

Final Report

On Farm Series | Cotton Research & Development Corporation

Final Report

Part 1 - Summary Details

CRDC Project Number: CRC75 **CRC 1.1.24**

Project Title: **Trichogramma incidence in cotton and grains growing regions of Australia – consequences for Helicoverpa management.**

Project Commencement Date: 1/7/05**Project Completion Date:** 30/6/06**Research Program:** On Farm

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Part 3 – Final Report Guide

1. Outline the background to the project.

IPM systems used across the major agricultural growing regions of Australia rely on information provided by growers and consultants regarding *Helicoverpa* egg and larval pressure, *Helicoverpa armigera* and *Helicoverpa punctigera* species identification, and the presence of beneficial insects. Preserving populations of beneficial insects in cotton and grains crops is appealing in IPM systems aiming to minimise the use of “hard” chemicals. A beneficial insect of particular interest is the *Trichogramma* wasp. As an egg parasitoid of *Helicoverpa*, it attacks developing larvae and prevents them from emerging and damaging crops.

This research proposed to generate and collate information on the frequency of *Trichogramma* across several regional growing areas of the east coast of Australia (i.e. Namoi Valley, Griffith, Victoria, Bundaberg, Darling Downs, Dawson-Callide Valleys), and collect information on its frequency in different crop types, and across the growing season. This project's aim was to enhance the effect of the beneficial wasp by collecting new information on its distribution, to provide an assessment of *Trichogramma* frequency with available crop management information and then attempt to correlate fluxes in beneficial populations with crop management events (eg. effect of a spray on *Trichogramma* population size, management of crop edges). For regions eager to include beneficial insects in their IPM systems, identifying the presence and levels of *Trichogramma* parasitism of *Helicoverpa*, and the effects of region, crop type and insecticide usage on their populations, were to provide guidelines for inclusion in future management development systems. Conversely, for regions utilizing Bollgard II cotton, characterising *Trichogramma* frequencies in refugia crops would provide valuable information about the efficacy of refugia in providing susceptible *Helicoverpa*.

Finally this project wanted to increase grower awareness of available natural sources for integrated pest management, as this research offered same day results on the proportion of *Trichogramma* parasitism in *Helicoverpa* eggs, and determination of *Helicoverpa* species identity, during the 12 month project. It was intended that with the speed that project results could be available it could increase grower awareness and enthusiasm for manipulation of beneficial activity on their properties, by providing real-time information on population status.

2. List the project objectives and the extent to which these have been achieved.

Obj No.	Objective	Achievement
1	Determine the frequency (in time and location) of <i>T. pretiosum</i> across the major grains and cotton growing regions of Australia	This objective was achieved and is evidenced by compiled results for the occurrences of <i>T. pretiosum</i> across the major grains and cotton growing regions of Australia, and the successful data reporting back to collectors within 24-48 hours of egg delivery.
2	Determine <i>Helicoverpa</i> species distribution and frequency within 48 hours of each egg collection event, allowing sample providers to use information in a timely manner if desired.	This objective was achieved and is evidenced by compiled results on the species distribution across the major grains and cotton growing regions of Australia, and the successful data reporting back to collectors within 24-48 hours of egg delivery.
3	Determine the effect that region, crop type, regional cropping patterns, and insecticide application have on <i>Trichogramma</i> frequency and <i>Helicoverpa</i> species distribution	Despite a detailed collection strategy (which covered all major growing regions), insufficient samples were able to be obtained for a statistical analysis of 'crop effects' on population. The only comprehensive sampling available to the project was thanks to Dr Sharon Downs and Dr Louise Rossiter
4	Collation of final Report to CRDC	Completed here.

3. Detail the methodology and justify the methodology used.

Dr Nicole Lawrence and Dr Leon Scott were responsible for the development and validation of the molecular diagnostic tests used in this project. The application of the diagnostics tests to field samples in this project have been successful. Dr Kirsten Scott had developed an extensive network of collaborators during her time managing the GRDC “Population genetics of *Helicoverpa* migration, recruitment and origins” and CRDC “Tracking *Helicoverpa* migration and the accumulation of insecticide resistance” projects. While many of these collaborators had initially indicated their willingness to provide *Helicoverpa* egg samples to the project, during the peak season many became too busy and the project thus suffered from too few samples in many regions. The exception to this was the comprehensive sampling that was supplied to the project by Dr Louise Rossiter and Dr Sharon Downs. These two scientist were outstanding in their dedication and efforts in supply of material to our project.

