

Program Two

Innovative Technologies



INTRODUCTION

Successful commercial cotton production relies on a number of chemical inputs. While progress has been made in adoption of transgenic plants, integrated pest management and best management practice, there remains an imperative to seek alternative management tools that minimise dependence on disruptive pesticides. This program reflects the need for innovative solutions to pest, weed and disease problems and the need for new tools to remediate or monitor environmental impacts. The program also includes fundamental work on the molecular genetics of cotton, which will aid in breeding for various characteristics, including pest and disease resistance and fibre quality.

There have been nine Cotton CRC funded projects and 15 CRDC projects addressing the aims and objectives of the Innovative Technologies Program. Five postgraduate students are associated with the CRC projects.

AIM AND OBJECTIVES

The aim of the program is to research and develop innovative technologies that provide an improved range of options for environmentally acceptable crop management and bioremediation.

Its objectives are to:

- rigorously evaluate the efficacy and environmental impacts of new transgenic plants
- develop and evaluate the use of attractants and repellents for *Helicoverpa* spp.
- identify and evaluate effective biocontrol agents for soilborne pathogens of cotton
- investigate the use of 'biofumigation' and 'systemic induced resistance' for improving the efficacy of disease control strategies
- develop more effective and user-friendly diagnostic kits for rapid detection of pests and diseases in plant tissues and in soil, and for pesticide residues and pest resistance
- investigate bioremediation techniques for pesticide contamination on cotton farms.

The use of new technologies emerging from this program will be developed in Program 3, in the context of sustainable farming systems, and flow into northern research projects in Program 1.

HIGHLIGHTS AND ACHIEVEMENTS

Evaluation and Management of Transgenic Cotton

This was the first season when combinations of the new Cry 2Ab gene were available. Results using a standard 5-day neonate bioassay demonstrated the high and consistent efficacy of Cry IAc/Cry 2Ab combinations and of Cry 2Ab alone.

Studies of INGARD varieties were continued at a number of locations. Field counts of larvae and laboratory bioassays confirmed previous findings of declining efficacy during crop development. The occurrence of differences in efficacy between tissues taken from different nodes on the plant was also confirmed.

Cotton tipworm, rough bollworm and cotton looper are susceptible to both Cry IAc and Cry 2A proteins. Tobacco looper had previously been shown to be relatively insensitive to Cry IAc. Further laboratory assays confirmed this observation and demonstrated that tobacco looper was highly susceptible to Cry 2Ab.

Previously experiments had shown that efficacy of Bt plants was influenced by temperature. In a second series of experiments Bt plants grown in a warm temperature regime (22 to 34°C) had 2 to 3 times higher efficacy than those grown in a cooler regime (14 to 26°C), confirming earlier results. Results from an experiment in which plants were exposed to changes in temperature regime were inconclusive with some cohorts showing increased efficacy and others decreased. Overall the work suggests the influence of temperature is complex.



Bruce Tuff (left) and assistant Lance Hayward, both from Busy Bee Narrabri, setting up laser moth monitoring equipment

Isolation of Genes Controlling Fibre Development in Cotton

The *Glabra 1* and *Glabra 2* genes from *Arabidopsis* encode transcription factors that are known to be involved in initiating single-celled hairs on stems and leaves. A *Glabra 2* homologue has been isolated from cotton. This gene is expressed in fibres throughout their development and also in other tissues including stems, leaves, roots and flowers. A gene construct that should specifically suppress the activity of the cotton *Glabra 2* gene has been made and introduced into cotton. The trans-

genic cotton plants will be assessed for changes in fibre development and fibre properties and the functional role of *Glabra 2* in cotton will be determined.

Semiochemical Approaches to Controlling *Helicoverpa* spp.

Two new, potentially more attractive blends of plant volatile chemicals have been identified using the olfactometer. These two blends performed better than the previously developed, standard, three-component blend. Techniques to study the behaviour of moths around lures have been developed and used to investigate potential close-range stimuli, which may be important in determining whether moths will contact or ingest a lure. Two types of ingestible formulations containing plant volatiles, a feeding stimulant and a toxicant have been developed and tested in field wind tunnels. A provisional patent application, "Attractants for moths", which covers the principles involved in formulating the most attractive blends, has been lodged with the Australian Patent Office. Expressions of interest in the project have been received from a number of semiochemical and insecticide companies.

