Australian Government

Cotton Research and Development Corporation

Annual, Progress & Final

REPORTS

Part 1 - Summary Details

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

CRDC Project Number: CRC69T

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☐ Due 30-September
(or within 3 months of completion of project)

Project Title: Travel: Oliver Knox – Pacific Rim Conference, Victoria, Canada

Project Commencement Date: 28/10/2005 **Project Completion Date:** 5/11/2005

Research Program: 1 People and Knowledge

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Signature of Research Provider Representative: _____

TRAVEL REPORT

Overseas trip report – 6th Pacific Rim conference. Victoria, BC, Canada

6th Pacific Rim conference, held at the Fairmount Empress Hotel, Victoria, BC, Canada from the 30th of October to the 3rd of November 2005. Travel commenced from Narrabri to Victoria, BC, Canada on 28th October, with the return trip started on the 5th November.

Dr Oliver Knox's attendance was facilitated through contributions from the CRDC, Cotton Community Catchments CRC and CSIRO Entomology.

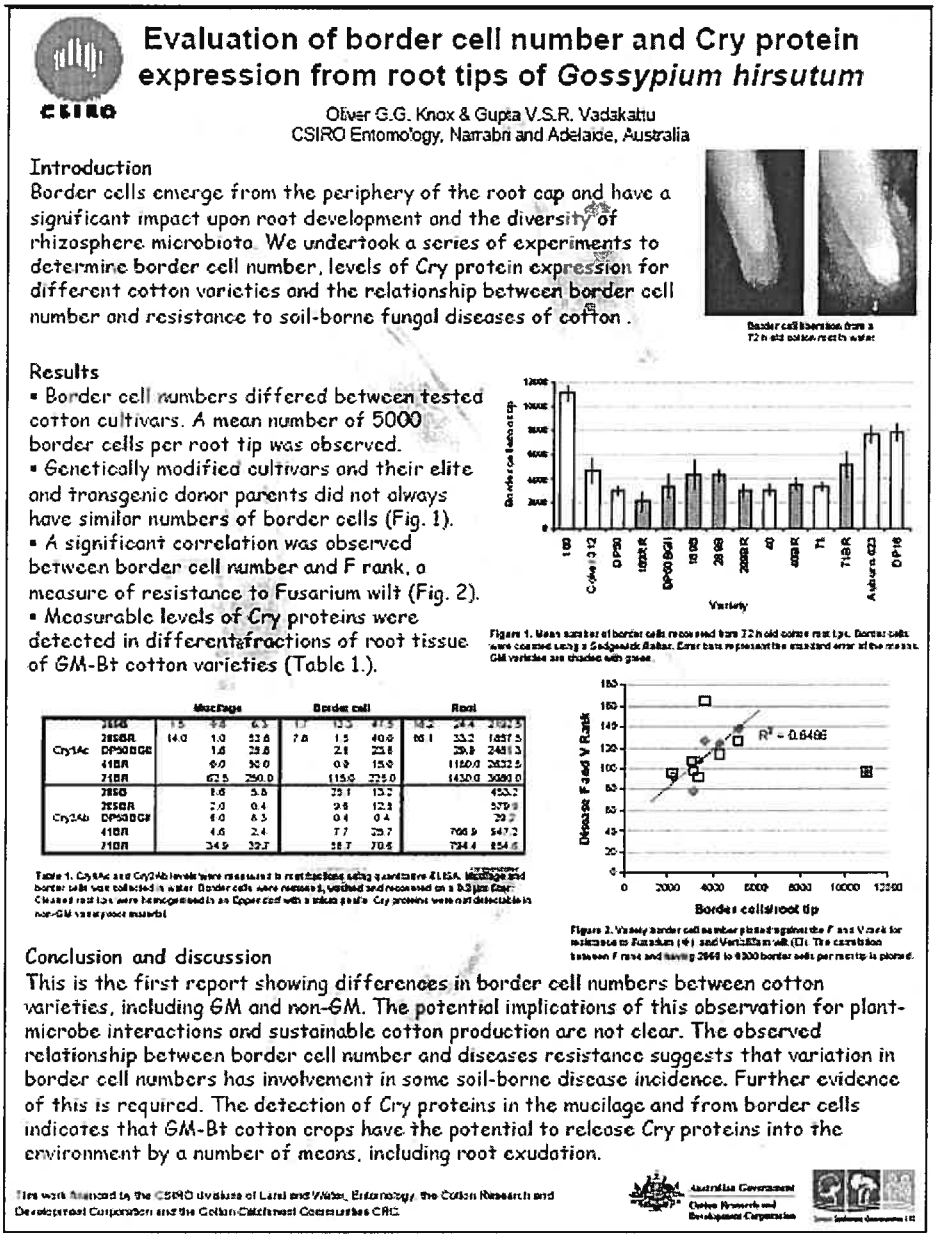
1. What were the:

