
		Myriocephalus stuartii	111	87.5
8/8/16	254 k N Mungerannie	*Senecio gregoriii*	84	100
		Myriocephalus stuartii	78	100
	35 k S Birdsville	*Myriocepohalus stuartii*	20	100
	1k S Birdsville (trap site)	*Goodenia* sp.	3	NA
9/8/16	Big Red sand dune	*Blennodia canescens*	14	50
	38 k W Birdsville	*Myriocephalus stuartii*	144	100
	9 k N Eyre Creek T/O	*Blennodia canescens*	1	NA
	2 k N Eyre Creek T/O	*Senecio gregorii*	216	100
	7k E Eyre Creek	*Rhodanthe floribunda*	151	50
10/8/16	47 k E Birdsville	*Cullen cinereum*	4	100
	85 k E Birdsville	*Calotis multicaulis*	0	
	108 k E Birdsville	*Myriocephalus stuartii*	0 (3)	
	36 k S Cordillo Downs T/O	*Rhodanthe moschata*	1	
	119 k S Cordillo Downs T/O	*Myriocephalus stuartii*	17	100
	9 k S Cordillo Downs	*Calotis multicaulis*	0	
		Cullen cinereum	0	
	40 k S Cordillo Downs	*Myriocephalus stuartii*	74	100
	77 k S Cordillo Downs	*Rhodanthe floribunda*	12	NA
11/8/16	21 k E Innamincka	*Myriocephalus stuartii*	119	100
	45 k E Innamincka	*Sclerolaena* sp. *	0	
		Rhodanthe stricta	0 (1)	
	152 k E Innamincka	*Senecio lautus*	7	100
	167 k E Innamincka	*Senecio gregorii*	10	100
	44 k NE Jackson	*Rhodanthe stricta*	0	
		Calotis multicaulis	1	0
	69 k NE Jackson	*Rhodanthe floribunda*	3	100
12/8/16	22 k E Eromanga	*Calotis multicaulis*	11	NA
	60 k E Eromanga	*Saltbush* sp. 1* (*Bassia longicuspis*?)	0	
		Saltbush sp. 2*	0	
	22 k S Quilpie	*Rhodanthe floribunda*	9	100
	5 k E Thargomindah	*Velleia glabrata*	2	NA
13/8/16	40 k E Thargomindah	*Nicotiana velutinum*	103	100
	45 k E Eulo	*Senecio lautus*	0	
	4 k E Cunnamulla	*Rhodanthe stricta*	0	
		Helipterum sturtianum	5	NA

Table 1. Summary of larval surveys

*plants not known to be hosts. Samples in brackets are where there was insufficient material for 5 x 20 samples. NA means not enough larvae survived to estimate percentage *H. punctigera*. Where percentage is less than 100, the remaining larvae were *Heliothis (=Neocleptria) punctifera.*

Summary and conclusions

The main conclusion from this trip is that in sandy country, especially around Birdsville, the Simpson Desert and Innamincka, there are very large populations of *H. punctigera* larvae. There are also lower density populations covering a wide area of the Birdsville Track and the stony downs country east of Innamincka. This suggests there are many potential immigrants for later in the spring, though conditions in many areas remain green and if there is continued rain in September and October, migrations may be delayed. Despite receiving good winter rain, the mulga areas still do not show extensive host growth. However, in areas where the rain was particularly heavy, such as around Thargomindah, there are more hosts than we have seen in many years, and this suggests that regeneration of host populations in some of these areas is happening, though it may take at least another good season of autumn/winter rain, and maybe several seasons, before it is complete.

It also appears that *Heliothis punctifera* populations are slowly re-establishing in western Queensland. Although the numbers are less than might have been expected after the May and June survey trips, which recovered substantial numbers of this species in South Australia, we caught more *H. punctifera* adults in the light trap than *H. punctigera*. Finally, we have not been able to clarify the origins of C4 *H. punctigera* from this trip. All the inland plants we collected proved to be C3, with the exception of large pigweed, and we are not certain of the host status of that plant. Additional samples on chenopods have not yielded any *H. punctigera* larvae, further suggesting that these plants are not major hosts. It therefore appears likely that, unless there is a major C4 host that we do not know about, the C4 *H. punctigera* are not coming from the inland.


(a) *Rhodanthe pygmaea* (a new host) 35 k N Hawker, (b) Orange Darling Pea, *Swainson stipularis* and *Cullen cinereum* in drainage line, 103 k N Marree


(a) *Rhodanthe floribunda* along Birdsville Track, 183 k N Marree (b) *Senecio gregorii* and *Myriocephalus stuartii* on sandhills with large numbers of larvae, 254 k N Mungerannie


(a) Extensive growth of *Blennodia canescens* and other brassicas, with some *S. gregorii*, near Eyre Creek. DBM larvae found here. (b) *M. stuartii* on sandhills near "Cordillo Downs"


(a) Host plant regeneration, mostly *Calotis multicaulis* in mulga, 22 k east Eromanga. (b) patchy *Rhodanthe floribunda* in mulga, 80 k NE Jackson


(a) No host plant regeneration in mulga, 17 k E Eromanga (b) *Rhodanthe stricta*, *Helipterum sturtianum* and medics on the Warrego floodplain, 4 k E Cunnamulla

CRDC Project Inland Trip Report
31 August, 7 October and 27 October 2016

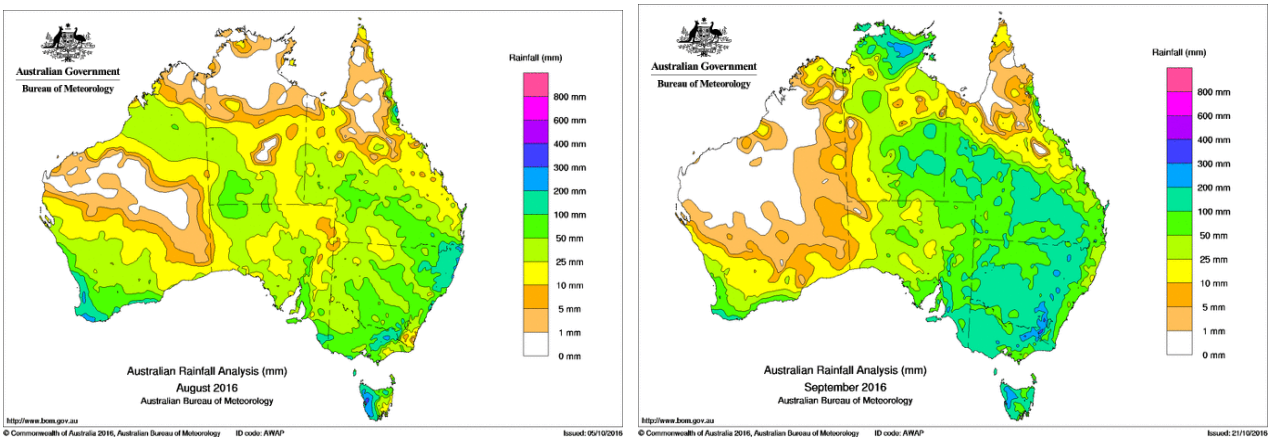
Peter Gregg and Alice Del Socorro

Objectives

This report describes three short surveys in southwestern NSW that were done in conjunction with other projects. One survey in the Cobar – Wilcannia – Broken Hill area was done on 31/8/16 while enroute to finish the GRDC project in South Australia. The second consisted of a single sample made on 7/9/16 while returning from South Australia and preparing for the spring moth busting experiment near Carrathool. The third consisted of two samples made during the return from this work on 27/10/16. The objectives were to follow the development of host plants and *H. punctigera* populations in southwestern NSW, as the spring progressed.

Background

In early August we had established the presence of large *H. punctigera* populations in northern SA and western Queensland, on *Senecio gregorii* and *Myriocephalus stuartii* (among many hosts). Host plants in the mulga areas of western Queensland, especially *Rhodanthe floribunda*, were patchy with evidence of recovery from the Millennium drought in some areas but not others. The need to travel through southwestern NSW for other projects gave us the opportunity to survey hosts in this area, which we have not previously investigated in mid to late spring. However time was very limited, so these surveys were each conducted during one day. Following the good rains in May and June (see June 2016 trip report) there had been further rain in August and especially September, which had led to extensive flooding in the Murrumbidgee and Lachlan valleys by the time of our October trip.



g. 1. Rainfall totals for (a) August and (b) September2016

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples (4 x 25 sweeps for the northern team) were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to identify species and determine parasitism levels. However the larvae from the 31 August trip were not reared due to the fact that we were working in the field in South Australia for the next week, with no access to constant temperature facilities.

Vegetation scores and the presence of host plants were recorded about every 20 km using methods described in previous trip reports. We did not use the GPS-enabled tablet computer running ArcMap. The survey route taken and sample sites are shown in Fig. 2.

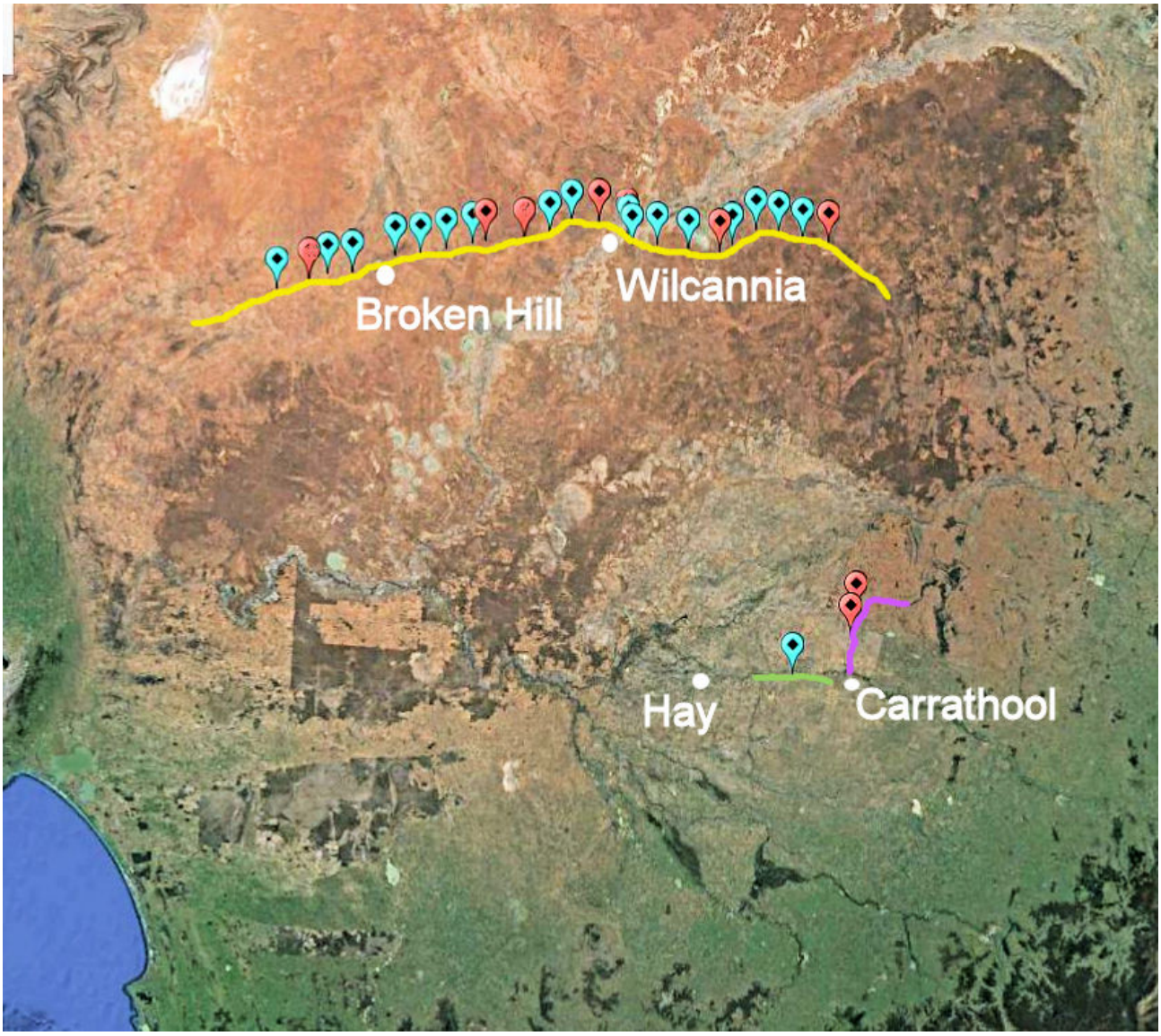


Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants. Where these have no dots there were no larvae found. Red markers with dots represent sample sites where larvae were found. The yellow track is 31/8/16, green is 7/9/16 and purple is 27/10/16.

31/8/16 Cobar to Peterborough

Beginning around Cobar, extensive areas of flowering *Rhodanthe floribunda* were seen. Growth of medics, mostly small burr medic *Medicago polymorpha* and cut leaf medic *Medicago lacinata*, was also extensive. There were numerous other hosts including *Helipterum jesseni* (now called *Hyalospermum semisterile*), *Rhodanthe moschata*, *Rhodanthe stricta*, *Craspedia uniflora* and *Erodium crinitum*, These conditions persisted through to Broken Hill, but after that hosts became patchy and pre-flowering, in saltbush dominated country. The Darling floodplain near Wilcannia had extensive areas of almost pure *R. floribunda*. Several samples yielded either only a few larvae, dominated by the very small and small size categories, or no larvae. However, at several sites many moths, of both *H. punctigera* and *H. punctifera*,

were seen flying among the hosts. The general impression was of an extensive area of *R. floribunda* and other hosts, more than we have seen previously even before the drought. These hosts were young and very green, were being rapidly colonised by heliothine moths and could support many larvae over the next few weeks.

7-9-16 Balranald – Hay – Carrathool – Griffith

No formal vegetation recording was adopted during this trip but extensive areas of *R. floribunda* in the budding or early flowering stage were noted from west of Balranald through Hay and Carrathool. At one site about 20 km west of Carrathool (exact location not recorded) a sample found no larvae, but many *H. punctigera* moths were seen flying. However, east of Carrathool the areas of *R. floribunda* became less obvious, with grasses, clovers, brassicas and medics dominating very green vegetation.

27/10/16 Carrathool – Rankins Springs

Two samples were undertaken following the spring moth-busting project on "Gundaline", during which pheromone catches and Magnet kills suggested that a predominantly *H. punctigera* population was widespread in the area. Much of the local vegetation was dominated by grasses and medics, but north of Carrathool some extensive areas of *R. floribunda* were found, and high numbers of larvae were found on them. Large numbers of hover flies were seen, as were some adult *H. punctigera*. Paterson's Curse was also abundant. However, going east towards Rankins Springs the *R. floribunda* disappeared.

The results of larval sampling are shown in Table 1.

Date	**Location**	**Host**	**Larvae/100 sweeps**	**% *H. punctigera***
31/8/16	180 k E Wilcannia	*R. floribunda*	3 (3)	NA
	90 k E Wilcannia	*H. semisterile*	6 (3)	NA
	64 k E Wilcannia	*R. floribunda*	0 (3)	NA
		H. semisterile	0 (3)	NA
	11 k E Wilcannia	*R. floribunda*	0	NA
	19 k W Wilcannia	*Craspedia uniflora*	2	NA
		Rhodanthe moschata	0	NA
	80 k W Wilcannia	*R. floribunda*	0 (3)	NA
	110 k W Wilcannia	*R. floribunda*	11	NA
	55 k W Broken Hill	*R. floribunda*	0	NA
7/9/16	20 k E Carrathool	*R. floribunda*	0	NA
27/10/49	25 k N Carrathool	*R. floribunda*	32 (2)	100
	42 k N Carrathool	*R. floribunda*	49 (2)	100
		E. plantagineum	8 (2)	100

Table 1. Summary of larval surveys

*plants not known to be hosts. Samples in brackets are where we did less than 5 x 20 samples. NA means not enough larvae survived to estimate percentage *H. punctigera*. Where percentage is less than 100, the remaining larvae were *Heliothis (=Neocleptria) punctifera.*

Summary and conclusions

The main conclusion from these trips is the remarkable areas of *R. floribunda* and (to a lesser extent) other native hosts that grow in southwestern NSW following good late winter and spring rain. These hosts were just being colonised in early September, but by late October were supporting large populations of larvae. It

seems likely that these populations could give rise to a late spring and summer generation that would affect local early cotton and other summer crops. If they are able to migrate northwards, for example on post-frontal - winds, they could also affect cotton regions in northern NSW, and this may be an explanation for the large spikes in *H. punctigera* activity in mid-summer that have been seen in recent years in these areas.

(a) (b)

(a) *R. floribunda* (white) and *H. semisterile* (yellow) in mulga, 31/8/16, 160 k east of Wilcannia.
(b) Extensive growth of *R. floribunda* on the Darling Floodplain, 11 k east of Wilcannia, 31/8/16

(a) (b)

(a) Patchy sub-story of medics with occasional *R. floribunda* and *R. stricta* in saltbush plains, 31/8/16, 37 k east of Broken Hill. (b) extensive growth of *R. floribunda* and medics in pastures, now being dominated by grasses and brassica weeds, 45 k north of Carrathool, 27/10/16

CRDC Project Inland Trip Report
5-12 June 2017

Peter Gregg and Alice Del Socorro

Objectives

The objectives of the trip were:
1) To survey the area in the centre of South Australia (Port Augusta to Coober Pedy) where significant rain had fallen about two months previously
2) To survey the area in south-west Northern Territory where similar rain had fallen. This area had not been surveyed since September 1991 under the old HIRG project.
3) To sample saltbushes and other chenopods to provide additional evidence of their status as host plants.

It was planned to leave the vehicle in storage at Alice Springs until further rain fell during winter, when we would fly back there and drive home through areas where rain may have fallen.

Background

There had been heavy rain in central Australia in December and January which had led to extensive grass growth, but little rain since then until April, when a rain band extended from the southwest corner of Northern Territory down through western South Australia (Fig 1a). May had been quite dry, and there had been very little rain since summer in western NSW, south-west Queensland or the north-east of South Australia (Fig 1b).

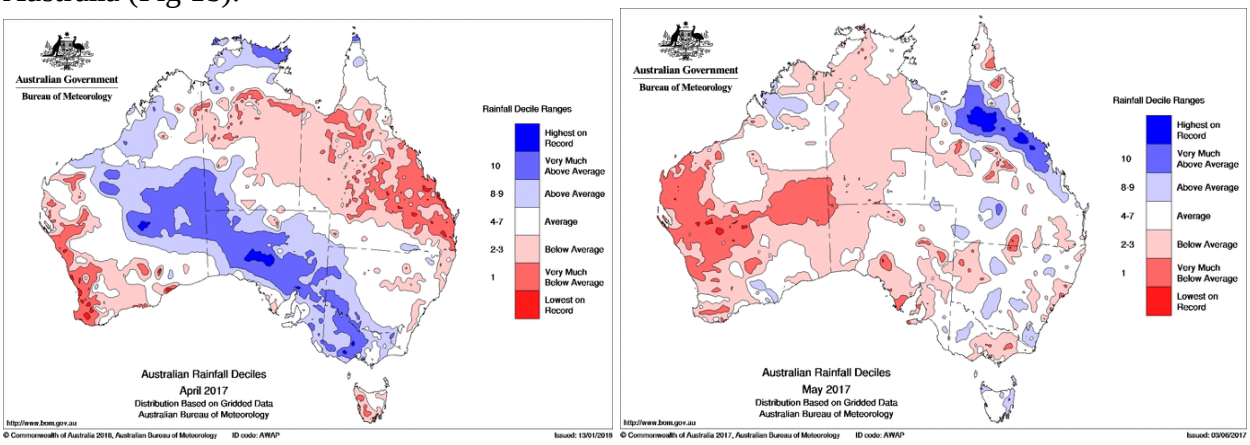


Fig. 1. Rainfall deciles for (a) April and (b) May 2017

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to identify species and determine parasitism levels. Vegetation scores and the presence of host plants were recorded about every 20 km using methods described in previous trip reports. The GPS-enabled tablet computer running ArcMap was updated with maps from the Northern Territory, and vegetation association numbers recorded with each vegetation assessment. The survey route taken and sample sites are shown in Fig. 2.

Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants. Where these have no dots there were no larvae found. Red markers with dots represent sample sites where larvae were found. Samples on chenopods are not shown as they were presumed to be non-hosts, but they are listed in Table 1.

5/6/17 Narrabri to Bourke

Conditions were extremely dry and the only host plants seen were small patches of medics in roadside table drains and drainage areas. No sampling was done.

6/6/17 Bourke to Tibooburra

We retrieved the pheromone traps from Bourke, where no collaborators had been available since 2014. Conditions continued very dry west of Bourke, with only very small medic patches close to Bourke, and one small patch of daisies (possibly small *Senecio gregorii*) west of Wanaaring. However, between Wanaaring and Tibooburra there were extensive areas where the ground was covered with dry daisy flowers, mostly *Rhodanthe floribunda*, which indicated that there must have been extensive host growth in the winter and spring of 2016 (Fig. 3), as we had found in both south-west Queensland and southern NSW at that time. No sampling was done.

7/6/17 Tibooburra – Broken Hill – Port Augusta

Conditions remained very dry, and the only hosts found were small patches of *Solanum* sp., mostly only on the roadside. No sampling was done, and from Yunta to Port Augusta no vegetation assessments were made as we were travelling in the dark.

8/6/17 Port Augusta – Roxby Downs – Woomera

In the area around Port Augusta some medics were seen among the saltbushes, but they cut out quickly as we headed north. No other hosts were seen until close to Woomera, when clumps of daisies, mostly *Brachyscome ciliaris*, were seen among the saltbushes, and in sandy country there were Malvaceous plants, especially *Abutilon otocarpum* and *Sida platycalyx*, but mostly too patchy for sweep netting. One sample near Roxby Downs on *A. otocarpum* yielded no larvae, as did a sample on a small patch of *Medicago laciniata* near Woomera. We sampled several times on saltbushes, including *Atriplex vesicaria*, *Maireana sedifolia* and *M. pyrimidata* but found no larvae. In places there was extensive growth of recently germinated saltbush seedlings, probably from the April rain, but they were too low to sweep.

At Roxby Downs we met the collaborators and retrieved moths from the pheromone traps for subsequent carbon analysis.

9/6/17 Woomera – Coober Pedy

North of Woomera clumps of *B. ciliaris* persisted briefly but none were seen from Glendambo out through Kingoonya and back north to the highway. We checked the traps at Kingoonya and retrieved moths for carbon analysis although very few were *H. punctigera*. We continued north to Coober Pedy using the back road to the highway. Occasional *A. otocarpum* and low broadleaf plants which may have included wild geranium were seen in this area, but no sampling was done until 80 km south of Coober Pedy, when another sample on *B. ciliaris* yielded no larvae. Several more samples on saltbushes (the same species as previously) also gave no larvae.

10/6/17 Coober Pedy – Erldunda

North of Coober Pedy conditions rapidly dried out, with no host plants seen. Further sampling was done on chenopods, including *Maireana astrotricha*, but no larvae were found.

11-12/6/17 Erldunda – Docker River

Conditions remained very dry with no host plants, other than a few patches of daisies *Minuria leptophylla* and *Brachyscome ciliaris*) between Curtin Springs and Yulara. A sample on *M. leptophylla* produced no larvae. West of Yulara the sandy country was heavily dominated by dry spinifex and buffel grass, originating from the summer rain (Fig. 4). It appeared that this had reduced host germination, with only a few Brachyscome clumps and a purple succulent seen between the grass clumps. No sampling was done. We camped 5 km west of Docker River.

13/6/17 Docker River – Erldunda

We returned via the same route as on the previous day, with no sampling being done. Alice flew home from Yulara while Peter continued on to Erldunda, sampling on saltbushes and *Sclerolaena* sp. with no larvae being found. A sample on *M. leptophylla*, 17 km east of Yulara, yielded three larvae in 60 sweeps, the only larvae found during the entire trip. Three samples were also made on a presumptive *Cassia* sp, called "firebush" by locals but unidentifiable because it was not flowering, with no larvae being found.

14/6/17 Erldunda – Alice Springs

Peter continued on to Alice Springs, where the vehicle was left in storage to await the return trip. Conditions were again extremely dry, with no hosts being seen. One sample on *Maireana astrotricha* produced no larvae.

The results of larval sampling are shown in Table 1.

Date	Location	Host	Larvae/100 sweeps	% *H. punctigera*
8/6/17	50 k N Port Augusta	*Atriplex vesicaria**	0	NA
	"	*Maireana sedifolia**	0	NA
	80 k N Port Augusta	*Maireana pyrimidata**	0	NA
	7k S Roxby Downs	*Abutilon otocarpum*	0	NA
	20 k S Roxby Downs	*Atriplex vesicaria**	0	NA
	"	*Tecticornia sp.**	0	NA
	5 k W Woomera	*Tecticornia sp.**	0	NA
	"	*Medicago laciniata*	0	NA
9/6/17	80 k N Pimba	*Atriplex vesicara**	0	NA
	"	*Maireana sedifolia**	0	NA
	199 k S Coober Pedy	*Maireana sedifolia**	0	NA
	"	*Maireana pyrimidata**	0	NA
	80 k S Coober Pedy	*Brachycsome ciliaris*	0	NA
10/6/17	39 k N Kulgera	*Maireana astrotricha**	0	NA
	"	*Sclerolaena* sp.*	0	NA
	60 k N Kulgera	*Maireana astrotricha**	0	NA
	66 k N Kulgera	*Tecticornia* sp.*	0	NA
13/6/17	17 k E Yulara	PG5006 (*Cassia* sp.?)*	0	NA
	"	*Minuria leptophylla*	3 (3)	100
	40 k E Yulara	PG5006 (*Cassia* sp.?)*	0	NA
	60 k E Yulara	PG5006 (*Cassia* sp.?)*	0	NA
	93 k E Yulara	*Sclerolaena cornishiana**	0	NA
	118 k E Yulara	*Maireana astrotricha**	0	NA
	60 k W Erldunda	*Maireana astrotricha**	0	NA
	40 k W Erldunda	*Maireana astrotricha**	0	NA
14/6/17	57 k N Erldunda	*Maireana astrotricha**	0	NA

Table 1. Summary of larval surveys

*plants not known to be hosts. Samples in brackets are where we did less than 5 x 20 samples. NA means not enough larvae survived to estimate percentage *H. punctigera*.

Summary and conclusions

Very few larvae were found on this trip, despite the fact that some parts of the trip covered areas where rain had fallen about two months previously. In western NSW it had been extremely dry and no host growth was expected. We did however find evidence that there had been extensive daisy growth in the previous winter, as we had seen then, in areas to both the north and south of the route we travelled. In the south of South Australia there was limited host growth, including *A. otocarpum*, *S. platycalyx* and *B. ciliaris*. There were also areas of low broadleaf plants, including saltbush seedlings, wild geranium and possibly daisies. We concluded that low temperatures restricted growth of these plants, and were unable to sample most of them because they were too low. Further north it became very dry. In the far west of the northern territory, although it had rained in April, we saw few hosts, probably because the extensive growth of spinifex and buffel grass after the summer rain had suppressed them. There were a few patches of daisies east of Yulara, and on one such patch three larvae were recovered in 60 sweeps, the only larvae found on the entire trip. Sampling on chenopods produced no evidence that they supported larvae, despite 16 samples being done on

9 species. The overall picture indicates that, unless rain falls soon, the inland will produce very few *H. punctigera* this spring.

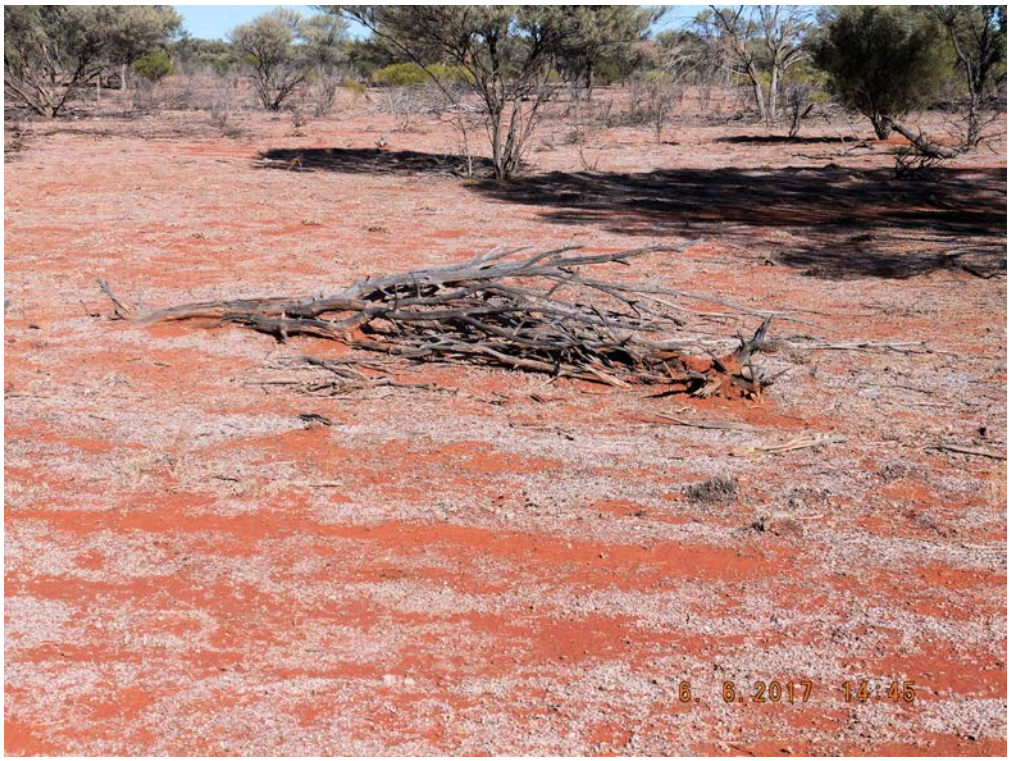


Fig. 3. Mulga 60 km west of Wanaaring, showing extensive ground cover of dry daisy heads, mostly *Rhodanthe floribunda*, indicating that this area must have supported many daisy hosts in the previous winter

Fig. 4 Mulga in the Peterman Ranges 40 km east of Docker River, showing heavy cover of buffel grass (*Cenchrus ciliaris*) which apparently smothered germination of host plants from the April rain. This area had supported many host plants in 1991, before buffel grass was introduced.

CRDC Project Inland Trip Report
8-24 August 2017

Peter Gregg and Alice Del Socorro

Objectives

The objectives of the trip were:

(1) To retrieve the vehicle from storage at Alice Springs and survey parts of central Australia

(2) To collect moths from collaborators for carbon analysis.

(3) To survey areas around “Jervois” and “Tobermory” in the eastern NT where rain had fallen

(4) As this was potentially the last inland field trip we wanted to further sample chenopods and other potential host plants that had not been sampled sufficiently in previous field trips.

The early parts of the trip (8-15/8/17) were done by Peter as a private trip. Alice flew into Alice Springs on 16/8/17 for the trip home.

Background

Following the rain in April in South Australia, May and June had remained dry throughout the inland, and in July only a small area of the eastern NT had received significant rainfall (Fig 1b). As this area had supported many host plants and large numbers of larvae on the only other occasion when it had been sampled (2000) we decided to target this region on the return trip. We also wanted to visit collaborators and collect any moths available from pheromone traps for carbon analysis.

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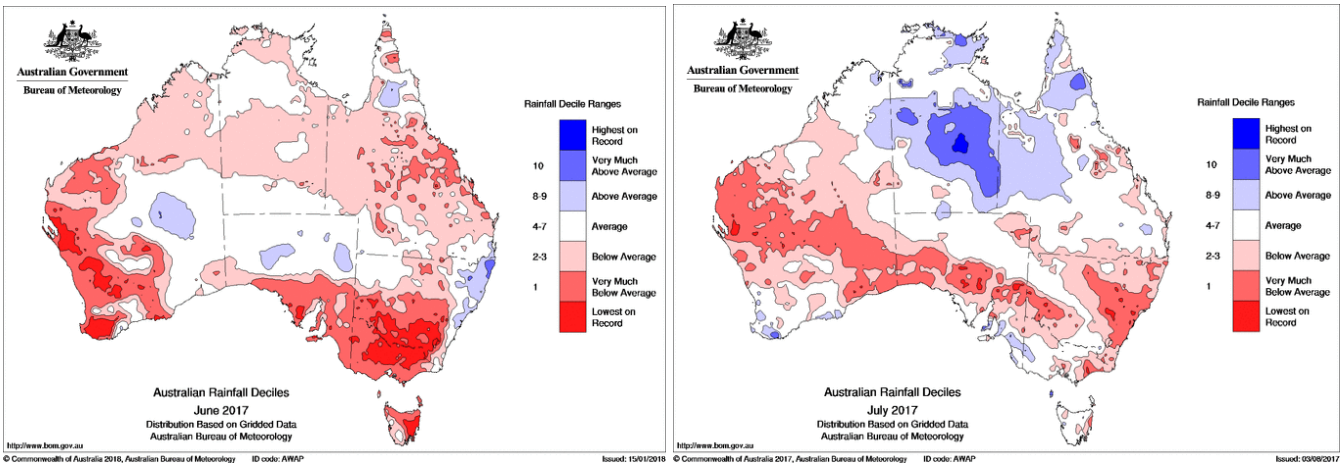


Fig. 1. Rainfall deciles for (a) June and (b) July 2017

Methods

Methods for sampling were the same as for previous inland surveys. At each site 5 x 20 sweep samples were made, and larvae were classified into very small, small, medium and large size categories. A minimum of 12 larvae per site (or all larvae, if less than 12 were recovered) were kept for rearing on artificial diet to identify species and determine parasitism levels. Vegetation scores and the presence of host plants were

recorded about every 20 km using methods described in previous trip reports. The GPS-enabled tablet computer running ArcMap was updated with maps from the Northern Territory, and vegetation association numbers recorded with each vegetation assessment. The survey route taken and sample sites are shown in Fig. 2.

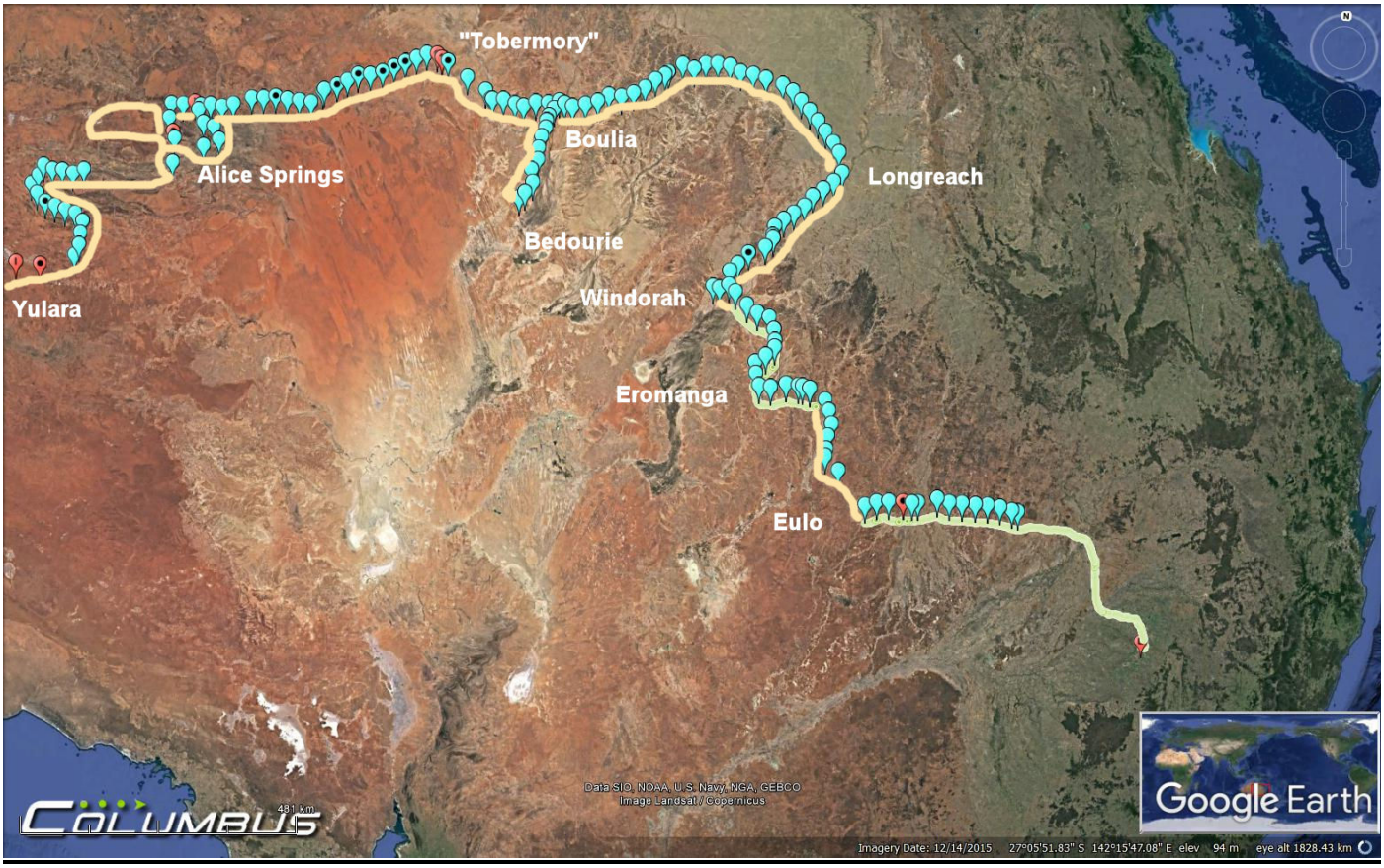


Fig. 2. Route and survey sites. Blue markers without dots are sites where no host plants were seen. Blue markers with dots are sites where hosts were found. Red markers represent sample sites on known host plants. Where these have no dots there were no larvae found. Red markers with dots represent sample sites where larvae were found. Samples on chenopods are not shown as they were presumed to be non-hosts, but they are listed in Table 1.

8-16/8/17 Around Alice Springs

Sampling and vegetation assessments were done on an opportunistic basis during a private trip around Alice Springs, Ross River and Yulara. In general this area was very dry, with no host plants present, as we had seen during the June 2017 trip. A small area of *Schoenia ayersii* was found near Yulara, and a few larvae were recovered from it, but on rearing they were found to be *Heliothis (Neocleptria) punctifera*, not *H. punctigera*. In the same area there were a few roadside Brachyscome ciliaris, but no larvae were found on them.