Mating Behaviour in *Helicoverpa armigera*: Influence of Host Plants

A study of female response to host-plant volatiles revealed that the presence of host plants does affect female behaviour. Generally moths spend more time calling in the presence of host-plant volatiles. Two- and three-day-old females (the age at which females of this species normally mate) were found to produce more pheromone when in the presence of host plant volatiles.

However, field studies using captive females and light and pheromone traps found no evidence that the type of host crop influenced mating success. There was nothing to suggest that any particular crop should be planted as a refuge for its effects on mating behaviour. Other factors are viewed as more important in selecting refuge crops (e.g. number of moths produced) or selecting trap crops (e.g. attractiveness for oviposition).

On-farm Production and Dissemination of Baculovirus for Pest *Helicoverpa* Control in Cotton – A Feasibility Study

Recent studies have shown that large quantities of a naturally occurring nuclear polyhedrosis virus can be produced on farm by strategically applying a commercial viral insecticide to host *Helicoverpa* populations. This project is investigating the feasibility of applying a technique new to cotton that uses populations of beneficials to spread this virus into cotton for improved *Helicoverpa* control.

Although feeding studies showed that two species of earwig had good potential as virus disseminators (in favouring virus infected prey and being capable of consuming several infected larvae in rapid succession), the pattern of virus defecation revealed that the virus is expelled far too quickly for these insects to be at all effective as virus disseminators in the field.

Other predator species, when combined, may be capable of transporting enough virus to have an impact on pest *Helicoverpa* in cotton. Trials are being planned for next season to investigate this question to determine the merit in continuing this research into a third year.

Molecular Diagnosis of Fusarium Wilt of Cotton in Australia

A collection of Australian and overseas isolates of the pathogen which causes *Fusarium* wilt (*Fov*) has been established. This collection is being extensively characterised using a number of different molecular techniques.

DNA sequence information that is unique to the two Australian strains of *Fov* has been identified and primers that specifically amplify the Australian strains have been developed. The PCR reaction conditions have been optimised for each of the sets of primers. At present the PCR tests for each strain are performed in separate reactions but the intention is to combine the two sets of primers into a multiplex reaction that will detect both strains in a single reaction.

The current focus of the laboratory work is to optimise PCR amplification directly from infested soil, infected plant material and contaminated seed.

Molecular Marker Systems for Breeding Cotton Cultivars with Enhanced Resistance to Verticillium Wilt Disease

Two AFLP (Amplified Fragment Length Polymorphism) markers linked to a gene encoding resistance to *Verticillium* wilt in cotton were identified. These markers (marker 8 and marker 63) lie on either side of the *Verticillium* wilt resistance gene. The technique is being optimised to reduce the complexity and time taken to obtain PCR-amplifiable DNA and to achieve a higher success rate of PCR amplification. Cotton species and breeding lines with known responses to infection with *Verticillium* wilt will be tested to determine the effectiveness of these markers for phenotype prediction.

Forty-seven putative defence response genes that are up regulated during *Verticillium* wilt infection have been partially characterised. Full sequence characterisation has been completed on six PR (Pathogenesis Related) protein genes. The gene activity profiles of PR protein genes are being analysed to understand their role in defence response mechanisms operating in wilt-tolerant cotton plants. Genes selected as indicators of the defence response mechanism could be employed as 'RNA' markers to confirm the presence of the genes encoding enhanced wilt tolerance.

Development of Molecular Marker Technologies in Cotton

The aim of this project was to apply DNA marker techniques to cotton and to determine if they could be used to enhance traditional breeding programs. The study sought DNA markers linked to three model traits that were chosen because they were easily assessed and thought to be controlled by single genes. The traits were okra leaf, brown lint and bacterial blight resistance.

No markers were found linked to either the okra leaf or brown lint genes. This was due to the extremely low level of polymorphism detected between the two parents used in the mapping cross. The lack of polymorphism could be due to the low genetic diversity of cultivated cotton or to the variation within parental varieties that obscures the variation between the parental varieties. A wider (interspecific) mapping cross was used to search for DNA markers linked to the bacterial blight resistance gene. One novel DNA marker was detected linked to the blight resistance gene – but fairly distant from it. More closely linked DNA markers are being sought.