a) major findings and outcomes

The poster, 'Evaluation of border cell number and Cry protein expression from root tips of *Gossypium hirsutum* (Oliver G.G. Knox and Gupta V.S.R. Vadakattu), was well received and generated plenty of interest amongst the 90 attending delegates as well as some interesting debate. The poster presentation has been converted to a short paper (attached) for inclusion in the conference proceedings, due for publication April 2006.

Of the meeting itself, this is probably the first conference I have ever attended where the keynote address, given by Miguel Altieri, managed to alienate the presenter from the majority of his audience. Dr Altieri's views on the requirement for the application of precautionary principals, that GM soybean is a cause of Amazon deforestation, that Bt crops provide no enhancement of diversity or abundance, and that GM crops are the product of commercial companies with no consumer requirement were all issues that were refuted in almost every talk that followed.

Of the subsequent sessions (there were no concurrent sessions at this meeting) there were several areas in which my understanding of Bt in the environment was greatly enhanced. I was particularly interested to note that after nearly 50 years of Bt use there is still much unknown about Cry proteins' mode of action. There was evidence presented to imply that the site and mode of action is either on the peritrophic, or the brush border membrane (BBM), or within the cells lining the BBM. There was also convincing data on Cry proteins working as either monomer or oligomeric structures, and in some cases other external factors were also demonstrated to be involved. The last of these points was of particular interest in light of several of the experimental results we have seen during the groups' current DEH funded project and I ended up having some interesting conversations with Kees VanFrankenhuyzen on this issue. There was available evidence to support them all and it made me wonder if they could all be right to some degree depending on protein and insect choice? The session on novel toxins featured a lot of work on parasporins. This group of proteins are closely related to the Cry proteins that appear to have a non-haemolytic anti cancer action. The future development of this area of Bt research should be truly fascinating. The session on public safety was short, but there was overwhelming evidence for the low environmental and human health concerns involved in using Bt and its derived formulations and technology. In relationship to this session were a number of posters. I was interested to note that two (Poletika and Storer & Ouakfaoui *et al.*) proposed measures involving risk assessment without trying to carry an actual assessment out, as we have recently completed with our Environmental Impact Quotient Assessment of cotton.



Evaluation of border cell number and Cry protein expression from root tips of *Gossypium hirsutum*

Oliver G.G. Knox & Gupta V.S.R. Vadakattu
CSIRO Entomology, Narrabri and Adelaide, Australia

Introduction

Border cells emerge from the periphery of the root cap and have a significant impact upon root development and the diversity of rhizosphere microbiota. We undertook a series of experiments to determine border cell number, levels of Cry protein expression for different cotton varieties and the relationship between border cell number and resistance to soil-borne fungal diseases of cotton.

Results

- Border cell numbers differed between tested cotton cultivars. A mean number of 5000 border cells per root tip was observed.
- Genetically modified cultivars and their elite and transgenic donor parents did not always have similar numbers of border cells (Fig. 1).
- A significant correlation was observed between border cell number and F rank, a measure of resistance to Fusarium wilt (Fig. 2).
- Measurable levels of Cry proteins were detected in different fractions of root tissue of GM-Bt cotton varieties (Table 1.).

Conclusion and discussion

This is the first report showing differences in border cell numbers between cotton varieties, including GM and non-GM. The potential implications of this observation for plant-microbe interactions and sustainable cotton production are not clear. The observed relationship between border cell number and diseases resistance suggests that variation in border cell numbers has involvement in some soil-borne disease incidence. Further evidence of this is required. The detection of Cry proteins in the mucilage and from border cells indicates that GM-Bt cotton crops have the potential to release Cry proteins into the environment by a number of means, including root exudation.

This work financed by the CSIRO divisions of Land and Water, Entomology, the Cotton Research and Development Corporation and the Cotton Catchment Communities CRC.

igure 1. The poster taken to the Pacific Rim conference. The poster is now on display in the ACRI Eastern corridor.