Our diagnostic test involved using a polymerase chain reaction on DNA from *Helicoverpa* eggs to generate bands on an agarose gel. These tests allowed us to identify eggs that contained *Trichogramma* parasites and also to distinguish *H. armigera* and *H. punctigera* species. Using our molecular diagnostic tests, we delivered information on *Trichogramma* parasitism, and *Helicoverpa* species just 4 hours after eggs are received in our laboratory. This allowed us to provide one-day answer to IPM questions where an overnight courier was used, and was a vast improvement on previous timeframes required for obtaining this information. In general between 20 and 50 eggs were found on sample materials and were picked and patched to 96 well format for DNA extractions and the PCR based diagnostic assays. All materials were archived by storage at minus 20 degrees C for any later use.

It should also be noted that this project was very negatively affected by not receiving its research funding until March 2006 (9 months after the project start date) particularly as it was a 12 month project finishing in June 2006. This funding situation made all aspects of this project more difficult to manage.

4. Detail and discuss the results including the statistical analysis of results.

Data collected during the testing stages of this research program are shown below:

Material Reference (DNA location)	Date Collected	Region	Site	Grower/Collector	Crop
Trich1 F1-H5	8-Dec-04	Darling Downs	Balmoral (near Pittsworth)	Robin Heath	conven.Cotton
Trich2A1-F7	6-Jan-05	Upper Namoi	Shangra-Le	Louise Rossiter	Bollgard (Ocro)
Trich2G7-D12+Trich3A1-H2	10-Jan-05	Lower Namoi	Yarral	Louise Rossiter	Bollgard round-up ready
Trich3A3-H6	6-Jan-05	Upper Namoi	Drayton/Gunnedah	Louise Rossiter	Bollgard
Trich3A7-H11	6-Jan-05	Upper Namoi	Battery Hill/Gunnedah	Louise Rossiter	Bollgard
Trich4A1-C3	11-Jan-05	Lower Namoi	Athelstone	Louise Rossiter	Siokra Cotton (Bollgard)
Trich4D3-G8	12-Jan-05	Lower Namoi	Whitewoods	Louise Rossiter	Bollgard
Trich4H8-D12	12-Jan-05	Lower Namoi	Dundee	Louise Rossiter	Bollgard
Trich5A1-A6	18-Jan-05	Gwydir	Bethel	Louise Rossiter	Conventional Cotton
Trich5B6-F9	18-Jan-05	Gwydir	Milo	Louise Rossiter	Bollgard
Trich5G9-D12	18-Jan-05	Gwydir	Auscott	Louise Rossiter	Conventional Cotton
Trich6 (A)	30/11/2005	Namoi (lower)	"Doreen"	Louise Rossiter	Maize
Trich6 (B)	30/11/2005	Namoi (lower)	"Doreen"	Louise Rossiter	Maize
Trich6 (C)	30/11/2005	Namoi (lower)	Leiches "Myal Vale"	Louise Rossiter	Sorghum

DNA Extracted	Number eggs at site	No. of <i>H.armigera</i> eggs (and as a % of amplified eggs)	No. of <i>H.punctigera</i> eggs (and as a % of amplified eggs)	No.Trichogramma Infected <i>H.armigera</i> (as % of amplified armigera)	No.Trichogramma Infected <i>H.punctigera</i> (as % of amplified punctigera)	No. Trichogramma Infected eggs of undetermined species (as % of total eggs collected)	Total No. Infected Trichogramma (as % of total eggs collected)
9-Dec-04	31	1 (3%)	30 (97%)	None Detected	None Detected	None Detected	None Detected
11-Jan-05	54	0	42 (100%)	N/A	20 (37%)	11 (20%)	31 (57%)
11-Jan-05	54	3 (7%)	41 (93%)	1 (33%)	5 (12%)	2 (4%)	8 (15%)
11-Jan-05	32	0	20 (100%)	N/A	3 (15%)	5 (16%)	8 (25%)
11-Jan-05	40	0	23 (100%)	N/A	0	4 (10%)	4 (10%)
13-Jan-05	19	2 (14%)	12 (86%)	2 (100%)	2 (17%)	1 (5%)	5 (26%)
13-Jan-05	44	3 (8%)	36 (92%)	2 (66%)	8 (22%)	1 (2%)	11 (25%)
13-Jan-05	29	1 (4%)	25 (96%)	0	10 (34%)	2 (7%)	12 (41%)
19-Jan-05	41	17 (52%)	18 (48%)	3 (18%)	6 (33%)	5 (41%)	14 (34%)
19-Jan-05	29	11 (52%)	10 (48%)	9 (82%)	2 (20%)	6 (21%)	17 (58%)
19-Jan-05	22	12 (67%)	6 (33%)	1 (8%)	0	4 (18%)	5 (27%)