Bioremediation Enzyme for Endosulfan Sulphate

Endosulfan sulphate is the toxic, water-soluble, breakdown product of endosulfan that persists in soil and water for several months. It is a significant contaminant of downstream water and the major component of the endosulfan residues found in contaminated beef.

A bacterium that can degrade endosulfan sulphate to a non-toxic compound has been successfully isolated. This bacterium is now being investigated as a source of a gene-enzyme system capable of detoxifying endosulfan sulphate.

ASSOCIATED CRDC FUNDED PROJECTS THAT CONTRIBUTE TO THE PROGRAM 2 OBJECTIVES

Evaluation of Transgenics

CSE 74C: Efficacy of Bt cotton plants and causes of variation in performance - Dr Joanne Daly

Semiochemicals

UNE 33C: Studies of slow-release formulations for semiochemicals in cotton pest management - David Britton

Novel genes

ANU 6C: Testing the tomato I-2 gene for its ability to confer *Fusarium* resistance in cotton - David Jones

ANU 4C: Cloning genes to manipulate cotton fibre cellulose production for improved fibre traits - Joanne Burn

CSP 104C: Evaluation of disease tolerance of transgenic cotton lines containing genes for putative antifungal proteins - Dr Helen McFadden

CSE 82C: Characterisation of a potential new insecticidal transgene - Erica Crone

Biofumigation, Biocontrol and Induced Resistance

DAN 121C: Disease of Cotton (VI) – Dr David Nehl

DAN 122C: Black root rot and slow early season growth of cotton – Dr David Nehl

DAN 123C: Controlling cotton seedling diseases and vascular wilts with micro-organisms – Dr Subbu Putcha

UQ 29C: Biology, ecology and utilisation of the Damsel bug as a predator in cotton – Mark Wade

DAQ 95C: In field development of novel options for *Helicoverpa* control in Central Queensland – Paul Grundy

CSD PATH: Controlling *Fusarium* wilt of cotton – Dr Stephen Allen, Mr Greg McNamara

Bioremediation

US 39C: Remediation of pesticides on cotton farms – Angus Crossan

Diagnostic kits

CRDC 123C: Bt gene test kit

LINKAGES

Important linkages within and outside the Cotton CRC are listed below for the key projects in Program 2.

Evaluation and Management of Transgenic Cotton

Monsanto, Cotton Seed Distributors Ltd, CRC for Weed Management Systems (Professor Rick Roush), University of Melbourne (Dr David Heckel), Queensland Department of Primary Industries (Dr Richard Sequeira, Dr David Murray)

Semiochemical Approaches to Control *Helicoverpa* spp.

Shanghai Institute of Entomology (Professor Jia-Wei Du), University of Queensland (Dr Craig Hull), University of Hamburg (Dr F. Ibarra), Lund University (Professor Christer Lofstedt), IPM Technologies Inc, USA, Biocontrol Ltd, Performance Feeds Ltd

On-farm Production and Dissemination of Baculovirus for Pest *Helicoverpa* Control in Cotton – A Feasibility Study

CSIRO Narrabri, Martin Dillon

Molecular Diagnosis of *Fusarium* Wilt of Cotton in Australia

Queensland Department of Primary Industries (Dr Joe Kochman, Dr Natalie Moore), CRC for Tropical Plant Protection (Dr Suzy Bentley), Cotton Seed Distributors Ltd (Dr Stephen Allen), SARDI (Dr Kathy Ophel-Keller, Dr Alan Mackay)

Project Number: 2.1.1 AC

Molecular Marker Systems for Breeding Cotton Cultivars with Enhanced Resistance to Verticillium Wilt and other Diseases

CSIRO Plant Industry, Narrabri (Dr Greg Constable, Peter Reid), Cotton Seed Distributors Ltd (Dr Stephen Allen), University of Sydney (Dr Bruce Lyon)

Isolation of Genes Controlling Fibre Development in Cotton

Department of Genetics, Adelaide University (Dr Sharon Orford and Associate Professor Jeremy Timms)

Bioremediation Enzyme for Endosulfan Sulphate

Orica Ltd and HRDC (Dr Irene Horne), Melbourne University (Dr Helen Billman-Jacobe), University of Nebraska (Professor Anthony Zera)