On 10/8/17 we left two pairs of pheromone traps for *H. punctigera* east of Hart's range, for subsequent collection on the way home.

17/8/17 Alice Springs to "Jervois"

Heading north from Alice Springs conditions were very dry, but there were a few patches of *Senecio magnificus*, mostly in roadside table drains. We sampled on one of these and recovered moderate numbers of larvae. Approaching Jervois the vegetation became greener, but was mostly dominated by grasses,

especially buffel grass. There were patches of *Brachyscome ciliaris*, but too scattered for sampling. We retrioeved the pheromone traps from near Hart's Range. No moths had been caught. We camped at "Jervois" and ran a light trap and three pheromone traps. No moths were caught in these traps either.

18/8/17 "Jervois" to "Tobermory"

Conditions remained fairly green, but mostly grass and perennial shrubs especially *Senna artemesioides* and *Eremophila* spp. Sampling on these produced no larvae. The only potential hosts were found about 160 km east of "Jervois", where a large patch of *Goodenia* spp., low and early flowering, was seen. No larvae were found on it. There was no sign of the large areas of daisies such as *Rhodanthe charsleyae, Schoenia ayersii* and *Senecio gregorii* which had been found in this area in 2000, following rain at a similar time of year. At "Tobermory" there was a small patch of *Cullen cinereum* which was being irrigated from a local creek. We sampled on this and found only two larvae per 100 sweeps. We ran a light trap and three pheromone traps overnight on this area, and another three pheromone traps approximately 7 km east of the Queensland border, where there were small patches of *Malvastrum americanum*. The light trap caught 15 *H. punctigera* but none of the pheromone traps caught any.

19/8/17 "Tobermory" to Boulia

Conditions dried out rapidly as we headed east from "Tobermory". We sampled on *Malvastrum americanum* near where we had run pheromone traps, with no larvae found. The Eyre Creek floodplain around "Glenormiston" was very dry, supporting no vegetation at all. From "Glenormiston" towards Boulia the Mitchell grass plains were also very dry, and no sampling was done.

20/8/17 Boulia – Bedourie - Boulia

We travelled to Bedourie to retrieve moths from the pheromone traps for carbon analysis. Conditions remained very dry and the only sampling was done on probable non-hosts, *Sclerolaena bicornis* and *Trichodesma zeylandicum*. No larvae were found. The Eyre Creek floodplains were very dry. We ran a light trap and two pheromone traps about 20 km east of Boulia, on the road to Winton. No moths were caught.

21/8/17 Boulia – Middleton-Winton-Longreach

Conditions continued extremely dry across the Mitchell grass plains and into the hilly mulga around Winton, with no host plants seen. Further sampling was done on chenopods, especially *Sclerolaena bicornis*, but no larvae were found.

22/8/17 Longreach to Windorah

Conditions remained very dry with no host plants, other than a few patches of roadside *Solanum quadriloculatum* near Jundah. Sampling on these and on various chenopods including *S. bicornis* produced no larvae. We ran a light trap and two pheromone traps 8 km east of Windorah, with one *H. punctigera* in the light trap but none in the pheromone traps. We retrieved moths from the collaborator for carbon analysis.

23/8/17 Windorah – Eromanga – Quilpie - Eulo

Conditions remained very dry, with no host plants seen. Sampling was done on *Pimelea simplex* east of Eromanga, with no larvae found. We checked the pheromone traps at Eromanga and recovered two moths with further moths being collected from the collaborator for carbon analysis.

24/8/17 Eulo-Cunnamulla-St. George

Conditions remained very dry with almost no vegetation under the mulga, and a complete absence of host plants on the Paroo and Warrego floodplains. About 5 km west of Cunnamulla we sampled on irrigated chickpeas and vetch, with large numbers of larvae found. A few of these proved to be *H. armigera* on rearing, but most were *H. punctigera*.

The results of larval sampling are shown in Table 1.

Date	**Location**	**Host**	**Larvae/100 sweeps**	**% *H. punctigera***
15/8/17	65 k E Yulara	*Schoenia ayersii*	8	0 (all *H. punctifera*)
	25 k E Yulara	*Brachyscome ciliaris*	0	NA
17/8/17	36 k N Alice Springs	*Senecio magnificus*	19	78
	60 k E Plenty Hwy T/O	*Senna artemesioides**	0	NA
18/8/17	15 k E "Jervois"	*Eremophila sp. (*PG4004)*	0	NA
	161 k E Jervois	*Goodenia sp.* (PG 4008)	0	NA
	"Tobermory"	*Cullen cinereum*	2	100
19/8/17	7k E Qld Border	*Malvastrum americanum*	0	NA
20/8/17	18 k S Boulia	*Sclerolaena bicornis**	0	NA
	75 k N Bedourie	*Sclerolaena bicornis**	0	NA
	157 k N Bedourie	*Trichodesma zeylandicum**	0	NA
21/8/17	6k E Boulia	*Sclerolaena bicornis**	0	NA
	60 k E Boulia	*Sclerolaena bicornis**	0	NA
	190 k E Boulia	*Sclerolaena bicornis**	0	NA
	140 k E Middleton	*Sclerolaena bicornis**	0	NA
22/8/17	176 k S Longreach	*Sclerolaena sp.* (PG 4011)*	0	NA
	16 k S Jundah	*Solanum quadriloculatum*	0	NA
23/8/17	67 k E Eromanga	*Pimelea simplex**	0	NA
24/8/17	5k W Cunnamulla	chickpeas	135	84 (rest *H. armiger*a)
	"	Vetch	12	71 (rest H. armigera)

Table 1. Summary of larval surveys

*plants not known to be hosts. NA means not enough larvae survived to estimate percentage *H. punctigera*.

Summary and conclusions

This trip suggested that there will be little immigration of *H. punctigera* from the inland to cropping areas in the coming spring. Even the area around "Jervois" and "Tobermory" where rain had fallen during July had few hosts. The comparison of this area with trips made before the Millennium Drought (2000) when daisies were widespread and abundant provides further evidence for long-term changes in host plants due perhaps to seed bank depletion. It could be that this trip was a little early and some hosts may germinate later, but we saw little evidence of this. Apart from a few hosts in this area, the rest of the inland was extremely dry and supported almost no larvae. Extensive sampling on chenopods again provided no evidence that these plants support *Helicoverpa* larvae. The large populations found on irrigated crops near Cunnamulla may be the progeny of immigrants from an earlier period when moths were leaving deteriorating conditions further west, or they may have come from cropping areas to the east. The presence of some *H. armigera* suggests

the latter explanation may be more likely, but in either case the presence of these larvae provides further evidence of the capacity of *Helicoverpa* to locate isolated patches of favourable environments over large distances.

Appendix 3

Carbon isotope status of *H. punctigera* moths from inland Australia, and likely host plants

For Final Report, Project UNE1502

Introduction

Recently Geoff Baker and Colin Tann (Project CRC1005) have reported the presence of *H. punctigera* moths with carbon isotope signatures indicative of feeding as larvae on plants with the C4 photosynthetic pathway. This is surprising because almost all the known hosts of *H. punctigera* have the C3 pathway. The C4 *H. punctigera* reported by Baker and Tann from cropping areas are mostly in the minority, and mostly occur in mid to late season.

This finding prompted us to examine the carbon isotope status of *H. punctigera* collected from inland pheromone traps.

Methods

We used the same carbon isotope analysis methods as Baker & Tann (2013; *Bulletin of Entomological Research* 103, 171–181), i.e. mass spectrometry at the University of New England, with the assistance of Leanne Lisle. For plants a small amount of leaf tissue (about 1 mg) was oven dried before assaying. For moths the insect was dried, the head was removed and about 1 mg of tissue used for analysis.

Sweep net samples were done using the same methods as applied to samples on other inland hosts, as described in Appendix 2.

Results and discussion

Sweep net sampling

The sweep net samples made during this project (2014 – 2017) are shown in Table 1. Samples were made on chenopods during four inland field trips in the winter and spring of 2016 and 2017. A total of 37 samples were made at 27 sites, covering the saltbush plains of western NSW and South Australia, the mulga country of Northern treritory and the grasslands and mulga of western Queensland. At each site 100 sweeps were made, in five replicates of 20 sweeps.

The most common chenopods sampled were in the two main genera of saltbushes, *Atriplex* spp. (especially *A. vesicaria*, bladder saltbush) and *Maireana* spp. (bluebushes, especially *M. sedifolia* and *M. astrotricha*). There were also several samples made on copper burrs, *Sclerolaena* spp. including *S. bicornis* and other species which were hard to identify because of lack of fruiting bodies, and on *Tecticornia* sp. (samphires) which are likewise difficult to identify to species.

In all these samples, totalling 3700 sweeps, not a single *Helicoverpa* larvae was recovered, even though in some instances there were larvae found on nearby daisies. These samples add to an extra 26 made during earlier projects, in which similar negative results were obtained, and suggest that if chenopods support *Helicoverpa* larvae at all, it is uncommon.

Table 1. Sweep net samples on chenopods, 2016 – 2017.

Host	**Location**	**Date**
Atriplex vesicaria	25 k W Coburn	12/5/16
Atriplex vesicaria	96k N Pt. Augusta	"
Maireana sedifolia	"	"
Atriplex vesicaria	1k E Pimba	13/5/16
Tecticonia sp.	"	"
Atriplex vesicaria	60k N Roxby Downs	"
Sclerolaena atrticulata	100 k N Roxby Downs	"
Chenopod 1	43 k N William Creek	14/5/16
Chenopod 2	"	"
Sclerolaena sp.	39 k N Roxby Downs	25/6/16
Atriplex vesicaria	50 k N Pt. Augusta	8/6/17
Maireana sedifolia	"	"
Maireana pyrimidata	80 k N Pt Augusta	"
Tecticornia sp.	20 k S Roxby Downs	"
Atriplex vesicaria	"	"
Tectico. nia sp.	5k W Woomera	"
Atriplex vesicaria	80 k N Pimba	9/6/17
Maireana sedifolia	"	"
Maireana sedifolia	199 k S Coober Pedy	"
Maireana pyrimidata	"	"
Maireana astrotricha	39 k N Kulgera	10/6/17
Sclerolaena sp.	"	"
Maireana astrotricha	60 k N Kulgera	"
Tecticornia sp.	"	"
Sclerolaena cornishiana	93 k E Yulara	13/6/17
Maireana astrotricha	118 k E Yulara	"
Maireana astrotricha	60k W Erldunda	"
Maireana astrotricha	40 k W Erldunda	"
Maireana astroitricha	57 k N Erldunda	14/6/17
Sclerolaena bicronis	18 k S Boulia	20/8/17
Sclerolaena bicornis	75 k N Bedourie	"
Sclerolaena bicornis	6k E Boulia	21/8/17
Sclerolaena bicornis	60 k E Boulia	"
Sclerolaena bicornis	190 k E Boulia	"
Sclerolaena bicornis	140 k E Middleton	"
Sclerolaena sp.	176 k S Longreach	"

Host plant carbon isotope analysis

We analysed 15 host plants collected during the August 2016 field trip. They included many of the most widespread and abundant hosts, capable of supporting many larvae. Two samples of each plant were collected. The following species were found to have the C3 profile: **Asteraceae: ,***Calotis cuneifolia* – purple burr daisy, *Calotis multicaulis* – woolly-headed burr daisy, *Myriocephalus stuartii* – poached egg daisy, *Rhodanthe floribunda* – large white sunray, *Rhodanthe moschata* – musk sunray, *Senecio lautus* – groundsel, *Senecio gregorii* – fleshy groundsel.

Fabaceae: *Cullen cinereum* – annual verbine, *Cullen pallidum* – woolly scurf pea, *Swainsona* sp. – Darling pea, **Goodeniaceae:** *Velleia glabrata* – pee-the-bed, *Goodenia fascicularis* – silky goodenia **Solanaceae:** *Nicotiana velutinum* – wild tobacco, **Geraniaceae:** *Erodium crintum* – wild geranium.

This result is not surprising because almost all species in these families are known to be C3. The few exceptions are species adapted to hot areas, mostly tropical. For this reason it was thought possible that some major inland host species might be (atypically) C4, but this does not seem to be the case. One plant, large pigweed, *Portulacca intraterranea* (Portulaceae) was identified as C4, but its host plant status is uncertain. Like many species in this family it has a prostrate growth habit which makes it unsuitable for sweep netting and while larvae have sometimes been observed crawling on it and some locals have claimed they feed on it, we do not know whether it will support complete larval development.

Carbon analysis of inland moths

The numbers of moths found to have C3 and C4 profiles from the inland are compared with *H. armigera* from the upper Namoi (Project UNE1301) in Fig. 1. The numbers of moths from various inland sites and dates that were classified as C3 or C4 are shown in Table 2.

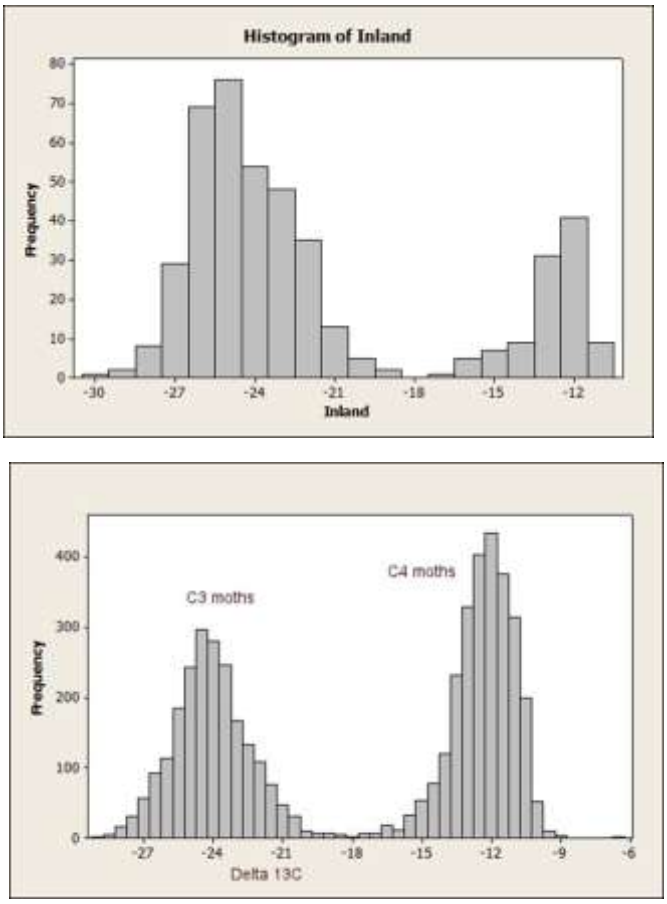


Fig. 1. Delta carbon values of inland *H. punctigera* moths (top) compared with *H. armigera* moths from the upper Namoi valley (bottom, data from Final Report for Project UNE1301)

At three sites, Roxby Downs, Bedourie and Eromanga, moths with the C4 profile were recovered. Two sites, Kingoonya and Windorah, had no C4 moths, but the numbers sampled from these sites were lower, especially in Kingoonya.

Table 1. Numbers of C3 and C4 moths recovered from pheromone traps at five inland sites, 2016-2017

Location	Date	N	C3	C4	%C4
Roxby Downs	19.5.17	61	52	9	14.8
Roxby Downs	23.5.17	73	64	9	12.3
Roxby Downs	30.5.17	25	25	0	0.0
Roxby Downs	6.6.17	10	8	2	20.0
Total		**169**	**149**	**20**	**11.8**
Kingoonya	22.4.17	4	4	0	0.0
Bedourie	8.6.16	100	32	68	68.0
Bedourie	3.10.16	100	94	6	6.0
Total		**200**	**126**	**74**	**37.0**
Eromanga	23.5.17	13	12	1	7.7
Eromanga	1.8.17	32	27	5	15.6
Eromanga	23.8.17	2	2	0	0.0
Total		**47**	**41**	**6**	**12.8**
Windorah	23.5.17	15	15	0	0.0
Windorah	4.6.17	3	3	0	0.0
Windorah	16.6.17	1	1	0	0.0
Windorah	18.7.17	4	4	0	0.0
Windorah	28.7.17	1	1	0	0.0
Windorah	22.8.17	1	1	0	0.0
Total		**25**	**25**	**0**	**0.0**
TOTAL		**445**	**345**	**100**	**22.5**

It is clear that C3/C4 discrimination using carbon isotope analysis yields similar results for *H. punctigera* as previously noted for *H. armigera* by Baker & Tann (2013) and by us in Project UNE1301: Delta C values higher than -18 indicate C4, and lower than -19 indicate C3 host origins, and there is little or no overlap between them. As with the results of Baker & Tann, we found C4 *H. punctigera* to be generally in the minority, with one notable exception: the moths from Bedourie in early June 2016 were mostly C4, but by October 2016 they had reverted to mostly C3.

Potential C4 hosts for H. punctigera in the inland include:

1. Chenopods, especially saltbushes. While we have never found larvae on these in our inland surveys despite extensive sweep netting (see Table 1), we have recently established through glasshouse feeding experiments at UNE that larvae will survive on two species of seedling saltbushes

including *Atriplex vesicaria*, which may be more tender and have less salt in their leaves than the mature ones we sampled in the field.

2. Succulents belonging to the families Portulaceae and Aizoaceae, which can be abundant in the inland. We have never sampled on these plants because they are too prostrate for sweep netting. However, anecdotal evidence from locals suggests that *Helicoverpa* larvae will feed on them, and we have recently shown that one species, pigweed (*Portulacca oleraceae*) will support larvae through to pupation.

3. A few plants in the Zygophyllaceae, especially *Tribulus* spp. While these occur in the inland they are not abundant. There is however one species, *T. terrestris* (caltrop) which is often abundant from spring through to autumn in cropping areas. It may be a source of C4 *H. punctigera* in cropping areas, but probably not in the inland.

The switch from C4 to C3 moths in spring 2016 in Bedourie (the only site for which we kept moths so early in the project) can be interpreted in the light of rainfall (Fig. 2) and host growth (see reports of field trips during that period: May, June, August and October 2016). It had been very dry during March and April, with little host growth except perennials such as chenopods. Rain in May to the west of Bedourie could have initially germinated mostly chenopods and succulents. Thus, larvae developing during April and May, to produce moths in early June, would have been restricted to such hosts, which are mostly C4. However, from May on, and especially in August-September when the October moths from Bedourie would have been developing, heavy rain produced extensive growth of daisies and legumes (C3 hosts), and this was reflected in the switch from C4 to C3 moths. This suggests that C3 plants are the preferred hosts for *H. punctigera*, but there are C4 hosts that can be utilised when the C3 hosts are not available.