2-Dec-05	48	46 (96%)	0	1(2%)	0	0	1(2%)
2-Dec-05	48	45(94%)	0	2(4%)	0	0	2(4%)
2-Dec-05	48	41(85%)	0	6(15%)	0	3(6%)	9(19%)

The Diagnostics

The DNA based diagnostic performed well in the diagnosis of both species identification and the levels of egg parasitism by *Trichogramma*. The diagnostic lends itself well to the laboratory and is now sufficiently simple in its design to be used outside a specialist molecular laboratory. In order for these diagnostics to be of use to industry, it needed to have high throughput, low inputs, and 24-48 hour turnaround from sample collection to results delivery. This has been achieved in this project. With the assays operating effectively the only impediment to this turnaround time was the delivery of samples to a central laboratory facility in Brisbane. While every effort was made to streamline this process the University of Queensland is not properly setup to deal with a commercially focused diagnostic service. While this is no real surprise, it is suggesting that a regionally located facility whose primary role is to perform diagnostics seems to be more appropriate. The skills to implement such a decentralisation are available through experience gained in this and previously funded research by CRDC.

The Results

The data that was collected on species composition showed a general trend in species composition showing *Helicoverpa punctigera* prominent early in the season (95% *H. punctigera*, 4% *H. armigera* and 1% *H. punctifera*) and remaining so until late November where *Helicoverpa armigera* became more prominent. Several collections from the Darling Downs however showed very high levels of *H. punctigera* (87%) as late as January. The most notable change in species composition in this study compared with other of previous years and project (UQ34C) is the presentation of an additional (putative) species *H. punctifera*.

The data collected on levels of parasitism early in the season showed zero to very low (2%) levels of *Trichogramma* parasitism. These rates of parasitism rose very slowly over the months before Christmas, peaking in January at around 40%, before then falling sharply presumably due to cultural regimens in cotton from which most collections were made. Dr Leon Scott and Dr Kirsten Scott designed a series of primers to determine species composition of *Trichogramma* parasites. Almost all *Trichogramma* found were *Trichogramma pretiosum*. Rates of *Trichogramma australicum* were found to be 1%-2% on the Darling Downs only.

5. Provide a conclusion as to research outcomes compared with objectives. What are the “take home messages”?

The project has highlighted a number of very important issues and has demonstrated several very useful points for industry to consider in its future investments in the area of diagnostic based decision support protocols. Firstly the project has clearly demonstrated that DNA based diagnostics are viable tools for helping in the control of *Helicoverpa* in a sustainable economic manner. Diagnostic turnaround times are currently within the timeframes needed for accurate decision making when choosing best practice strategies for control, and can deliver this information in a very cost effective way.

Secondly the project has highlighted the need to decentralise the diagnostic capabilities. The center of expertise is focused in Brisbane at the University of Queensland. This has created a number of difficulties, which include inadequate sample receipt facilities. While the University is well resourced in terms of its laboratory capabilities it does not have a commercial focus and as such can and does stumble over sample "chain of custody" issues. This is not likely to improve which prompts the notion of decentralisation of this expertise in diagnostics to several regional laboratories. The diagnostic are now of a robust and reliable nature such that with a few simple and inexpensive pieces of equipment, someone with a grade 12 education could perform these assays with a high degree of precision and accuracy. The *Trichogramma* parasitism and *Helicoverpa* and *Trichogramma* species identification diagnostic assays are also now greatly enhanced for their specificity and sensitivity.