Evaluation and Management of Transgenic Cotton

AIMS AND MILESTONES

The aims and milestones for this project were as follows:

- Commence field evaluation of new lines of Bt transformed varieties expressing Cry IAc and Cry 2Ab proteins using replicated small plots at several locations to quantify seasonal patterns of efficacy of field grown plants against *Helicoverpa* species.
- Quantify the concentration of Bt proteins, nitrogen, tannins and sugars in plant samples collected from different plant parts, stages of development and varieties expressing combinations of Cry IAc and Cry 2Ab to establish relationships to field patterns of efficacy.
- Conduct efficacy screening of transgenic cotton lines to provide breeders with additional information in the selection of lines for progression to improved varieties
- Complete studies of the efficacy of Cry 2Ab cottons against minor lepidopteran pests, specifically cotton tipworm, cotton leaf perforator, *Spodoptera* and loopers.
- Quantify the impact of Bt plants on growth and adult fitness of *Helicoverpa* spp.
- Test variations in efficacy in response to stress between Bt cotton varieties, constructs and promoters. Investigate the effect of stress factors individually on different Bt varieties. Factors to be tested include different temperature regimes and insect damage (Canberra).
- Complete a series of publications on the transgenic cotton research during the past 6 years.

STAFF

Dr G. P. Fitt, CSIRO Entomology, Narrabri
Ms C.L. Mares, CSIRO Entomology, Narrabri
Dr G. Baker, CSIRO Entomology, Canberra
Dr R. Mahon, CSIRO Entomology, Canberra
Ms K. Olsen, CSIRO Entomology, Canberra

PROGRESS

This was the first season when combinations of the new Cry 2Ab gene were available. Field experiments were completed with four varieties (Sicala 40, Sicala V3, Sicot 289, Siokra V16), expressing both Cry IAc and Cry 2Ab genes (previously referred to as Cry

X) from *Bacillus thuringiensis*. One variety, Sicala V2, was available with only Cry 2Ab, while a wide range of varieties expressing Cry IAc was included.

Laboratory bioassays demonstrated the high and consistent efficacy of Cry IAc/Cry 2Ab combinations and of Cry 2Ab alone (Figure 2). We have shown previously that such efficacy in a bioassay would result in no survival under field conditions. The small numbers of larvae that survived to five days never averaged 0.5 mg in weight compared to 1.25 to 2.0 mg for those on INGARD plants and 3.75 mg for those on control varieties. There was no significant difference in efficacy or larval weight between different varieties with the two Bt genes, in contrast to our previous experience with INGARD varieties.

We also continued studies of INGARD varieties at a number of locations. Field counts of larvae and laboratory bioassays confirmed previous findings of declining efficacy during the crop development (Figure 3). We have also confirmed differences in efficacy of tissues taken from different nodes on the plant. Efficacy increases down the plant, with leaves at node 8 having twice the efficacy of those from node 3.

Much effort is now directed to quantifying concentrations of Cry IAc in INGARD varieties using ELISA. An earlier technique has been further adapted to provide high and consistent protein extraction and determination of Bt using antibodies produced by Dr Danny Llewellyn (CSIRO Plant Industry). A number of samples from previous seasons have been assayed. These include tissues from a range INGARD varieties sampled in earlier experiments samples from different tissues within plants and samples from commercial crops. Bt concentration has been shown to correlate well with efficacy revealed from bioassays.

A number of new experiments have been conducted. One experiment took advantage of floods in the Namoi Valley in November 2000. A crop of INGARD cotton (Nucoatn

37), submerged by floodwater, was studied to identify any changes in efficacy or Bt levels. Figure 4 shows there was little difference in Bt concentration between plants which had been totally immersed in water for two days, those which had been flooded but not immersed (moderate) and those which had not been flooded at all (dry). Clearly we do not yet understand the influence of stresses on the expression of Bt transgenes. Bt levels in squares were about 50% of those present in leaves.