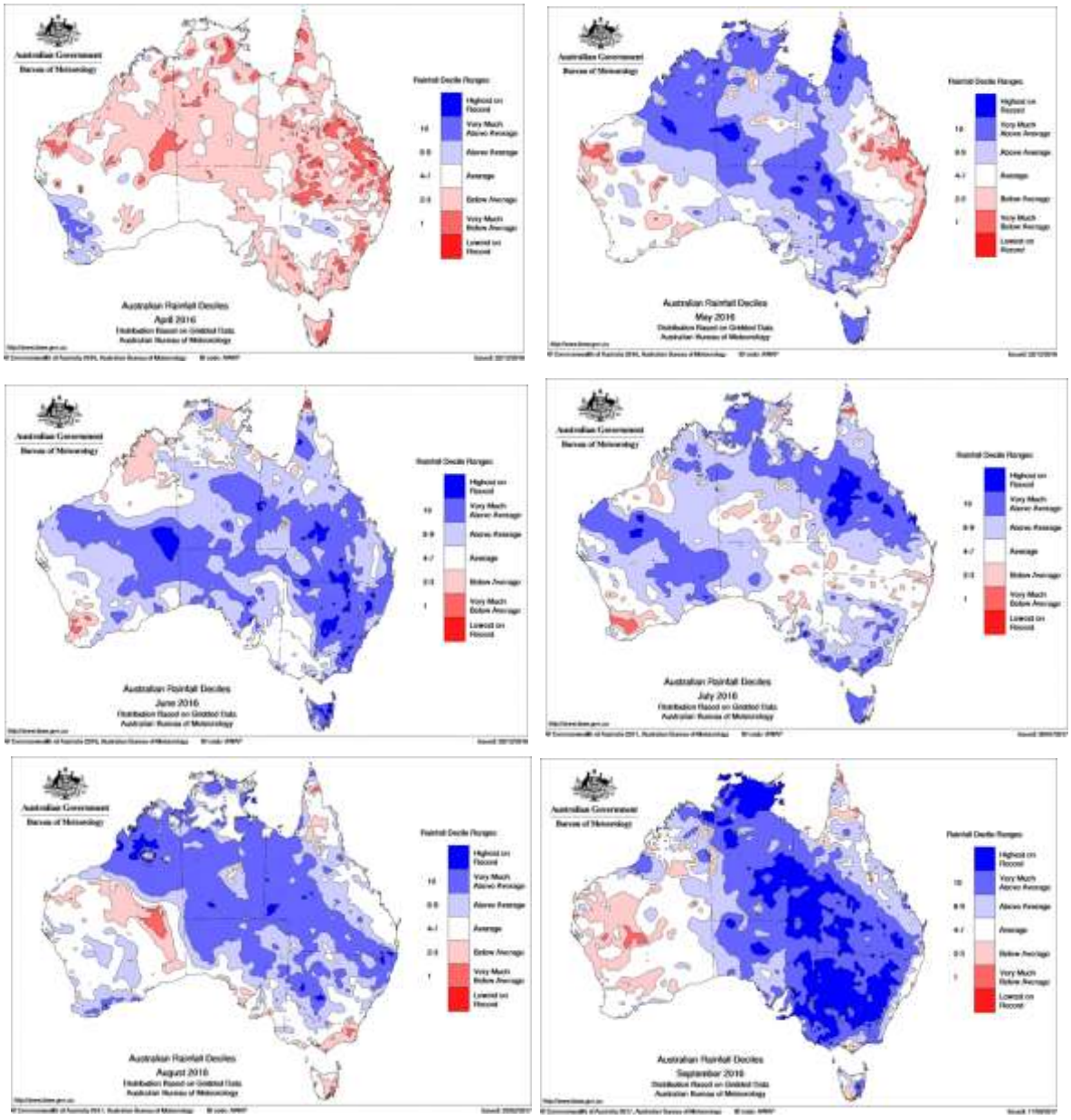


Fig. 2. Rainfall deciles from April 2016 to September 2016 (Bureau of Meteorology)

Appendix 4. Emergence cage studies for Final Report, Project UNE1502

Chapter 3 from Kris Le Mottee's PhD thesis

Chapter 3: Field studies on *Helicoverpa punctigera* overwintering

3.1 Introduction

3.1.1 Overwintering of *H. punctigera* in field crops

Chapter 2.1 set the scene for the state of the literature on diapause in *Helicoverpa punctigera*, in that there are few laboratory studies to reference. There are more field studies on overwintering and diapause in *Helicoverpa* spp*.,* though only a few specifically deal with diapause in *H. punctigera*. Murray (1991) established a *H. punctigera* culture from adults caught in a mercury-vapour light trap in Toowoomba, then reared them on artificial diet continuously in an open-air insectary from 1985-1988. A large proportion these individuals sampled during mid-to late- April were in diapause based on eye spot development (Murray, 1991). This suggested that diapause before late March is unlikely, but increasingly likely as photoperiods drop below 12.5L:11.5D (Murray, 1991), a conclusion also supported by the laboratory studies from Cullen and Browning (1978) and the results of Chapter 2.

In the past, the vast majority of *Helicoverpa* pupae found overwintering in northern New South Wales have been *H. armigera* rather than *H. punctigera* (Fitt and Daly, 1990). Wilson (1983) only found overwintering *H. punctigera* pupae in one year, at only one site, out of nine years of study in the Namoi Valley area. Fitt *et al.* (1989) found the distribution of adult *H. punctigera* (via pheromone trap catches) was clumped, with moths often found in small scale patches of 1-2km wide. The difficulties of simulating field conditions with regard to changing photoperiods and fluctuating temperatures under laboratory conditions have led Murray (1991) to use late-stage field-collected larvae in field cages or open-air insectaries. However, the lack of late-stage larvae in the field during the photoperiods critical for diapause induction has limited the success of these strategies (Cullen and Browning, 1978).

The use of refuge crops such as pigeon pea to manage Bt resistance (Chapter 1.4) may have led to the apparent increase in the numbers of *H. punctigera* overwintering in cropping

areas (Chapter 1.5), and as a result there is sufficient cause to attempt overwintering studies to monitor the incidence and fate of overwintering pupae.

Although studies have recorded *H. armigera* overwintering in the immature stages in western Queensland, it is much less common than *H. punctigera* (Gregg *et al.*, 1989). In contrast, *H. punctigera* populations often survive the winter months in large numbers in inland regions including western Queensland (Gregg *et al.*, 1989, Gregg, 1995, Oertel *et al.*, 1999). Late autumn/winter rainfall in some areas of inland western Queensland has been shown to be predictive of spring populations of *H. punctigera* (Oertel *et al.*, 1999, Maelzer and Zalucki, 2000), though more recently Baker *et al.* (2011) questioned this correlation. Rainfall in May, June and July germinates *H. punctigera* host plants, which in turn allow the build-up of populations of *H. punctigera* which can overwinter in both the larval and pupal stages, and migrate as adults in spring (Gregg, 1993, Oertel *et al.*, 1999).

As spring host plants dry off in inland western Queensland, *H. punctigera* moths are carried into cotton growing regions by westerly and north westerly winds (Gregg, 1993, Gregg *et al.*, 1993, Oertel *et al.*, 1999, Gregg *et al.*, 2001). These ecological patterns indicate a need to investigate overwintering of pupae in this region, which has not been directly attempted previously, though there are many observations of overwintering larvae (Zalucki *et al.,* 1994).

3.1.2 Methods in overwintering studies

One simple way of estimating the abundance of pupae in the field is the direct excavation of soil containing *Helicoverpa spp.* pupae, down to a depth of about 10cm (Titmarsh *et al.*, 1991). This method is less physically demanding than alternatives such as soil sieving, and has been used by several researchers in the past (Slosser *et al.*, 1975, Caron *et al.*, 1978, Wilson, 1983, Lopez and Hartstack, 1985).

Emergence cages are a non-destructive method for estimating pupal abundance. By confining a small area with a cage, all moths that emerge may be captured and counted, with the time of emergence recorded. This method estimates adult survivors rather than actual overwintering pupal numbers (Titmarsh *et al.*, 1991). Field cages have been used to

study various aspects of the ecology of *Helicoverpa/Heliothis* moths in the USA (Caron *et al.*, 1978, Roach, 1981, Lopez *et al.*, 1984) and in Australia (Del Socorro and Gregg, 2001, Duffield and Chapple, 2001, Mahon and Young, 2010, Rogers and Brier, 2010, Sigsgaard *et al.*, 2002), although of these studies only Duffield and Chapple (2001) studied *H. punctigera*. These cages are often used in conjunction with pheromone traps when observing overwintering, as pheromone trap catches can be compared with emergence of moths within the cages (Lopez *et al.*, 1984). Previous techniques for emergence cages were not entirely appropriate for our needs. For example, Wilson (1983) used pyramid emergences cages, 1m x 1m x 1m, but these cages are not suitable for pigeon pea, the most numerous refuge where the highest *H. punctigera* populations are found (Baker and Tann, 2014). Pigeon pea refuges can be as tall as 2m, so a new emergence cage design was needed. Wilson (1983) also placed glass vials containing *H. punctigera* larvae and diet in the ground to monitor emergence, but this method does not simulate natural behaviour of H. punctigera well.

In order to demonstrate that *H. punctigera* does overwinter, direct observation of *H. punctigera* pupae emerging as adults is needed, rather than indirect monitoring of pheromone traps, where any *H. punctigera* adults detected in the spring could be immigrants rather than moths emerging from diapausing pupae.

3.1.3 Aims

The work described in this chapter aimed to:

1. Develop methods for sampling overwintering *H. punctigera* in the Namoi Valley and in far western Queensland.
2. Determine if *H. punctigera* are diapausing in Namoi Valley cotton refuges or in native host plants in far western Queensland.
3. Determine the timing for emergence of *H. punctigera* that are overwintering in the Namoi Valley and in far western Queensland.

There have been no studies on any aspect of *H. punctigera* overwintering as pupae in the western Queensland area, and no published studies directly investigating the abundance or

timing of *H. punctigera* emergence. In this chapter I aimed to investigate how *H. punctigera* overwinters in inland western Queensland as well as in the cotton regions in the Namoi Valley in NSW. This was attempted using field cages to examine the timing of adult emergence, monitored manually as well as using nearby pheromone traps and temperature probes to record temperatures during the field experiments. When emergence cages failed to generate adult moths to sample, first self-contained emergence cells were attempted, and then a pupae-digging method was adopted to allow direct assessment of overwintering pupae.

3.2 Materials and Methods

3.2.1 Study areas

Field studies to investigate overwintering in *H. punctigera* were conducted in the Namoi Valley in NSW and in inland Queensland (Fig. 10). There were four sites in the Namoi Valley: "Kilmarnock" and "Milchengowrie" in Boggabri, NSW, "Drayton" and "Boondah" in Breeza, NSW. In inland Queensland, field studies were done at "Monkira" and "Cluny" near Bedourie, Queensland. Table 9 summarises the location of each study site along with the methods used to explore overwintering in *H. punctigera.*

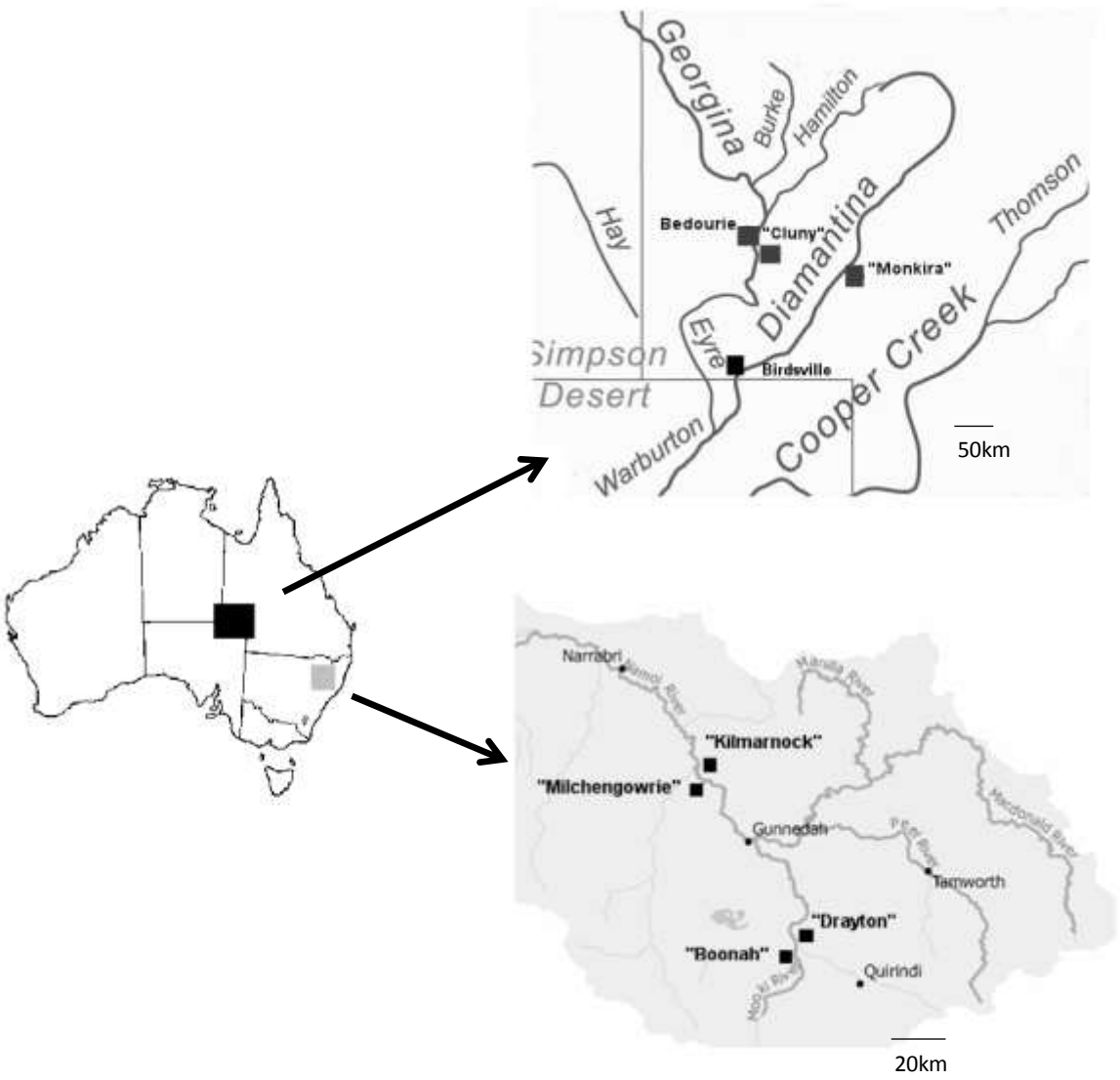


Fig. 10. Map displaying field study sites in inland Queensland (top) and the Namoi Valley (bottom) (Murray-Darling Basin Authority, Diamantina Shire Council)

Table 9. Summary of field sites used to study H. punctigera *overwintering from 2011-2014.*

Year	Start Date	Location	Decimal Coordinates	Methods
2011	10/03/11	Namoi Valley, ACRI	30.21S, 149.60E	Emergence cages

2011	10/07/11	Inland Queensland, ”Cluny” Station	24.51S, 139.59E	Emergence cages
2012	09/03/12	Namoi Valley, “Milchengowrie”	30.85S, 150.13E	Emergence cages
2012	09/07/12	Inland Queensland, “Monkira” Station	24.84S, 140.56E	Emergence cages
2013	26/03/13	Namoi Valley, “Kilmarnock”	30.85S, 150.13E	Emergence cages, Emergence cells
2013	14/07/13	Inland Queensland, “Cluny” Station	24.51S, 139.59E	Emergence cages, Emergence cells
2014	13/04/13	Namoi Valley, “Drayton”	31.27S, 150.44E	Emergence cages, pupae digging
2014	13/04/13	Namoi Valley, “Boondah”	31.31S, 150.49E	Emergence cells

3.2.3 Pheromone trapping

Universal canister pheromone traps (sometimes referred to as AgriSense traps, produced by Suterra LLC, Oregon, United States baited with lures produced by Suterra LLC, NSW, Australia and sourced from Entosol Pty Ltd, Roselands, NSW, Australia) were used to monitor the activity of *H. punctigera* moths in the study areas. For the inland studies two pheromone traps were placed at each field site for the duration of each emergence cage study. For comparison, data covering the same periods from similar traps that had been operating for some years in inland regions (Birdsville and Bedourie, P. Gregg and A. Del Socorro unpublished) and data from the Namoi Valley obtained from available grower literature (Cotton Seed Distributors, 2012, Cotton Seed Distributors, 2013) and recently published pheromone trapping data (Baker and Tann, 2014) were used.

3.2.5 Emergence cages

Several designs of emergence cages were used for sequential years of field studies, each improving on the designs of the previous year.

The first design of the emergence cage used screen tents (Tasman 2, Oztrail, Eagle Farm, Queensland, Australia) (Figs. 11b and c), with the bottom cut out to expose the inside of the cage to the soil. These cages were placed around pigeon pea (*Cajanus cajan* L.) crops (Namoi Valley studies, Fig. 11b) or wild host plants (inland Queensland studies, Fig. 11c)). The host plants used in inland Queensland are described below in section 3.2.11. Once the cage was placed over the host plants the soil was piled around the edges of the cage to prevent access of *Helicoverpa* spp. larvae or natural enemies into or out of the cage. The capture apparatus was set up at the top of the tent. This apparatus consisted of an 80mm powder funnel attached to the lid of a 100 ml plastic culture vial using a rubber washer. The apparatus was held up by tensile cord tied to a metal bracket, supported by an iron pole hammered into the ground. Cloth tape was used to secure the funnel to the top of the cage. A solar powered garden LED light (Solar Stake Stainless Steel OX0633, Uncle Bills Imports, Silverwater, NSW, Australia) was disassembled and reassembled with an ultraviolet LED (5mm LED Waterclear 60mcd, Cree Inc, Durham, North Carolina, USA) for use as the lure at the top of the cages.

The second iteration of the apparatus used a larger screen tent (Tasman 4V, Oztrail, Eagle Farm, Queensland, Australia), without the fly. The Tasman 4V tent had twice the area of the Tasman 2, allowing twice the number of host plants inside. The Tasman 4V had a cross-section at the top, which the capture mechanism could be attached to with plastic ties, replacing the supporting tensile cord, washer and iron pole combination (Fig. 12b).

The third iteration of the apparatus had a PVC plate custom made by the Science Engineering workshop at the University of New England, Armidale NSW. This plate had 5mm holes, two per corner, to secure the plate to the tent. The funnel was held in place, and held at the top of the tent, by a small screw thread. The culture vial lid was securely fastened to the plate with a small plastic insert that fitted into the lid exactly. The PVC plates could fit either the Tasman 2 or the Tasman 4 tents.

Fig. 11. First iteration of field emergence cages used in the Namoi Valley and inland Queensland. Fig. a) shows the detail on the capture mechanism, two plastic culture tubes glued together, with an ultraviolet LED wired into a solar-array that activated after dark. A 45mm powder funnel was attached to the bottom culture tube via the lid of the culture tube and a rubber washer. One-way glass fibre mesh prevented moths from escaping the culture tube. Fig. b) shows the offset cages that the capture mechanism was used in, at Boggabri NSW. Fig. c) shows how the capture mechanism was attached to the cages, via an iron post, tensile cord and metal bracket.

Fig. 12. Second and third iterations of emergence cage design. Fig. a) shows that the post, tensile cord and bracket were discarded in favour of strapping the capture mechanism directly to the cages, Fig. b) shows the larger cages over multiple rows, and c) shows how a custom-designed PVC sheet was strapped to the edge of the cages using aluminium brackets, to secure the catchment cup and keep it upright, even in strong winds. A TinyTag dual probe data logger was also attached to at least two cages. (Photo: P. Gregg).