Additionally, the poster of Maureen O'Callaghan *et al.* and Melanie Douville *et al.* were of particular interest. Maureen's poster looked at detection of *Bacillus thuringiensis kurstaki* after applications of recommended, 100 times and 1000 times field strength. Her results showed that even at 1000 fold levels of application the bacteria could only be detected in the environment for a couple of weeks post application. Also of interest, although not on the poster, was she told me that free living bacterial feeding nematode populations significantly increased under this level of application, although the reasons and consequences of this were unclear. Melanie's poster also dealt with Bt persistence in the environment, but looking at the protein and not a producing organism. Although her work was with corn, it was interesting to discover that they have never been able to recover Bt proteins from the water or soil associated

with a Bt corn crop. They have now developed a DNA based assay to monitor the presence of the Bt producing genes, but this does not reflect protein levels.
Of the remainder of the sessions the presented paper by Illimar Altosaar, highlighting the potential to use Rice in place of bacterial fermentor, was not only immensely interesting, but his vision into downstream process applications was truly inspiring. My question to Illimar on the potential knock on effect of removing rice straw from a low input sustainable farming system, in which the straw is currently burned or ploughed in, resulted in a much lengthier discussion that evening. Finally, the session on applications in agriculture was of interest, mainly due to what little I know of the spray industry in cotton. To hear about the size of the areas being sprayed in forestry control programmes, the small volumes of formulation being used (1.5 l/ha), the high tech aircraft mounted weather instrumentation, and the transferral and application of the knowledge from 50 years of forestry research to farm fields in Asia was fascinating.

b) other highlights

A number of new contacts were made, some of which have requested copies of published material. A few contacts were made that have led to offers of assistance in sourcing previously published documents pertinent to some of the issues relevant to ongoing projects. Of the Bt work presented at the meeting I had the impression that work in which our group is engaged is ahead of the game and that the Australian cotton industries measures taken in securing the safe integration of GM technology to the farming system is second to none.

2. Detail the persons and institutions visited, giving full title, position details, location, duration of visit and purpose of visit to these people/places. (NB:- Please provide full names of institutions, not just acronyms.)

The purpose of attendance at the conference was to present a poster on the soil microbiology that has been carried out on the genetically modified cotton, expressing the Cry1Ac and Cry2Ab Bt genes. It was also hoped that the conference would prove a source of useful information, contacts and discussion on new and emerging techniques being developed for investigating Bt and environmental interactions.

Itinerary

1. Depart Narrabri 08:35 on 28/10/2005
2. Arrive Victoria, BC, Canada 21:50 on 28/10/2005 (via Sydney, Los Angeles and Vancouver)
3. 6th Pacific Rim conference starts 16:00 on 30/10/2005
4. Conference finishes 11:45 on 03/11/2005
5. Depart Victoria 12:00 on 5/11/2005
6. Return to Narrabri 19:10 on 7/11/2005 (via Vancouver, Los Angeles and Sydney)

3. a) Are there any potential areas worth following up as a result of the travel?

The paper on the work presented in the poster has been approved and submitted and should be available in print and on-line in April 2006.
Drs Vadakattu and Watson, CLW DEH report was forwarded to Maureen O'Callaghan, AgResearch, New Zealand, who in return has provided copies of some of her transgenic crop studies.
Publications on Bt protein and spore/crystal/protein mix efficacy have been sourced and are proving to be useful for the DEH project that the research group is undertaking.

Samples of the Valent BioSciences ELISA quick sticks have been trialled and found to be less efficient than the SDI ones currently sourced from Brett Ross at CSD.
Attendance at the 7t Pacific Rim conference in 2007 would be desired and provide an ideal opportunity to present the findings of the current project as well as the Environmental Impact Quotient assessment that has been undertaken.

b) Any relevance or possible impact on the Australian Cotton Industry?

The potential for Bt accumulation in the environment was addressed at this conference in a number of posters (as outlined above) and found to be largely unsubstantiated. Although the self mulching vertisols common to this area differ from those in the reported experiments, it will be reassuring if these reports are found to be similar for Bt derived from the Australian cotton crop.
The work of Drs Gunning and Moores on resistance in *H. armigera* to Cry1Ac **AND** Cry2Ab should be considered of potential concern to the industry if it can be substantiated. However, this was in direct conflict with the work that Dr Downes reported in which no cross resistance to both proteins has been found in any of her screened populations. It was interesting to hear, that after 50 years of use of Bt formulations, there is still much debate about the actual mode of action of Bt proteins.

4. How do you intend to share the knowledge you have gained with other people in the cotton industry?

Reports have been submitted to Entomology and will be forwarded to CRDC and the Cotton Catchment Community CRC once completed (this document). A summary will be made to the CSIRO staff at the January programme meeting.
All useful knowledge and collaborations will be integrated into the current projects involved in Bt monitoring and the outcomes of these projects will be extended to growers, consultants and other industry personal through talks and publications as per the norm.