Research has shown that Cotton tipworm, rough bollworm (*Earias huegeli*) and cotton looper (*Anomis flava*) are susceptible to both Cry IAc and Cry 2A proteins. Tobacco looper (*Chrysodeixis argentifera*) had previously been shown to be relatively insensitive to Cry IAc. Further laboratory assays have confirmed this but demonstrated that tobacco looper is highly susceptible to Cry 2Ab. Further work is needed with *Spodoptera* spp. and *Earias* to confirm the range of activity of the Cry 2Ab protein.

Finally we have conducted a series of experiments in controlled environments seeking to understand the factors that lead to variable efficacy of Bt varieties in the field. All work involved use of a standardised leaf disk bioassay technique, which had been thoroughly validated previously.

Figure 2. Bioassay survival of neonate *H. armigera* larvae on node 4 leaves from control varieties and those expressing Cry IAc and Cry 2Ab genes, alone or in combination. Plant grown at PBI, Narrabri, 2000-2001.

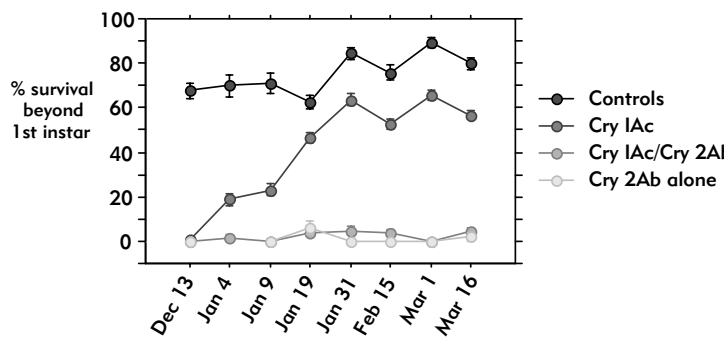


Figure 3. Seasonal bioassay efficacy of control varieties and all commercial INGARD varieties (Field 18, ACRI, 2000-2001)

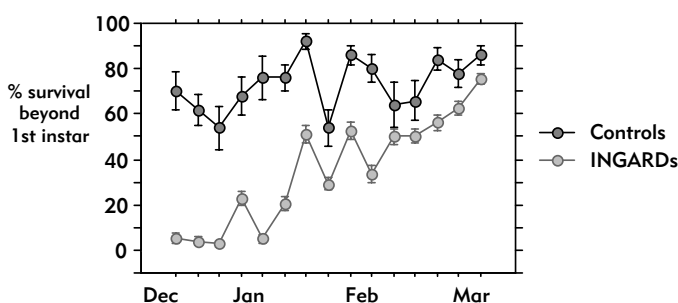
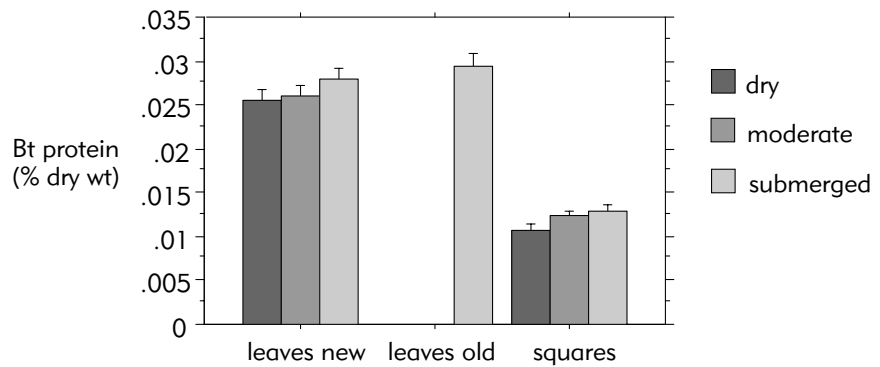


Figure 4. Concentration of Bt protein (expressed as % dry wt) in tissues of INGARD plants exposed to varying levels of inundation (November 2000). "Dry" plants were not affected by floodwater, "moderate" plants sat in floodwater for a period, but were not covered, while "submerged" plants were almost totally immersed in floodwater for at least 2 days.



The research has further demonstrated an effect of temperature on the efficacy of Bt cotton. Plants grown in a warm temperature regime (22 to 34°C) had 2 to 3 times higher efficacy than those grown in a cooler regime (14 to 26°C), confirming earlier results. An experiment in which plants were exposed to changes in temperature regime produced inconclusive results with some cohorts showing increased efficacy and others decreased. Overall the work suggests the influence of temperature is complex.