3.2.6 Data loggers

Dual-probe TinyTag Plus 2 TGP-4520 data loggers (Gemini Data Loggers, Chichester, UK) were used to monitor temperatures in field trials. Each data logger had one probe in the soil at 10cm below the soil, at the level where pupae were likely to be. The second probe on a

cage was secured at 1.5m above ground level to capture the air temperature. Secondary probes were used to explore differences between sites or to collect data at other locations (Table 9). In “Milchengowrie”, 2012, probes were placed 1.5m above the ground and in the soil beneath the cage. “Monkira”, 2012 also had probes in the same positions, but had two additional probes inside the soil of the cage and at the top of the cage, to allow comparison inside and outside the cage. “Kilmarnock” 2013 had probes in the same position as “Milchengowrie”, 2012, but the probe suffered water damage and the data was unrecoverable. “Cluny” 2013 had probes outsides of cages, in the soil and 1.5m in the air, but also in the soil of the cages and inside the self-contained emergence cells. “Boondah”, 2014 had a probe inside the self-contained cells, and a probe outside the cage in the soil (Table 9).

3.2.7 Self-contained emergence cells

In an attempt to improve the survival of larvae in emergence cage studies, a new protocol was developed, isolating a single *H. punctigera* larva inside a cell made of 40 mm PVC pipe, with a reservoir of artificial diet to sustain it over its development (Fig. 13a). The reservoir was filled with artificial diet and then sealed with either parafilm or beeswax, then placed inside the PVC cylinder encased with fine metal gauze. The cylinder was then half-filled with soil and the lid (a drain cowl) was placed over the top (Fig. 13b) with a single larva on the top. The cell was then placed with 80 others, inside a Tasman 2 tent with a data logger attached. At “Cluny” in 2013, temperature probes were placed inside a cell (a hole was cut in the bottom of the cell to allow access) and 10cm below the surface at an equivalent depth to the cell. Each cell was considered a full replicate, as each cell was independent of the others.

Table 10. Data logger locations and probe locations. Probes 1 and 2 were from the primary data logger and 3 and 4 from a secondary probe inside the cages.

Year	Region	Location	Probe 1	Probe 2	Probe 3	Probe 4
2012	Namoi Valley	"Milchengowrie" Farm 30.85S, 150.13E	10cm below surface	1.5m in the air		
2012	Inland Queensland	"Monkira" Station S24.84, 140.56E	10cm below surface		1.5m in the air	Soil surface
2013	Namoi Valley	"Kilmarnock" Farm S30.85, 150.13E	Logger failed	Logger failed		
2013	Inland Queensland	"Cluny" Station 24.51S, 139.59E	10cm below surface	1.5m in the air	Inside cell	10cm below surface
2014	Namoi Valley	"Boondah" Farm S31.31, 150.49E	Inside cell	10cm below surface		
2014	Namoi Valley	"Drayton" Farm S31.27, 150.44E	10cm below surface	1.5m in the air		

3.2.8 Bugdorm® cages with emergence cells

In the "Boondah" study in 2014, Bugdorm® 2 2120 Insect Rearing Tents, (MegaView Science Co., Ltd., Taichung, Taiwan) (Fig. 14) were used in combination with the emergence cells (Fig. 13a) described above. Emergence cells were buried in 10cm of soil, heat treated at >200°C for 8h to reduce the survival of potential pathogens, with a single first instar, laboratory-reared *H. punctigera* larva was placed in each cell.

3.2.9 Pupae digging

Pupae digging was done to collect *Helicoverpa* spp. pupae from the soil. To do this, the surface soil was cleared away, and the surrounding soil was carefully turned over and layered 2cm at a time, down to 10cm in depth (Titmarsh *et al.*, 1991). Often emergence tunnels dug by the pre-pupating larvae were visible, and a pupa could then be extracted from the end of the tunnel. In some cases where tunnels were not visible, pupae had to be retrieved from the loose soil.

3.2.10 Namoi Valley studies

The first Namoi emergence study was at the Australian Cotton Research Institute (ACRI) at Narrabri in 2011, the second at "Milchengowrie" at Boggabri in 2012, the third at "Kilmarnock" at Boggabri in 2013, and the final one at "Boondah" at Breeza in 2014 (Table 10).

In the Namoi Valley studies, crops of pigeon pea, grown as refuges in association with Bt cotton (Baker *et al.*, 2008), were first sampled using sweep nets. Emergence cages were placed over selected flowering crops with green foliage in order to provide sufficient nutrition to complete larval development. To facilitate plant growth and keep the foliage green, the cage enclosure was irrigated with 20mm of water spread evenly across the cage. Emergence cages were then seeded with *H. punctigera* larvae. Each cage was seeded with either 50 larvae caught from the surrounding pigeon pea crop, or with 50 larvae from the laboratory culture (see Chapter 2.3.2 for details), on the dates shown in Table 10. Two different cages (one with wild larvae and one with laboratory-reared larvae) were randomly chosen to receive a data logger, with one temperature probe outside the cage at 1.5m above ground and one probe inside the cage soil at 10cm below ground. Each type of cage was replicated four times, to allow comparison between wild larvae and field-caught larvae.

Prior to setting up emergence cages in 2014, it was necessary to first locate sites of *H. punctigera* larval populations. Each scouting location was subject to 5 x 20 sweeps using a 38 cm diameter net fitted with a bag made of polyganza. The numbers and size class (very small, small, medium and large) (Flower *et al.*, 2010) of *Helicoverpa* spp. larvae per 20 sweeps were recorded to compare the relative abundance at each site. *Helicoverpa* spp.

larvae were collected and sorted to species by the criteria of hair colour on the first segment behind the head, using a hand lens. Black hairs in larvae above 20mm are indicative of the caterpillar being *H. punctigera* (Bailey, 2007). While this method was less reliable than examining the pupal cremaster spines to accurately determine species (Kirkpatrick, 1961), it was the only one available to separate the larval stages.

Another approach that was tried in the Namoi valley involved the direct collection of overwintering *H. punctigera* pupae. In 2011, 2012 and 2013, not enough late-season *H. punctigera* were found to attempt this, but in 2014 late season numbers were higher. In mid-April ten farms in the Namoi valley area were surveyed for larvae and overwintering pupae. The 2014 year of fieldwork was the most successful year for finding *H. punctigera* larvae. Almost every location that was sampled in 2014 was better than in previous years, where the ratio of *H. punctigera* larvae to *H. armigera* was generally less than 5%. "Drayton" Farm (31.19S, 150.42E, Fig. 10) was selected as the field site when the surveys discovered 2-3 times as many putative *H. punctigera* larvae in the pigeon-pea refuge crop compared to the other farms (Table 11). Pupae were collected from "Drayton" each week by digging 50-100 pupae from the soil, and separating the *H. punctigera* pupae from *H. armigera* based on the cremaster spines (Kirkpatrick, 1961).

Fig. 13. a) Self-contained single-rearing unit constructed from PVC plastic and a drain cowl. b) The bottom of the cell was buried 8cm in the soil. The inner 'cup' was filled with artificial diet, preserved with a layer of wax or parafilm A single Polycalymma stuartii *flower containing a* H. punctigera *larva was placed in each cell. c) Each cell was placed inside a screen tent. (Photos b & c: P. Gregg).*

Fig. 14. Location and cage used in 2014 study at "Boondah".

Pupae were held in a 50mm x 20mm cylindrical Perspex tube, with the lid fitted loosely, for transport back to the laboratory. Pupae were then incubated at either 19 or 25°C, and

monitored for development and emergence each week. Individual pupae were kept in 30ml plastic soufflé cups (Chapter 2) with moist vermiculite for rearing. Half of the pupae collected were reared at 19°C and the other half at 25°C. The state of diapause was checked every 72h based on eye spot and emergence time (see Chapter 2 for details on diapause determination).

3.2.11 Inland Queensland studies

For the inland Queensland studies, native host plants were sampled for larvae using sweep nets as described by Zalucki *et al.* (1994). Emergence cages in inland Queensland were placed over host plants where *H. punctigera* were already present prior to the seeding of cages, though the numbers already present were much lower than the numbers that were added to the cages. Cages were seeded with larvae on the dates shown in Table 10. Since the area was remote, distant from the University of New England, and sparsely inhabited, it was necessary to choose sites where a local collaborator, from a nearby cattle station, could be recruited to monitor the cages. In 2011, the flowering daisy *Gnephosis arachnoidea* Turcz. was the most abundant source of *H. punctigera*, and was used as a field site when a collaborator at "Cluny" station was found. In 2012, the only source of *H. punctigera* found was on *Cullen cinereum* (Lindl.) J.W. Grimes at "Monkira" station, where a field site and a collaborator were established. In 2013, the poached egg daisy, *Polycalymma stuartii* (F.Muell. & Sonder) was abundant along the sand dunes next to "Cluny" Station, with most flowers found to be supporting 1-3 *H. punctigera* larvae, so cages were set up nearby and the same collaborator from 2011 was enlisted. Temperature probes were placed at the soil surface, 10cm below the surface and at 1.5m above the surface. The 2013 inland Queensland study used 90 emergence cells, placed at "Cluny" Station, which were to be checked for emergence by a local collaborator every week.

In the inland Queensland studies, pupae in the soil were only excavated when the cages were removed at the end of the study periods (06/10/11, 16/09/12 and 11/11/13 respectively). All plant materials in the cages were carefully extracted to search for moth wings or other evidence of predation on emerging adults. The soil underneath the cages was broken apart using a shovel and examined for evidence of pupal tunnels. Finally the soil was

put through a sieve to find whole pupae and pupal cases. Any emergence from the cages and catches in the pheromone traps were monitored by a local collaborator during the study period.

3.3 Results

3.3.1 Namoi Valley studies

The Namoi Valley studies were hampered in all years by very high mortality in the late larval stages. Studies conducted in 2011 (ACRI) and 2012 ("Milchengowrie") yielded no moths in the emergence cages. When soil underneath the cages was dug no pupae were found, nor were any pupal tunnels or pupal casings found. Two different cages in 2012 had larvae hanging from the roof of the cage with symptoms indicative of death by infection with NPV (Poinar and Thomas, 1978). The 2013 study ("Kilmarnock") also did not yield any live adult moths, but 12 *H. punctigera* pupae were extracted from the soil underneath the cages. The fate of these pupae, after incubation at 25°C in the laboratory, is shown in Table 13.

The laboratory-reared larvae used in the single-cell emergence cages in 2013 all died as small-medium larvae. Another cage housing the 50 self-contained cells did not show any adult emergence, and only 2 dead pupae were recovered. Ants were discovered in two of the cells inside the cage, feeding upon the remains of a moist larva which had most likely died of NPV.

Table 11. Pupae collected from emergence cages at "Kilmarnock", 2013.

Laboratory-reared	**Field-collected**
1 killed by parasitoid	1 killed by parasitoid
3 killed by fungal pathogen	3 killed by fungal pathogen
2 emerged alive 1 killed in extraction	1 emerged alive

To conduct pupae digging in 2014, based on the number of *H. punctigera* candidates obtained from sweep net sampling, “Drayton” was chosen as the site for the study (Table 12).

Table 12. Numbers of H. punctigera *larvae found at different Namoi Valley farm fields over 80 sweeps in 2014.*

Farm and field	**Coordinates (Lat,Long)**	**Number of *H. punctigera* in 80 sweeps**	**Percentage of *H. punctigera* on larval criteria**
“Gabo” 13	31.11S, 150.48E	10	13%
“Kilmarnock” W9	30.85S, 150.21E	15	19%
“Kilmarnock” C	30.86S, 150.11E	23	29%
“Milchengowrie” P1	30.85S, 150.13E	2	3%
“Milchengowrie” B7	30.83S, 150.09E	1	1%
“Milchengowrie” W6	30.81S, 150.08E	22	28%
“Milchengowrie” W5	30.92S, 150.20E	5	6%
“Drayton” A	31.19S, 150.42E	39	49%

Table 13. Pupal survey data from ~4h searches at "Drayton", 2014. Pupae recovered at different dates were split between 19°C and 25°C and the fates of H. punctigera *pupae were recorded.*

	Date	12-05-14	22-05-14	29-05-14	25-06-14	09-07-14	Total
25°C	Alive	3	0	0	4	1	8
	Nonspecific death	0	3	1	2	0	6
	Diptera parasitism	0	3	2	2	1	8
	Hymenoptera parasitism	0	0	0	0	0	0
	Fungal pathogen	0	0	1	0	0	1
19°C	Alive	2	4	1	1	2	10
	Nonspecific death	1	1	1	3	0	6
	Diptera parasitism	0	1	1	2	0	4
	Hymenoptera parasitism	1	0	0	0	0	1
	Fungal pathogen	0	0	0	1	0	1
	Total	7	12	7	15	4	45
	Count of *H. armigera*	-	109	70	66	92	
	Total	-	121	77	81	96	
	Percentage of H. punctigera	-	10%	9%	19%	4%	

In the pupal digging studies at "Drayton" in 2014 the ratio of *H. punctigera* larvae surviving to pupae compared to *H. armigera* was much lower than the ratio of larvae collected by sweep netting (a maximum of 17% compared to 49%; Table 13 and Table 12). Pupae obtained from "Drayton" (Table 13) were all initially in stage A of development (Chapter 2.3.1), but the pupae reared in the laboratory at 25°C all developed to stage E within 5 days while those at 19°C remained in stage A. This suggests that all of the pupae were in diapause when collected, but exposure to 25°C at all times of collection broke the diapause and caused development to continue. Of the *H. punctigera* pupae extracted from "Drayton", 12 died of non-identifiable causes, while the 13 parasitised pupae found showed 92% Diptera (Tachinidae) and 8% Hymenoptera (Ichneumonidae) parasitism (Table 13).

3.3.2 Inland Queensland studies

"Cluny" Station, 2011

The study at "Cluny" station in 2011 had only nine adults emerged from the cages (Table 14). The moths emerged in the week leading up to 21/08/11. When the soil beneath the cages was dug up on 6/10/11 pupal cases from which moths had emerged were found, and there were some emergence tunnels without cases. This suggested that adult moths emerged from their pupae, but were not captured in the collecting apparatus with the light. Several moth wings fragments were found in cages 3 and 4, but they were beginning to disintegrate and it was not possible to quantify them.

Table 14. Pupal emergence obtained by assessing the soil beneath the field cages, "Cluny" Station, 2011. n = 50 per cage.

Cage	Moths emerged	Pupal cases	Emergence tunnels without pupal cases
1	2	6	0
2	1	3	8
3	6	18	2
4	0	20	0

"Monkira" Station, 2012

The 2012 "Monkira" study had a total of 64 moths emerged from the cages. There were two peaks of emergence 19/7/12 and 27/9/12, but many moths also emerged over the intervening period (Fig. 15). When the soil was dug up on 15/9/12, many pupal cases were recovered, but there were no live pupae.

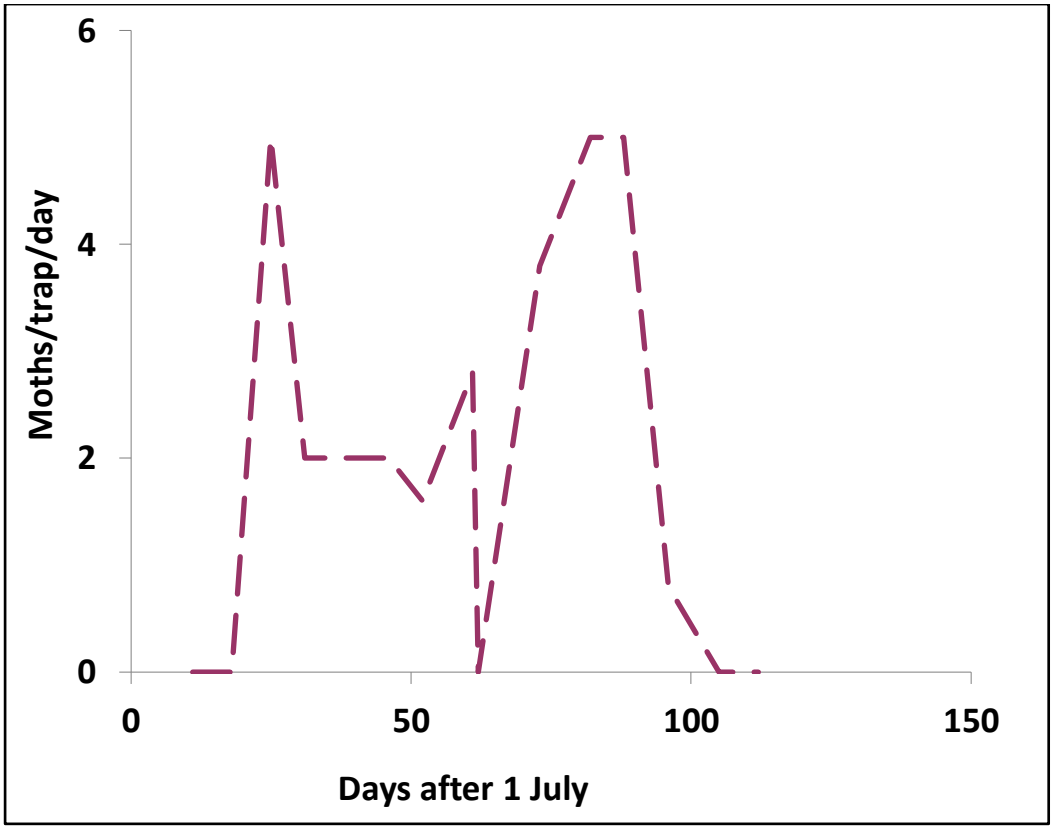


Fig.15. Emergence of H. punctigera *adults from cages at "Monkira" station, 2012.*

"Cluny" Station, 2013

The collaborator was unable to check the emergence of moths in the field cages during 2013, so no data on moth emergence timing were available. When the cages were examined on 11/11/13, of the 90 emergence cells used, 18 contained moths that had successfully emerged, while 72 failed to emerge. The screen-tent emergence cages did not have any moths in the catchment container at the top, although sieving the sand beneath the cages did recover some *H. punctigera* dead adults and pupal cases (Table 15).

Table 15. Results of soil excavation beneath field emergence cages in" Cluny", 2013.

Cage	Adults (dead)	Pupal cases	Whole pupae (dead)
1	0	0	0

2	0	6	0
3	1	0	0
4	0	0	8

3.3.3 Summary of results

The variety of methods used over the field seasons of 2011-2014 had varying successes and failures (summarised in Table 16). Each year, the methods/apparatus were improved in order to improve the chances at collecting data, but all studies in the Namoi Valley using emergence cages and emergence cells did not yield live moths, even when the same apparatus did in inland Queensland.

Table 16. Summary of overwintering field studies, 2011-2014.

Year	Location	Methods	Results
2011	Namoi Valley	Emergence cages	No data. 100% mortality in emergence cages.
2011	Inland Queensland	Emergence cages	Emergence data/pheromone trap data collected. Local rat plague may have reduced emergence. 9 moths emerged in the cages.
2012	Namoi Valley	Emergence cages	No data. 100% mortality in emergence cages.
2012	Inland Queensland	Emergence cages	Emergence data/pheromone trap data collected. 64 moths emerged.
2013	Namoi Valley	Emergence cages, Emergence Cells	No larvae emerged in cages or cells, 3 emerged alive from soil under cages.
2013	Inland Queensland	Emergence cages, Emergence Cells	No timing data when emergence occurred due to collaborator failure. Emergence cells had 18 moths emerge out of 90 cells in total.
2014	Namoi Valley	Emergence cages, Emergence Cells, Pupae digging	Emergence cells unsuccessful, pupae digging yielded 18 live pupae all in diapause.