The presence of *Trichogramma* parasitism in the 2005/06 growing season was similar to that of previous years with *Trichogramma pretiosum* being the major parasite species. An additional species *H. punctifera* (putative) was found for the first time in egg lays at low levels on the Darling Downs.

A formal process of commitment to collections and appropriate acknowledgement of contributions made is needed to improve the number of assays performed allowing a proper statistical analysis to be performed. We were unable to make comparative assessments statistical analysis of spray regimens, crop type, and management practice due to a lack of industry wide collections.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

This projects diagnostics facilitate strategies for *Helicoverpa* management, for area wide management groups across a large portion of Australia's Cotton growing regions. This has the potential to reduce pesticide usage, which simultaneously addresses the economic, environmental and social outputs for the corporation.

7. Provide a summary of the project ensuring the following areas are addressed:

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

None of our technical achievements in this 12 month project were of commercial consequence.

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

These assays are now sufficiently robust that they can be performed by non-experts in a simple laboratory or even a kitchen with a few inexpensive equipment items. It is the recommendation of this research team that that these assays should be regionalised. This will improve further the speed of the 360 degree turn around of collection to information by significantly shortening the sample transit times. This regionalisation offers an excellent commercial opportunity for an innovative person or persons in the various regional areas and would enable a commercially driven expansion of diagnostic services beyond *Trichogramma* by using many of the assays developed by the University research groups resourced by CRDC.

c) are changes to the Intellectual Property register required?

No changes are required for the Intellectual property register.

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

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

Regional laboratories

Regional laboratories are essential to implant DNA based diagnostic into management practices for the cotton industry. Relatively simple and inexpensive setups can be implemented.

Outline the need:

A shed or kitchen with reliable electricity

a fridge/freezer

power pack/gel rig

PCR machine

Detection system

Cost \$8,000 equal to the cost of one spray application. Possibly shared between several growers in an Area Wide Management group.

Training:

Dr Kirsten Scott's team has run training for these kinds of diagnostics and could run these at regional locations. It would take approximately 1 week to acquire all the skills needed and these courses could be run 4-5 times a year or as needed.

Advantages:

- 6-8 hour turnaround
- Test could be done 24 hours a day, 7days a week
- Cheaper
- more flexibility in choosing what information is want/needed
- The grower can be in total control and using these assays will have environmental benefits.
- It also provides a commercial opportunity to regional areas for an innovative person to build a viable business.

Assessing the need:

Field days topics including what are the diagnostics, what they can and cannot do, who is interested and who will do them?

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

N/A

9. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

The application of this diagnostic would have an immediate effect on reducing cropping costs through reduction of unnecessary spraying. This result would benefit the cotton industries image by using information rich integrated pest management program. The likely follow on of a reduction in sprays is also an environmental achievement.

Part 4 – Final Report Executive Summary

The offering of diagnostic services in the determination of species identification and *Trichogramma* parasitism in *Helicoverpa* eggs provided submitters with valuable insight into the distribution and prevalence of *H. armigera* and *H. punctigera* activity as well as the levels of *Trichogramma* activity in these populations of *Helicoverpa*.

The data collected on species composition showed a general trend in species composition showing *Helicoverpa punctigera* prominent early in the season (95% *H. punctigera*, 4% *H. armigera* and 1% *H. punctifera*) and remaining so until late November where *Helicoverpa armigera* became more prominent. Several collections from the Darling Downs however showed very high levels of *H. punctigera* (87%) as late as January. The most notable change in species composition in this study compared with other of previous years and project (UQ34C) is the presentation of an additional (putative) species *H. punctifera*. Data collected on levels of parasitism early in the season showed zero to very low (2%) levels of *Trichogramma* parasitism. These rates of parasitism rose very slowly over the months before Christmas, peaking in January at around 40% before then falling sharply presumably due to cultural regimens in cotton from which most collections were made. Almost all *Trichogramma* found were *Trichogramma pretiosum*. Rates of *Trichogramma australicum* were found to be 1%-2% on the Darling Downs only.

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