Other experiments have examined the effect of insect damage on the efficacy of Bt cotton at pre-squaring and flowering growth stages. Laboratory bioassays of plants previously damaged by *Helicoverpa* larvae showed a threefold increase in efficacy of four Bt varieties compared to controls. Similar increases in efficacy following feeding damage were also seen in fruiting plants. Such changes have yet to be confirmed in field grown plants.

Conventional cotton plants also responded to insect damage. Larvae reared on undamaged leaves for seven days were 4 to 20 times heavier than those reared on damaged leaves, and mortality was also lower. Thus the response of Bt varieties following damage is likely a combination of changes to the expression of Bt (or presentation of the toxin) and changes in secondary plant compounds.

**Project Number:
3.3.1**

Isolation of Genes Controlling Fibre Development in Cotton

AIMS AND MILESTONES

The aims and milestones of this project are to:

- Augment our knowledge of cotton fibre biology at the molecular level by isolating and characterising genes that have a regulatory role in the initiation and development of cotton fibres. The cotton homologues of two *Arabidopsis* genes, *Glabra 1* and *Glabra 2*, are initial targets. Other genes encoding transcription factors will also be identified.
- Assess the role of these genes by developing transgenic plants that have reduced levels of expression of the gene products.

STAFF

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Dr Danny Llewellyn, CSIRO Plant Industry, Canberra

Dr Liz Dennis, CSIRO Plant Industry, Canberra

PROGRESS

This project has tried to isolate genes from cotton that are involved in controlling production of cotton fibre with the long term aim that they might be used to engineer the quality or yield of fibres in transgenic plants. We have initially targeted two types of regulatory or master control genes similar to genes known in other species to control leaf and root hairs which are similar structures to the seed hairs of cotton that have developed into cotton fibres. One type of gene is represented by *Glabra 1*, which is a MYB type regulatory gene found in *Arabidopsis*. This gene is critical for leaf hair development.

We have isolated and characterised a set of cotton genes encoding MYB class transcription factors that are expressed in cotton fibres. These genes were isolated from a cDNA library constructed from cotton ovules collected at the early stages of fibre initiation. Ten of the MYB genes are previously unknown cotton genes but they have similarity to MYB genes isolated from other plants, most of unknown function. A cotton *Glabra 1* homologue was not amongst those isolated from cotton, despite extensive attempts. It may be that *Glabra 1* is specific to the brassica family, as we were able to use similar approaches to isolate *Glabra 1* homologue from two other brassicas, canola and the brassica weed, hoary cress. All our attempts to isolate a *Glabra 1* equivalent from other plants were unsuccessful, however, one gene has sequence features in common with *Arabidopsis Glabra 1* and *Arabidopsis GA MYB* thought to have a function in the gibberellin hormone controlled growth of leaf hairs. The *GA MYB* gene has been shown in barley and *Arabidopsis*, to be a regulator of genes controlled by the hormone gibberellin. Gibberellin is a necessary hormone in cotton fibre initiation and elongation. Therefore, the cotton *Glabra 1 GA 'hybrid'* gene is a candidate for further analysis. Direct functional analysis of the gene, will be assessed by generating transgenic cotton plants with reduced levels of the gene product, and this work will be ongoing.

The second type of regulatory gene, cloned in collaboration with University of Adelaide researcher, Dr Sharon Orford, was the cotton *Glabra 2* gene. This gene controls later stages of leaf hair growth in *Arabidopsis* and might also have a role in cotton fibre growth. A *Glabra 2* genomic clone was isolated at CSIRO and a construct, which suppresses the level of the *Glabra 2* gene product has been engineered and introduced into cotton. Mature transgenic plants harbouring the construct will be assessed for changes in fibre development and fibre properties to confirm the function of this gene in fibre development.

An increased understanding of the role the *Glabra 2* gene and MYB genes have in fibre biology will assist in the development of transgenic cotton with altered fibre traits.