3.3.4 Meteorological data during the overwintering trials

The temperature probe data showed how extreme the inland conditions were on the soil surface over the study period. The 2012 “Monkira” study had up to 50°C of temperature fluctuation in a single day (Fig. 16). Maximum temperatures commonly exceeded 40°C, even though the experiment was conducted during winter. However, it should be noted that

these readings were from probes that were placed in the foliage and would have been exposed to sunlight for some time during the day. They are therefore not directly comparable with temperatures that would have been recorded in a Stevenson screen, or with temperatures that might have been experienced by larvae which would have been able to seek shade. Minimum temperatures were much lower. Even at the surface of the soil, minimum temperatures still went below 0°C on some nights. At the soil surface, some of the more extreme upper temperatures were reduced, but lower winter temperatures were not reduced. Inside the cage and 10cm into the soil, temperatures were relatively cool but stable, and temperatures remained in a range that would maintain diapause rather than breaking it (Fig 18).

At “Cluny” Station in 2013, there was a similar pattern of extreme temperature fluctuation each day in the air, but once again, much of this was ameliorated by the soil. An important distinction is that in 2012 soil temperatures at the field site in “Cluny” rose above 25°C almost every day, above the threshold where diapause is broken (Fig. 17). In 2012 the effect of the cages on soil temperature was measured directly, with 1-2°C difference between soil in the cage and outside the cage (Fig. 22). Temperatures inside the cage emergence cells at “Cluny” were within 2-3°C of the surface temperatures inside the cages (Fig. 18), but the cage itself still provided an insulating effect (Fig. 22). The soil temperatures in “Monkira” were low enough to induce diapause, while the temperatures in “Cluny” were not.

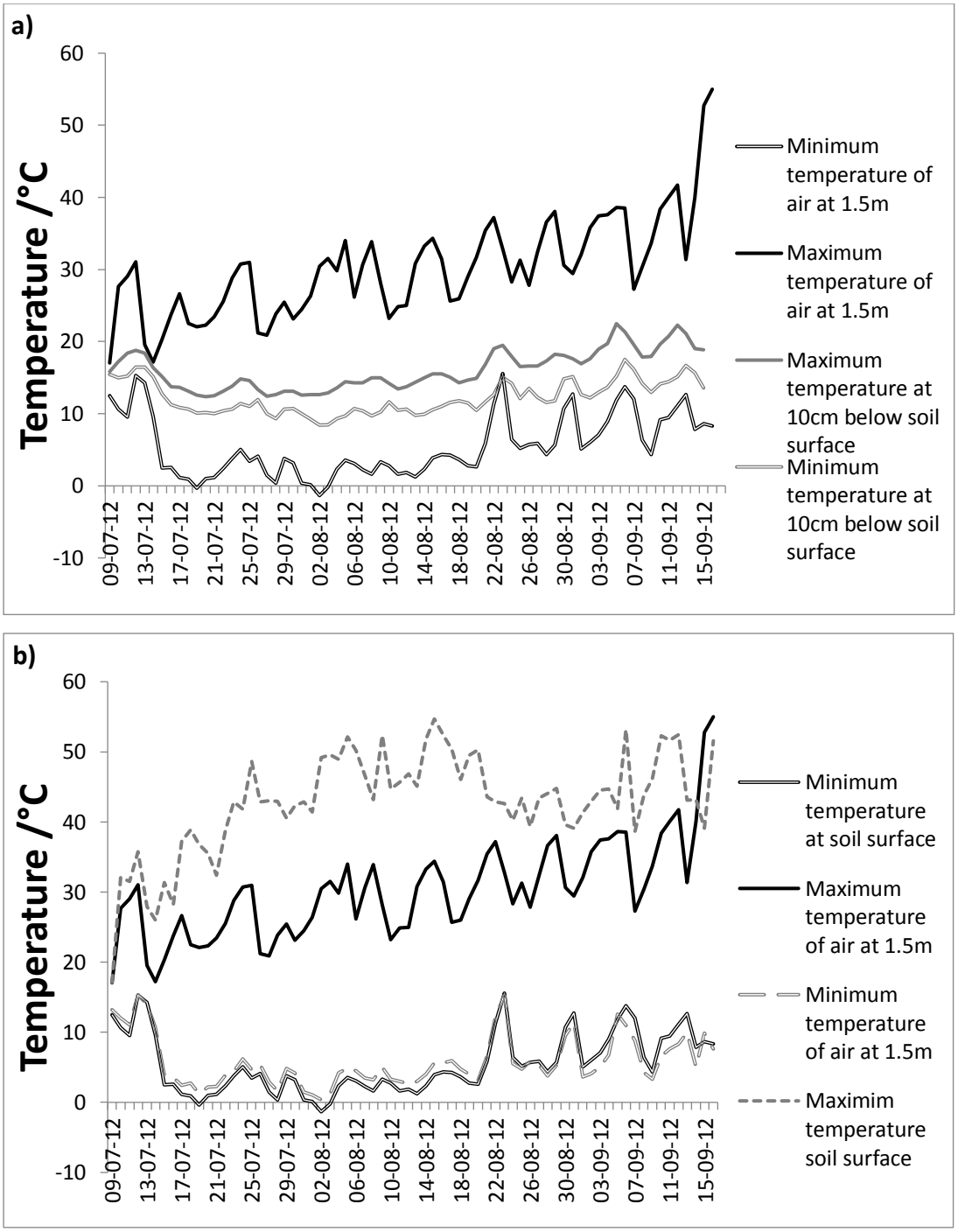


Fig. 16. Daily maximum and minimum temperatures at "Monkira", 2012. Maximum daily temperatures 1.5m and 10cm below the surface (a), and soil surface, air 1.5m above cages (b).

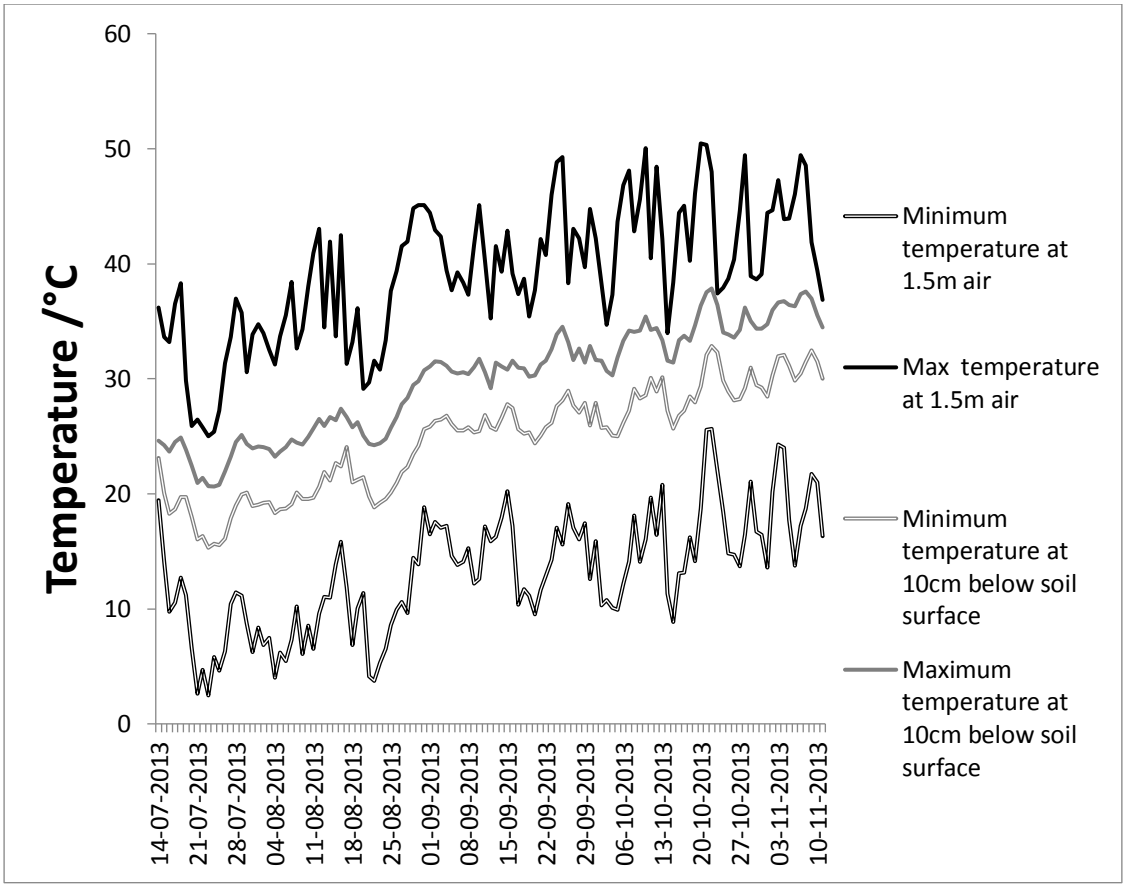


Fig. 17. Daily maximum and minimum temperatures at "Cluny", 2013. The maximum and minimum temperatures collected at 1.5m outside the cages were much higher and lower respectively than the maximums and minimums inside the cages at 10cm below the soil surface.

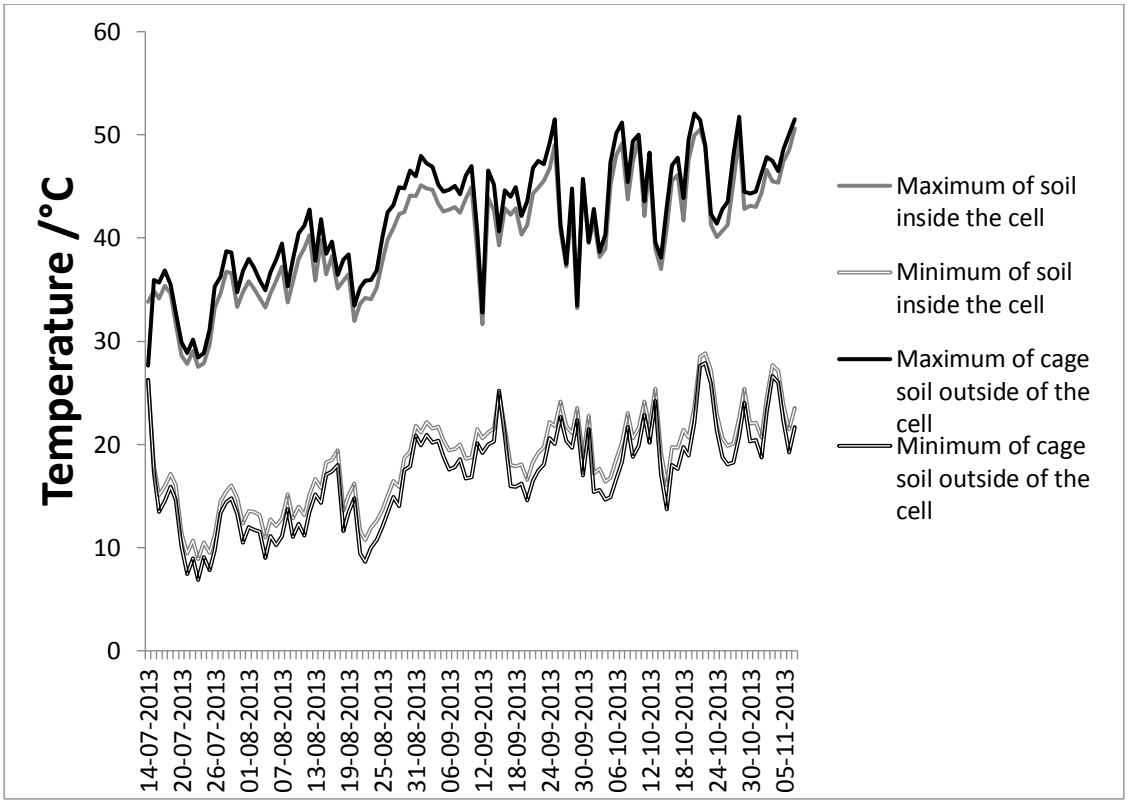


Fig. 18. Daily minimum and maximum soil temperatures inside emergence cages and inside emergence cells at "Cluny", 2013. There was a slight insulation effect of the cells, but overall there was little difference between the soil in the emergence cages, and the cells inside the emergence cages.

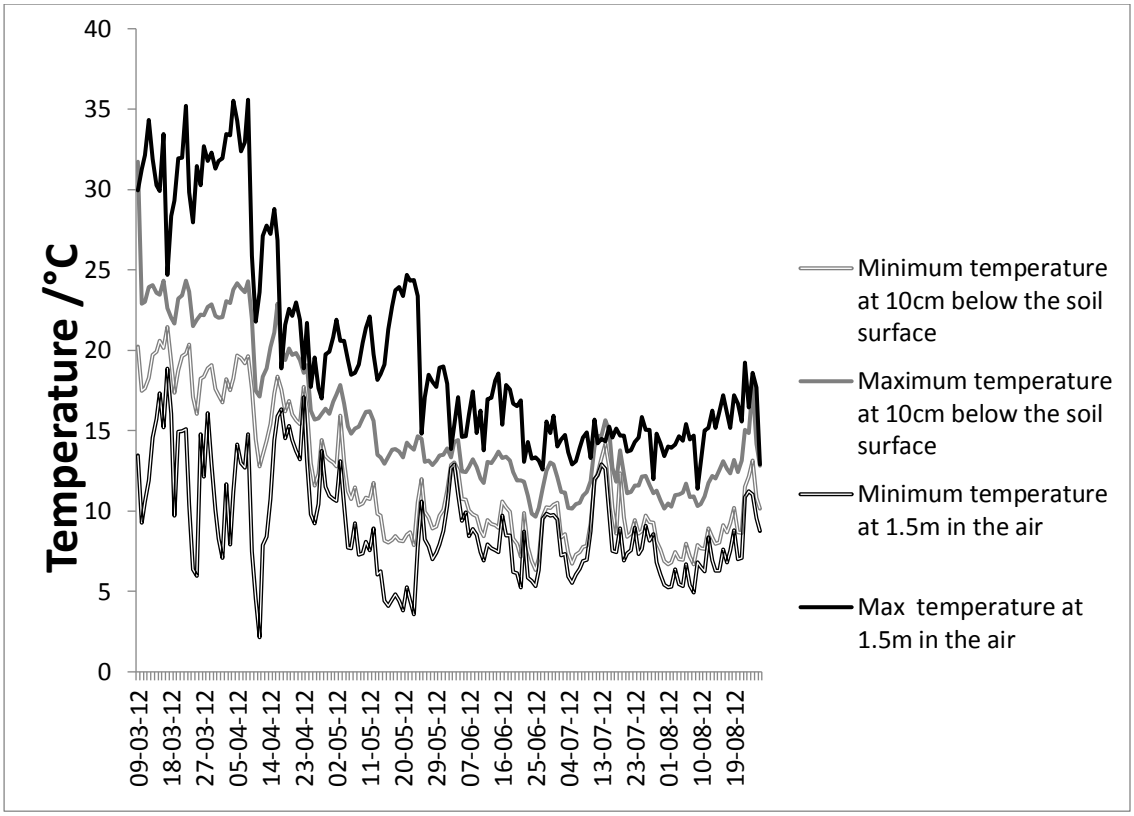


Fig. 19. Daily maximum and minimum temperatures during the emergence study at "Milchengowrie" in, 2012.

Air temperatures at "Milchengowrie" in 2012, where no larvae and very few pupae survived, had over 20°C of fluctuation on certain days, with temperatures going as low as 2°C from the start of recording on 9/3/12 through to 10/4/12 (Fig. 19). Maximum surface air temperatures in "Drayton", 2014, stayed above 20°C until 7/5/14, where temperatures dropped below a maximum of 15°C and remained there until 25/8/14 (Fig. 20).

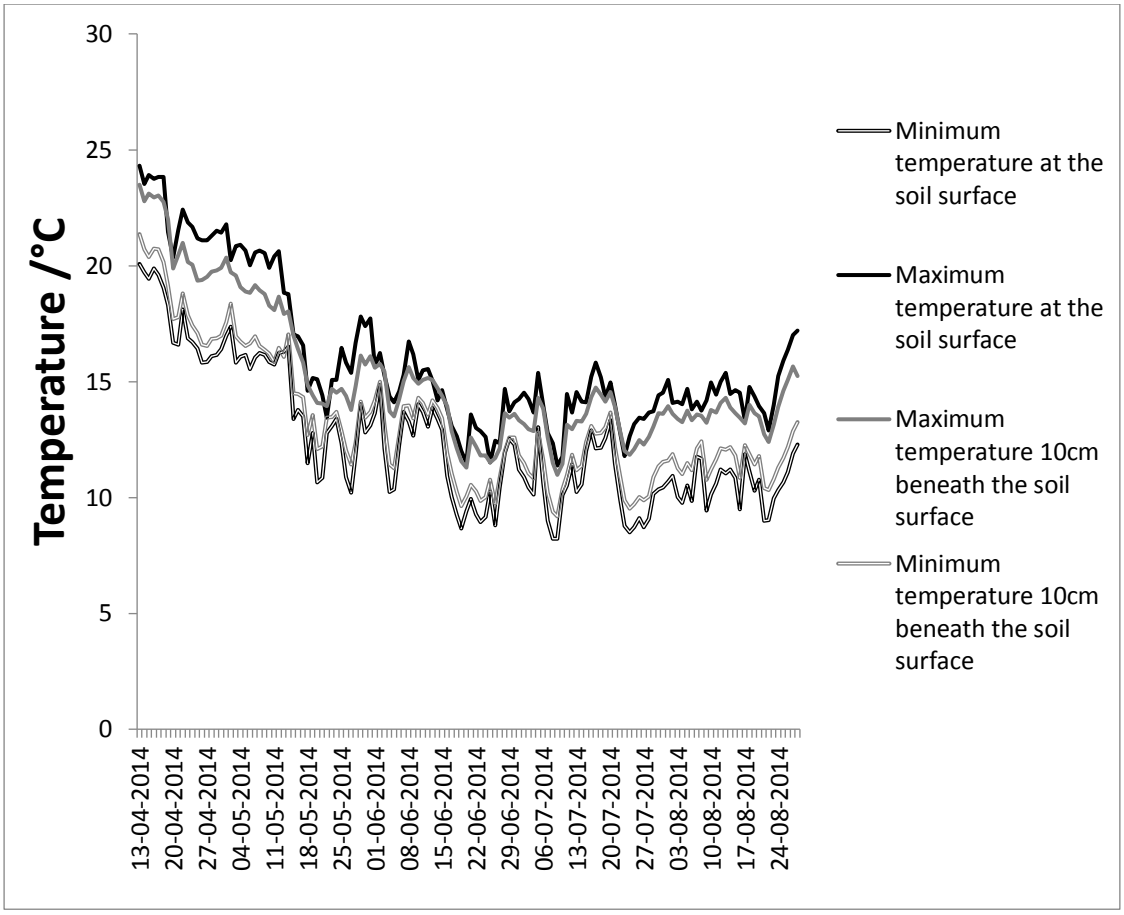


Fig. 20. Soil temperatures at "Drayton" farm, the site of the 2014 pupae digging.

Despite the geographical closeness to "Drayton", "Boondah" had lower temperatures overall, and greater daily temperature fluctuations but followed the same general temperature trends (Fig. 21). The new field cages and the soil did not seem to have a large effect on daily temperature fluctuations relative to the surface, particularly with the lower temperatures.

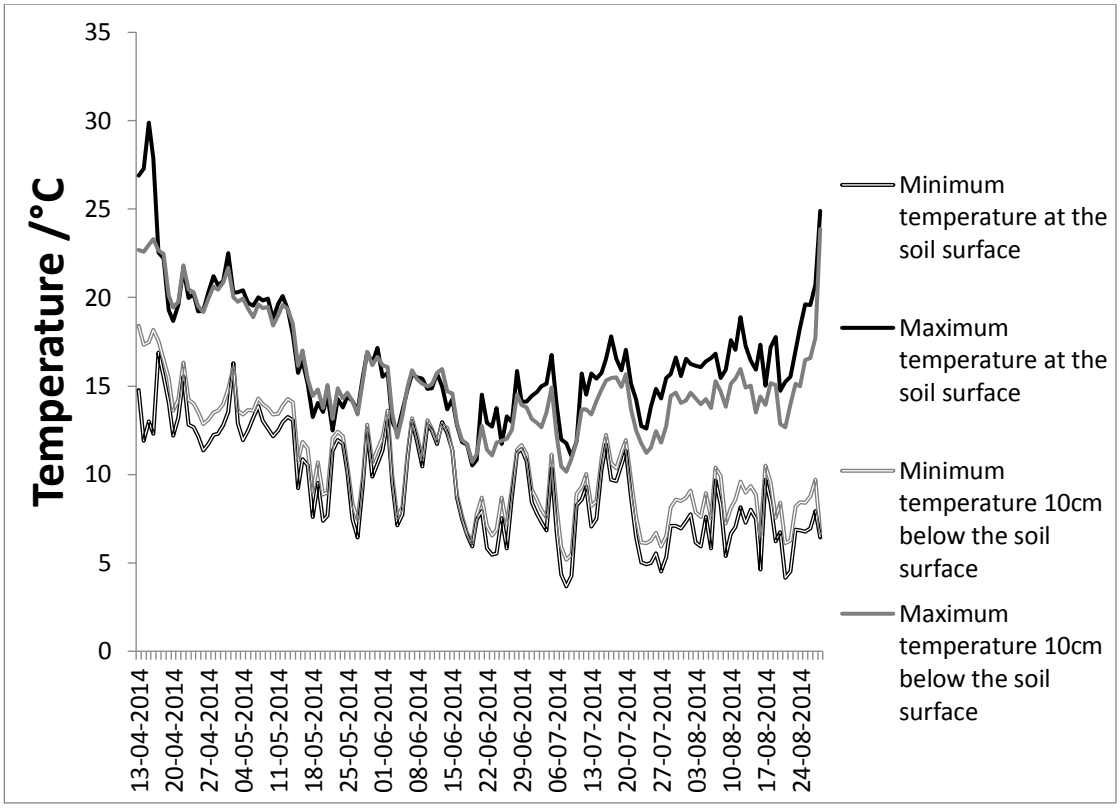


Fig. 21. Soil temperatures at "Boondah", where the 2014 emergence cells were placed. The effects of the cage and the soil did not insulate the soil in the cage more than 1-5°C.

The effects of cages on soil temperatures can be seen by comparison of probes inside and outside the cages at at "Monkira" 2012 and "Cluny" 2013 (Fig. 22). In general cage effects were relatively small. At "Monkira" the maxima were similar except on a few hot days in late winter and spring, where the peak was reduced probably due to shading. However there was a consistent increase in minimum temperatures of 1-2^{o}C, suggesting an insulating effect of the cages at night.

Conversely, at "Cluny" there appeared to be an insulating effect of 1-2^{o}C for maximum temperatures, but very little effect on minimum temperatures.

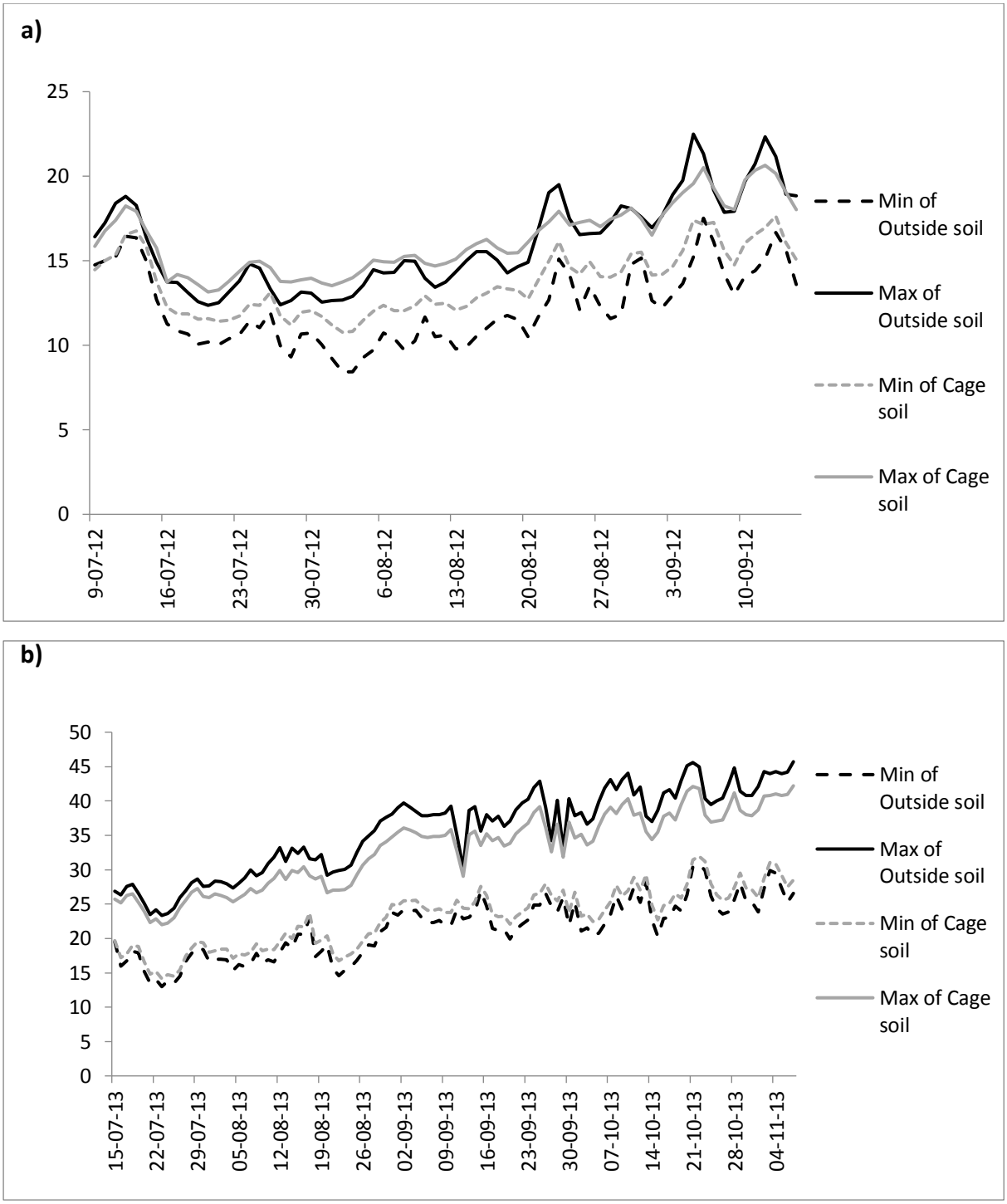


Fig. 22. The effect of emergences cages on soil temperature at 'Monkira' 2012 (a) and 'Cluny' 2013 (b)

3.4 Discussion

3.4.1 Evaluation of emergence cage methodologies

Emergence cage methods were developed for both local and inland field studies, and improvements were made each year aimed at increasing their effectiveness in field overwintering studies of *H. punctigera*. However, with the high mortality of larvae and pupae in the Namoi Valley, it appears that none of the emergence cages, nor the single emergence cell method, were able to sufficiently monitor large enough numbers of *H. punctigera* to obtain reliable data on the timing of emergence of local overwintering adults. In contrast, the same cage methods had much greater success in the field studies in inland Queensland. It is plausible that larval mortality factors such as potential predators, parasitoids and pathogens may be less important in the non-cropping inland Queensland areas compared to Namoi valley cropping regions. Compared to overwintering survival in inland Queensland, the best that can be said for these emergence cage methods in the Namoi Valley is that they served to demonstrate high natural mortality in the immature stages during the autumn, and they contained too few overwintering *H. punctigera* to provide any useful information on the extent and timing of diapause induction and termination, and subsequent moth emergence. Based on the meteorological data (Fig. 17, Fig. 18 and Fig. 19), it may have been better to bury the cells deeper than they were, as there may have not been enough insulation to truly capture soil temperature conditions, and this was a possible source of larval mortality in both the “Cluny” 2013 and “Boondah” 2014 studies.

3.4.2 Diapause in 2014 “Drayton” pupae

All of the 18 surviving pupae obtained (split between 19°C and 25°C) from pigeon peas at “Drayton”, were initially in diapause, but those held at 25°C broke diapause and continued development while those held at 19°C did not. This initially seems at odds with the results of Cullen and Browning (1978) and the conclusions of Chapter 2. This apparent contradiction may be resolved by considering the stages in which diapause might be induced, maintained or terminated, using the process in *H. armigera* as a possible model.

I postulate that pupal development in *H. punctigera* proceeds along several paths with multiple stages, depending on environmental conditions (Fig. 23). In the first path, diapause is induced and maintained at low temperatures. In the second path, diapause is initially induced but broken at higher temperatures, and finally in the third path, conditions are in the optimal range and no diapause is induced. In *Helicoverpa zea*, once diapause has been induced, a period of cold exposure is needed to 'reactivate' the prothoracic glands, to complete development to adult stage (Tauber *et al.*, 1986).

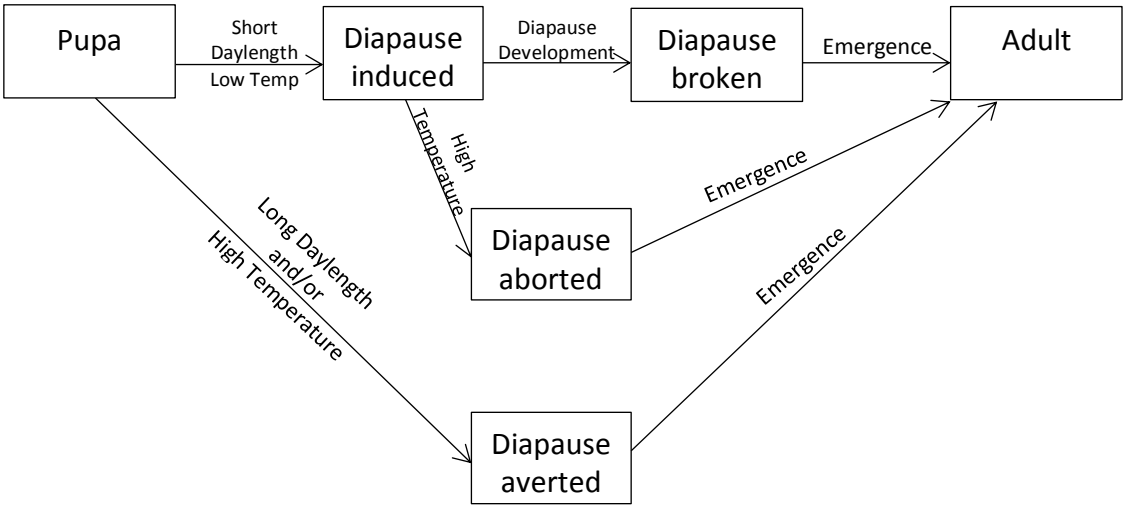


Fig. 23. The possible routes of development to adult for a H. punctigera *pupa, including standard development, diapause, and diapause aborted by high temperatures, based on comparisons with Cullen and Browning (1978) and the data in Chapter 2.*

The specific temperatures required for each of these 'high' or 'low' temperatures in Fig. 23 have not been accurately defined at this time, but based on the results of Chapter 2 and Cullen and Browning (1978), some values can be proposed. Chapter 2 explored the onset conditions for diapause, with a temperature of 19°C and a photoperiod of 12L:12D producing the greatest amount of diapausing pupae. However, most diapause-inducing conditions produce some proportion of pupae not in diapause, so there appears to be no 'hard' set of conditions where every pupa is immediately switched into diapause development. Cullen and Browning (1978) found that diapausing pupae could have their diapause aborted early by exposure to 28°C, while Chapter 2 found that 25°C was not high enough to reproduce this response. A temperature regime of 25°C was enough to avert diapause in nearly all pupae, if diapause was not already induced (Chapter 2, Table 3). A

month into the study (13/5/14), temperatures at “Drayton” dipped below a 19°C maximum, and the maximum did not rise above 17°C from 31/5/14 over the study period, ending 28/8/15 (Fig. 20). With the ~11h photoperiod and low temperatures in May, 80-100% of pupae would be in diapause if they were not already in that state before May. The 18 surviving pupae from “Drayton” were all in diapause when collected from the field, and based on the 4 surviving *H. punctigera* pupae collected on the first sampling trip (12/5/14), the conditions prior to that trip were sufficient to induce diapause. Daily maxima were already dropping (and staying) below 25°C.

3.4.3 Survival of *H. punctigera* in Namoi Valley field trials

The proportion of *H. punctigera* surviving the winter in the Namoi Valley area has historically been extremely low, with only 2/2182 *Helicoverpa* pupae recovered from cotton, pigeon pea and sunflower being *H. punctigera* compared to *H. armigera* from 1987-1988 (Fitt and Daly, 1990). The limited proportion of overwintering *H. punctigera* in local fields was also observed by Wilson (1978). More recent studies of *Helicoverpa* species show that the abundances of adult and pupal *H. armigera* and *H. punctigera* in Namoi Valley cotton refuges have dropped considerably from between the Ingard® era (1996-2004) to the Bollgard II® era (2005-onwards) (Baker and Tann, 2014). Whether this is due to changes in migration, seasonal differences or an increase in natural enemies since broad spectrum pesticides are no longer used in cotton is still not clear (see Chapter 1.4). However, despite the overall decrease, there are suggestions that the proportion of *H. punctigera* relative to *H. armigera* has recently increased (Baker and Tann, 2014). My data from the Namoi Valley in 2014 support this suggestion. This fits with earlier data that suggested that *H. punctigera* numbers increased in some years (Wilson, 1983). The single good year of data collection (2014) may (tentatively) suggest that when and where conditions are favourable higher *H. punctigera* populations may be present than have previously been recorded by authors such as Fitt and Daly (1990) and Wilson (1983), but this incidence is either extremely patchy or subject to factors that we have not fully studied. Wilson (1983) noted that in one year where *H. armigera* populations were low, *H. punctigera* populations comprised 80% of larvae caught on late-grown cotton, although this has not been the case over the four years of field studies in this project.

The change in ratio of *H. punctigera* to *H. armigera* larvae caught compared to the same ratio for pupae collected suggests that far fewer *H. punctigera* larvae survive to the pupal stage than *H. armigera*, and this may help explain the lack of success in the Namoi valley field cage trials. However, an alternative possibility is that the methods for identifying *H. punctigera* larvae over *H. armigera* are unreliable, and some of the larvae collected from pigeon peas might have been incorrectly identified as *H. punctigera* when they were *H. armigera*.

In a dataset spanning from 1996 to 2003, Baker *et al*. (2008) observed that there was considerable variability in the abundance of *Helicoverpa (H. armigera* and *H. punctigera*) in both space and time. High abundance of *Helicoverpa* in the 1998-1999 growing season was likely due to heavy rains in winter-early spring generating abundant spring host-plants, which would have encouraged early plantings by farmers, and generated ideal sequence of host plants for *Helicoverpa* populations to develop on (Zalucki and Furlong, 2005, Baker *et al.*, 2008, Oertel *et al.*, 1999).

3.4.4 Biotic factors affecting overwintering survival

The relative lack of studies on natural enemies (pathogens, predators and parasitoids) of Australian *Helicoverpa* spp. in the pigeon pea refuges makes it extremely hard to quantify the direct impact of natural enemies on *Helicoverpa* populations in the field (Seymour and Jones, 1991). However, with the introduction of Ingard® and Bollgard II® Bt cotton, pupal parasitism in *Helicoverpa* has steadily increased as a result of refuge crops and reduced pesticide use (Baker and Tann, 2014).

Over 15 potential tachinid parasitoids and 8 ichneumonid parasitoids of *H. punctigera* have been described in Zalucki *et al.* (1986). My observations on parasitism at "Drayton" in 2014 are consistent with the results of Baker and Tann (2014), where the most common parasitoids of *Helicoverpa* spp. pupae were tachinid flies along with the ichneumonid wasp, *Heteropelma scaposum* (Morley). Of 67 *Helicoverpa* spp. pupae collected on pigeon pea at Getta Getta (North Star) in the Macintyre Valley in February 2008, 63% were parasitised while only 21% survived (Baker and Tann, 2014).

In addition to the parasitoids, there are a number of predatory insects from a number of diverse Orders including Dermaptera, Orthoptera, Heteroptera, Neuroptera and Coleoptera, as well as spiders from Orders Araneida, all of which prey upon *Helicoverpa* spp. (Zalucki *et al.*, 1986). It is very difficult to estimate levels of predation, or ascribe predation to a particular species, because predators often leave no evidence of their activities (Seymour and Jones 1991). However, in "Milchengowrie" in 2012 for the first two weeks of May, 10-20 brown lacewings (*Micromus tasmaniae* Walker) were caught inside the catchment containers of emergence cages. Both larvae and adults of *M. tasmaniae* are predators, and feed on small *Helicoverpa* larvae (Samson and Blood, 1980). Given the difference in size between *M. tasmaniae* adults and larvae, and late stage *H. punctigera* larvae already present in the field, it is unlikely that field-caught larvae were predated upon by *M. tasmaniae*. The laboratory-reared individuals however, were smaller and might have been attacked. The presence of other predators was also possible. Predators, such as spiders hiding in the cracks in the soil, might have provided additional predation pressure. Little evidence of pupal predation was found overall, with very few 'headless' pupal casings found (as opposed to pupal cases from emerged moths), suggesting that much of the predation was confined to earlier larval stages. Studies on related *Helicoverpa* spp. have shown that mortality due to predation and parasitism is at its highest in eggs and early instar larvae, suggesting pupal mortality is less of an issue (Sansone and Smith, 2001, Zalucki *et al.*, 2002, Mohapatra and Sahu, 2005, Pustejovsky and Smith, 2006).

There are several pathogens which kill *Helicoverpa* spp., including naturally occurring *Bacillus thuringiensis*, nuclear polyhedrosis viruses (NPV) and fungal pathogens. NPV may be a large contributor to mortality of *H. punctigera*, with some NPV strains being effective enough to be formulated as biopesticides against *Helicoverpa* species (Teakle *et al.*, 1986, Dhaka *et al.*, 2010). Predators which feed on *Helicoverpa* spp. cadavers can spread NPV via excreta, further contributing to *Helicoverpa* spp. mortality (Cooper, 1981). Sampling *Helicoverpa* spp. larvae in refuges using sweep nets may have potentially been a source of NPV contamination. If cadavers hanging from a host plant are caught in a sweep net, it may inadvertently saturate the netting of the bag, potentially exposing all insects caught in the net to NPV particles. While there is no evidence for or against this hypothesis, it might help

account for the high mortality of experimental insects in the Namoi studies in 2011, 2012 and 2013.