Project Number: 3.2.5

Development of Molecular Marker Techniques in Cotton

AIMS AND MILESTONES

The aims and milestones of this project are to:

- apply molecular marker techniques to cotton
- determine the level of polymorphism within *Gossypium hirsutum* cultivars and between *G. hirsutum* and *G. barbadense* cultivars
- search for markers linked to the okra leaf, brown lint, and bacterial blight resistance genes
- use previously mapped markers to identify chromosomal locations of the okra leaf and bacterial blight resistance genes
- assess feasibility of application of molecular marker techniques to cotton breeding programs.

STAFF

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Dr Danny Llewellyn, CSIRO Plant Industry, Canberra

Dr Liz Dennis, CSIRO Plant Industry, Canberra

Dr Bruce Lyon, University of Sydney, Sydney

PROGRESS

Molecular marker (or DNA marker) techniques allow the genes controlling agronomically important traits to be mapped. This involves detecting DNA markers that are linked to or "flag" the location of a particular gene. Once these genes are mapped, the DNA markers can be used to infer the presence or absence of a particular gene without the need for testing for the trait controlled by that gene. This can speed up the breeding process, as agronomic traits do not have to be measured directly, but can be identified at the seedling stage. For example, if a DNA marker was found that flags a disease resistance gene, the presence of that gene could be detected as soon as DNA can be extracted from a plant, and without exposing the plant to the causal pathogen.

The aim of this project was to apply DNA marker techniques to cotton and to determine if they could be used to enhance the traditional breeding programs. The success of a marker project depends on the ability to detect differences (or polymorphisms) at the DNA level between the two parents of a cross. The more polymorphism between the two parents, the more likely it is to detect a DNA marker linked to a particular gene. In this study, DNA markers linked to three model traits were sought. The traits were okra leaf, brown lint and bacterial blight resistance. These traits were chosen as they were easily scored, and were thought to be controlled by single genes.

No markers were found linked to either the okra leaf or the brown lint gene. This was due to the extremely low level of polymorphism detected between the two parents used in the mapping cross. The lack of polymorphism between the two parents could be due to the low genetic diversity of cultivated cotton, but could also be attributed to the variation found *within* parental varieties, which obscures the variation *between* the parental varieties. A wider mapping cross was used to search for DNA markers linked to the bacterial blight resistance gene. The level of polymorphism detected between the two parental varieties was correspondingly higher. One novel DNA marker was detected linked to the bacterial blight resistance gene. This marker was fairly distant from the bacterial blight resistance gene, but the screening of more DNA markers may identify a marker closer to the resistance gene.

DNA markers can be of use to enhance cotton breeding programs, but the traits targeted for DNA marker assisted selection will have to be carefully chosen, and the construction of mapping populations controlled to maximise the chance of detecting DNA markers linked to the targeted trait.

**Project
Number: 2.2.1 AC**

**On-Farm Production and Dissemination of Baculovirus for
Pest *Helicoverpa* Control in Cotton – a Feasibility Study**

AIM AND MILESTONES

The aim of this project is to contribute to the integrated management of pest *Helicoverpa* species in cotton through research into the feasibility of a novel technique for the on-farm production and dissemination of viral insecticides.

Year 1 milestones are to conduct:

1. Laboratory studies to assess the feasibility of managing populations of arthropod predators as mobile disseminators of NPV in cotton.
2. Field trials to assess late season pigeon pea trap crops for use as on-farm *Helicoverpa* baculovirus production systems.

STAFF

Dr Andy Richards, Research Scientist, CSIRO Entomology, Canberra

Ms Janelle Scown, Technical Officer CSIRO Entomology, Canberra

PROGRESS

This project is investigating the feasibility of applying a technique new to cotton that uses late season trap crops designed to concentrate populations of pests to magnify production of a naturally occurring viral insecticide and then employ populations of beneficial insects to spread this virus into cotton for improved management of *Helicoverpa*.

Field and laboratory experiments were used to:

- assess candidate species for use as virus disseminators
- evaluate late season pigeon pea trap crops for virus production.

For the first of these two milestones, we evaluated two species of earwig that are abundant in cotton, highly mobile and fairly resilient to chemical spray drift to determine their food preference and feeding rate, and the pattern of virus defecation from their gut.