With a wide range of potential predators and pathogens present, it is understandable why there was poor survival in the Namoi Valley field cage studies. In comparison, higher survival in inland Queensland suggests lower levels of activity by natural enemies of *H. punctigera* there. In Australia there is general tendency for areas of patchy or intermittent rainfall to give rise to pest outbreaks (Drake, 1994). These patchy rainfalls cause a desynchronisation between pests and natural enemies so they may never reach an equilibrium, leading to the chance of pest outbreaks becoming relatively high (Drake, 1994). This is a pattern that fits inland Queensland *H. punctigera*, which build up on vegetation germinated by patchy rainfall in the winter (Gregg *et al.*, 1995), seemingly largely unaffected by predators or parasitoids, before migrating into the cropping areas in the spring.

The 2011 inland Queensland study took place during a documented (and very rare) plague of the native long-haired rat, *Rattus villosissimus* (Waite) (Arthur and Harris, 2011). Rodent-sized access tunnels into the inside of two of the emergence cages were discovered, which may account for the large number of pupal cases inside the cages, along with the relatively low adult catches or lack of adult cadavers (Table 15). Such an occurrence is quite rare and rodent predation is not typically considered as an important factor in *H. punctigera* mortality in cropping areas.

3.4.5 Abiotic factors affecting survival

There are limited cold hardiness metrics on the life-stages of *H. punctigera.* There is an estimated developmental threshold of 10°C, compared to *H. armigera*, with a developmental threshold of 11-14°C (Allsopp *et al.*, 1991). However, without data on lower lethal temperature, time to death or other cold-hardiness metrics for *H. punctigera*, it is hard to determine whether or not *H. punctigera* can survive colder temperatures than *H. armigera*, or whether temperatures in the Namoi Valley ever approach lower lethal limits. Diapausing *Helicoverpa zea* pupae exposed to cold temperatures drop from surviving 115 days at 5°C to surviving 67 days at 0°C (Morey *et al.*, 2012), a change that could potentially be reflected in *H. punctigera* and prevent much of the overwintering population from

surviving if temperatures are too cold for too long. In the case of the 2014 study at "Boondah" Farm, the larvae did not reach pupal stage, all dying as small and medium larvae sometime in mid-July. Although the laboratory-reared larvae had an optimal artificial food source, they did not develop to pupal stage in time, so were not in their cold-hardy pupal stage when the cold weather conditions hit (Fig. 21). The CLIMEX model for ecological suitability of an environment states that when an organism is below its developmental minimum, it accumulates cold stress, with an excess of stress killing off an individual (Maywald and Sutherst, 1991). *H. punctigera* larvae in these field studies would likely be subjected to extended cold-stress when the temperature went below their developmental threshold, and eventually succumb to it. Larvae at "Boondah" were exposed to greater variability and lower daily average soil temperature and daily minimums than those at "Drayton" (Fig. 20).

The 2012 study at "Milchengowrie" had considerably more variable daily fluctuations (Fig. 19) than "Drayton", 2014 (Fig. 20), though the most severe fluctuations were ameliorated by the soil. Temperature alone was likely not severe enough to kill the experimental insects.

The insects in the cells at "Boondah" in 2014 experienced rather extreme temperature minimums and maximums throughout the study period. Although exposed to extreme temperature variability, typically as high as 20°C each day, this was unlikely to be the cause of the deaths of the experimental insects. Temperature fluctuations of this magnitude are unlikely to be lethal unless the upper or lower end of the range approaches lethal levels. One possible cause may have been that insects reared in the lab at 25°C were exposed to low temperatures soon after being placed in the cells (Fig. 21). The extended cold stress from this exposure may have caused them to die. It should be noted that a smaller type of screen cage was used for this study compared to previous years, which appears to provide less insulation from temperature extremes.

Given the temperatures at both 2014 sites, "Boondah" and "Drayton" (Fig. 20 and Fig. 21), it is almost certain that any pupae present in the soil would have not continued their development beyond the diapause stage throughout the winter, because temperatures never approached 25°C. The inland Queensland field studies showed differences between sites, and the soil types at "Cluny" and "Monkira" were also quite different from each other.

The soil around “Cluny” is sandy, and the host plants in the area grow sparsely on nearby sand dunes. In “Monkira”, the soil is firmer and floodplain vegetation can be found there. The vegetation cover on sand dunes is typically much lower than on floodplains, and this is a potential reason for the difference between the temperatures at these two sites, with less shade at “Cluny” and higher temperatures. In both cases however, the soil protects any overwintering *H. punctigera* from the extremes of air temperature in inland Queensland. The differences in temperature from the different soil types may have had different effects on inducing diapause. The temperatures at “Cluny” were high enough to prevent diapause (Fig. 17) but at “Monkira”, they were low enough to potentially induce diapause (Fig 16).

The emergence cells, buried in the soil as well as inside a screen-tent, also insulated the pupae from external extremes, though the effect was not as great as a pupa buried fully in the soil of an emergence cage (Fig. 18). Note that Fig. 18 is from a different data logger to Fig. 17, which experienced slightly different temperature conditions.

3.4.6 Overwintering, adult emergence and migration

Some tentative conclusions about the relationship between emergence and local pheromone trap catches in inland areas and in the Namoi Valley can be drawn from comparisons shown in Fig. 24 and Fig. 25.

In 2011, the beginning of pheromone catches at “Cluny” corresponded with the limited emergence in the cages. However, the pheromone catches at Bedourie (about 25 km away) began before, and continued well after, emergence had stopped. This may suggest that moths originating from other surrounding areas were caught at Bedourie. Another possibility is that earlier and/or later emergence from the cages at “Cluny” was not detected because the moths were taken by predators (e.g. rats) before they were captured in the containers at the top of the cages.

In 2011, pheromone catches in the Namoi valley were well synchronised with those at Bedourie, and also followed the peak of emergence in the “Cluny” cages. Given the large differences in soil temperatures between the Namoi and the inland (compare Fig. 19 and Fig. 20), this suggests that this early spring peak in pheromone catches in the Namoi was

due to migration from warmer areas, such as inland Queensland, rather than local emergence (Fig. 24).

The 2012 study at “Monkira” showed slightly different results. Emergence was prolonged over a period of about 100 days, although the cages had been seeded on one day, with larvae of similar sizes. This suggests some type of “bet-hedging” mechanism, possibly related to diapause, that would allow a fraction of the population to emerge quickly and utilise any host plants remaining in late winter and early spring, while another fraction emerged later and probably emigrated. Lower temperatures suggested that diapause could potentially be induced in floodplain soils found at “Monkira”, but not on the sand dunes at “Cluny” (Section 3.4.5 above). “Monkira” is roughly equidistant from both Birdsville and Bedourie, but Birdsville is in the same river catchment, and in 2012 that catchment flooded, whereas the one at Bedourie did not. Birdsville is therefore a more appropriate comparison site for this year. Despite the low numbers at Birdsville, there was synchronisation between early emergence at “Monkira” and Birdsville pheromone trap catches. However, later emergence at “Monkira” was not reflected in pheromone catches at Birdsville. As in 2011, there was a close synchronisation between a second peak in pheromone trap catches in western Queensland and in the Namoi valley around weeks 12-13 (early September), despite wide variation in soil temperatures, suggesting that in the Namoi early season catches may be associated with immigration rather than local emergence. However, the earliest peak of emergence in inland Queensland (mid to late July) was not mirrored in the Namoi. A possible explanation for these observations is the difference in temperatures between inland Queensland and the Namoi Valley. Temperatures during the first (July) peak may have been high enough to allow emergence of non-diapausing individuals in inland Queensland, which did not migrate from that region. Temperatures in the Namoi Valley did not allow pupae to complete diapause development.

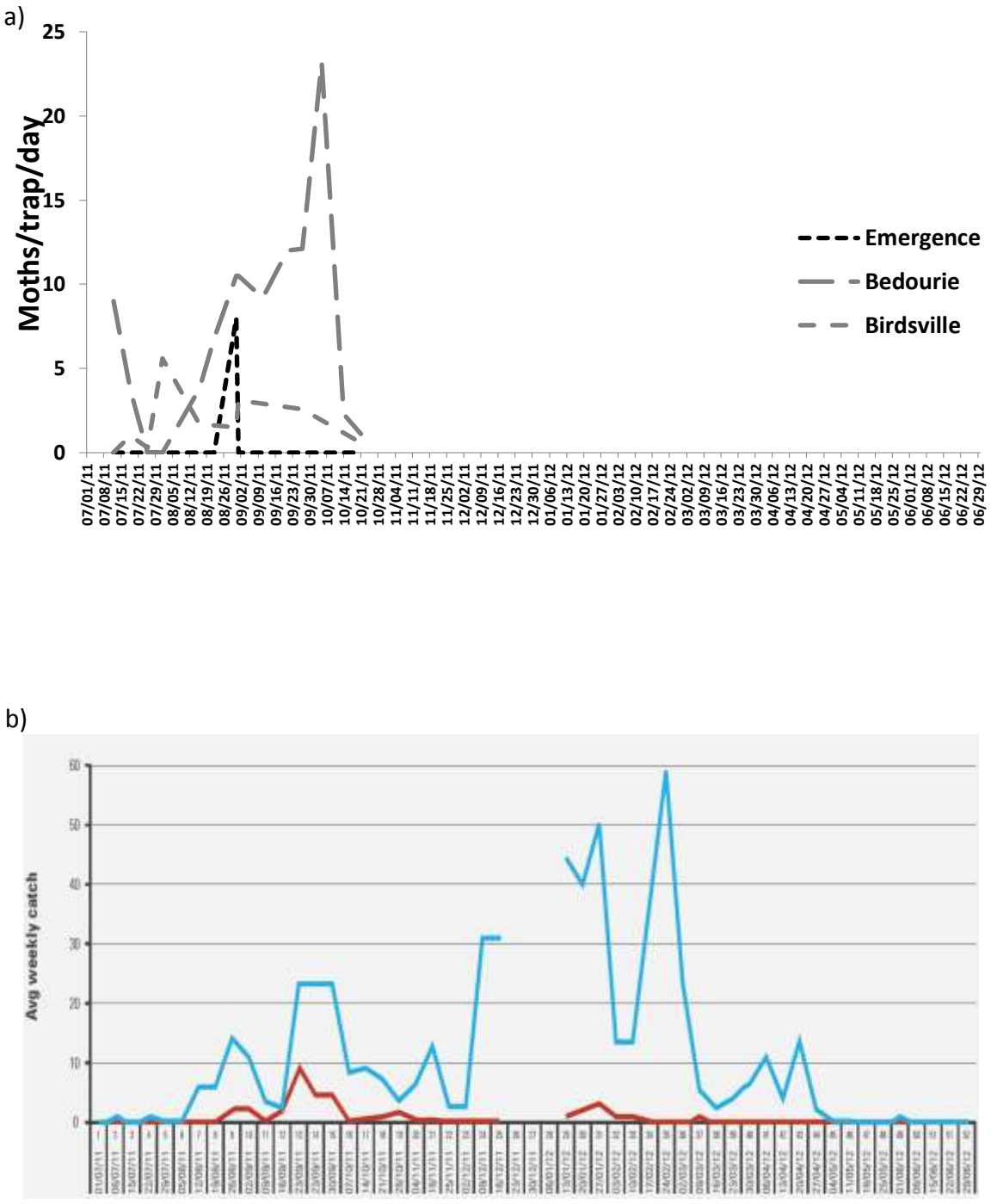
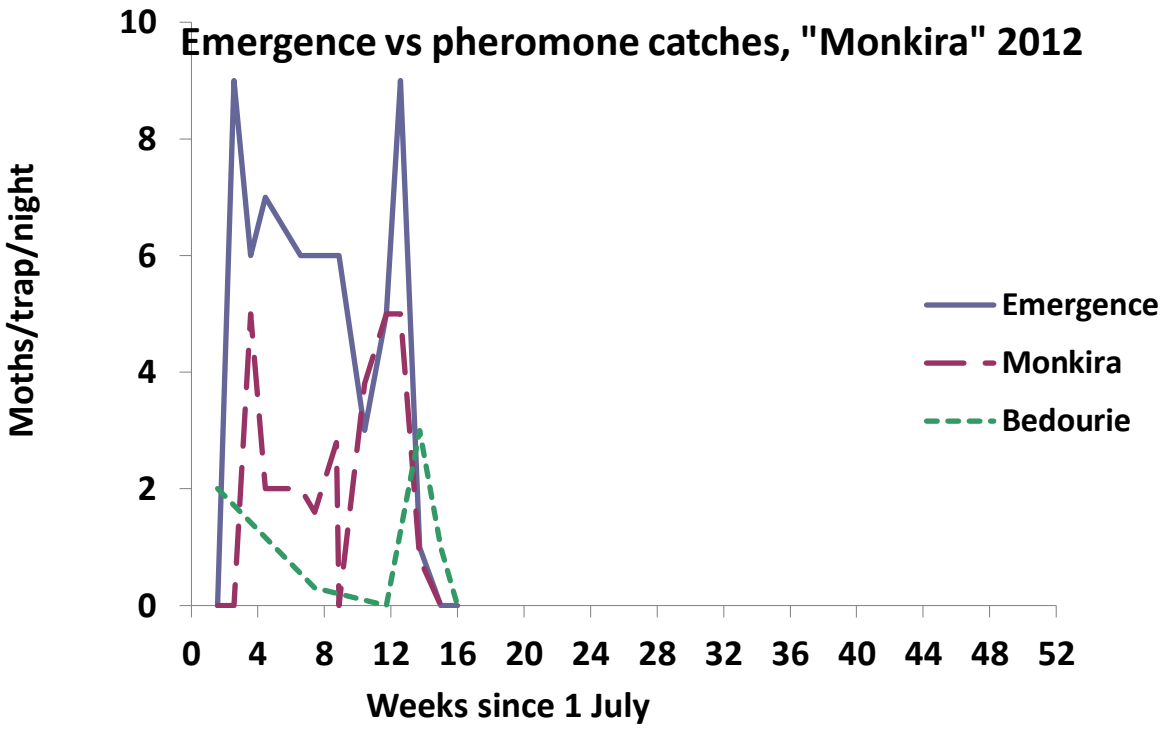


Fig. 24. Emergence of H. punctigera *adults from cages at "Cluny" along with pheromone trap catches at the nearby town of Bedourie and Birdsville, QL (top), and from the Namoi (bottom, red line) over the same period, aligned on the same temporal scale, 2011 (Cotton Seed Distributors, 2012).* Numbers for H. armigera *are also shown over this period (blue line).*

a)



b)

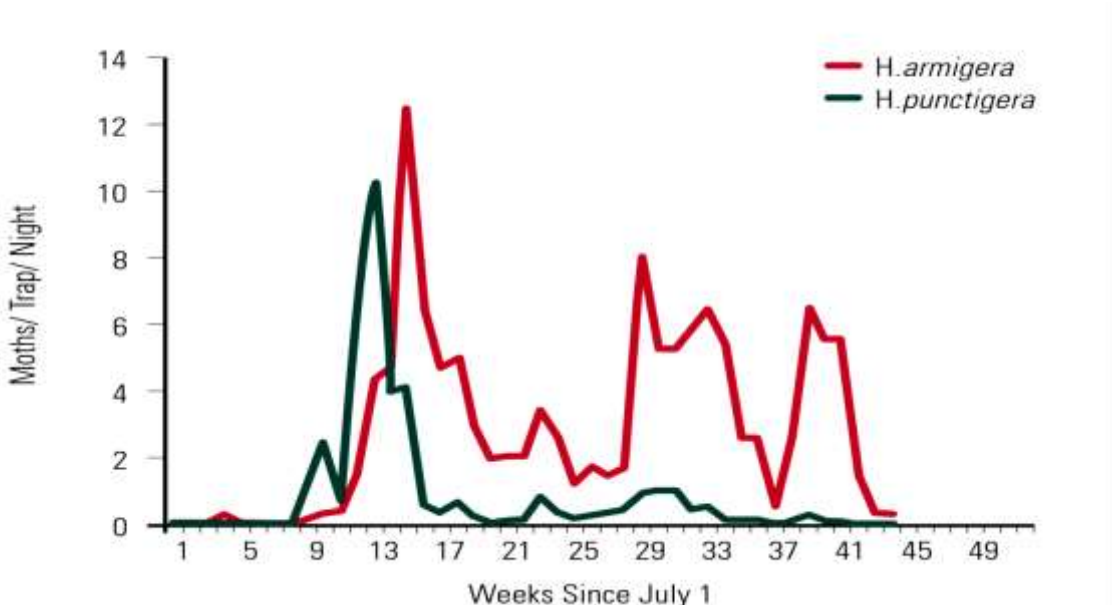


Fig. 25. Emergence of H. punctigera *adults from cages at "Monkira" along with pheromone trap catches at "Monkira" and the nearby town of Birdsville, QLD (a), compared with Namoi Valley pheromone trap catches over the same period, on the same scale, 2012 (b)(Cotton Seed Distributors, 2012).*

3.4.7 Summary

There are a variety of factors that may have contributed to larval mortality in the 2011-2013 Namoi Valley emergence cages, and thus greatly limited the number of pupae for study. These include rainfall affecting the availability of host plants (Baker *et al.*, 2008), temperature reducing the survival of pupae in the soil (Morey *et al.*, 2012), a changing cotton landscape (Ingard®/Bollgard®) which reduced insecticide use, thus increasing the abundance of predators and parasitoids while reducing the abundance of *Helicoverpa* (Baker and Tann, 2014). The unpredictable ratio of *H. punctigera* to *H. armigera* also made finding a suitable site for a study extremely time consuming. All of these factors potentially contributed to the failure of these field trials and emphasise the need for long-term studies.

Despite these setbacks, the same methods performed better in inland Queensland, where three years of field data were collected. Results in 2011 and 2013 were adversely affected due to a rat plague in 2011 and the collaborator not checking the cages in 2013. Nevertheless, emergence cages provided useful insights into the timing of overwintering moth emergence, and combined with local and remote pheromone trap data, the results suggest that immigration from inland Queensland made a substantial contribution to early season catches in the Namoi region.

Regular pupae digging, which provided immediate access to diapausing *H. punctigera* was the most effective method for obtaining *H. punctigera* pupae in the Namoi, at least in 2014. This method, combined with incubation at two temperatures, suggested that a high level of diapause was induced in late season larvae. Diapause was broken by exposure to low temperatures by early winter, but emergence was delayed because temperatures remained too low until spring.

Temperature probe data suggests a role of vegetation and soil types in the incidence of diapause in overwintering *H. punctigera* in inland Queensland. Pupae in the soil under sand dunes with sparse vegetation are less likely to be in diapause than those in the floodplains, where heavier vegetation cover may play a role in keeping soil temperatures below the threshold for diapause induction. This, and the prolonged emergence at “Monkira” in 2012, suggests that a fraction of the overwintering population emerges early, in mid to late winter, and does not emigrate, while another fraction emerges in late winter to early spring,

and does emigrate. This is in contrast to the Namoi Valley, where temperatures drop below the diapause-inducing threshold in May, and pupae found in the soil under refuge crops would be expected to be in diapause, and there is little emergence until early to mid-spring.