Although feeding studies showed that these species had good potential as virus disseminators (they favour virus infected prey and are capable of consuming several infected larvae in rapid succession), the pattern of virus defecation revealed that virus is expelled far too quickly after ingestion for these insects to be at all effective as disseminator virus to other parts of a farm. By themselves, other predator species in cotton such as ladybird beetles, spiders, red and blue beetles, sucking bugs and lacewings, are unlikely to have the necessary attributes of abundance, mobility and foraging behaviours for virus dissemination but it is possible that combined these species may be capable of transporting enough virus to have an impact on pest *Helicoverpa* in cotton. Trials are being planned for next season to investigate this question to determine the merit of continuing this research into a third year.

**Project Number:
2.2.13 AC**

Mating Behaviour in *Helicoverpa armigera*: Influence of Host Plants

AIMS

The aims of this project are to:

- determine whether mating success in *Helicoverpa armigera* is influenced by the surrounding host plants
- determine the underlying mechanisms involved if host plants are found to influence mating success
- train a postgraduate student as a future research entomologist.

STAFF

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Olivia Kvedaras, Postgraduate student, University of New England

Dr Chris Moore, Principal Chemist, Queensland Department of Primary Industries

Dr Alice Del Socorro, Research Officer, University of New England

Mr D. Alter, Professional Officer, University of New England

PROGRESS

It is well documented in the literature that reproductive success in animals depends on maturation rate, fecundity and mating success. Larval nutrition affects maturation rate and fecundity, and adult nutrition (nectar) affects longevity and fecundity. In *H. armigera* it is not known if host plants affect mating behaviour and therefore mating success.

Mating is energetically expensive and risky, so investment in mating could be related to how close suitable larval hosts are. Previous work has shown increased pheromone production in the presence of host plants (McNeil 1989, Raina 1992) in other species, but does this apply to a highly polyphagous, mobile species like *H. armigera*, and does increased pheromone production mean increased mating success?

The aims of this study were to examine the effects of host plants and their volatiles on maturation rate, mating behaviour and mating success in *H. armigera*. These results will help us understand the ecology of *H. armigera* in multi-crop environments, and how different crops can be used in management (e.g. refuges, trap cropping). We conducted laboratory experiments to determine whether host plant volatiles affected sexual maturation of females, female calling behaviour, female pheromone production and male responses to pheromones. We then looked at field experiments to determine whether mating success was dependent on the host crop surrounding the moths.

The results of female response to host plant volatiles revealed that the presence of host plants does affect female behaviour. Generally, moths spend more time calling in the presence of host plant volatiles. Two- and three-day-old females were found to produce more pheromone when in the presence of host plant volatiles. This is the age at which females of this species normally mate.

Laboratory studies of male behaviour in the presence of host plants in a wind tunnel indicated that there are few effects of host plants on male response to female sex pheromones. We also looked at the effect of two compounds on male response to female sex pheromone in the field. Previous studies have suggested that male response to female sex pheromone is increased by Z-3 hexenyl acetate (*H. zea*) and phenylacetaldehyde (*S. frugiperda*). We found for *H. armigera* that there is evidence that plant volatiles detract from responses to pheromones in the field not enhance them.

Finally we looked at the mating success of female *H. armigera* in the presence and absence of host plants in the field. Host plants included cotton, sunflower, sorghum, soybeans and maize, as well as fallow land. Virgin wing-clipped females were placed in mating trays in three or four crops each night. Male and female abundance was measured by light and pheromone traps in each crop. Females were not more likely to be mated in any one crop than another, despite differences in abundance between crops.

**Project Number:
2.2.3 AC**

The implications for management suggest host plants do not greatly affect the mating behaviour and mating success of *H. armigera*. Therefore, other factors are viewed as more important in selecting refuges (e.g. number of moths produced) or trap crops (e.g. attractiveness for oviposition).

Semiochemical Approaches to Control of *Helicoverpa* Spp.

AIMS AND MILESTONES

The aims of this project are to:

- develop improved attractants based on plant volatile chemicals, for adult *Helicoverpa* spp., especially females
- conduct large-scale field trials of attract-and-kill techniques using female attractants or pheromones or both.

Milestones for Year 1 are to:

1. Develop improved female attractants from lab and field trials.
2. Characterise moth responses around lures using night vision and wind tunnel studies.
3. Determine best formulations to use in large-scale field trials, including insecticides.
4. Conduct negotiations with commercial companies and file patent claim(s) if warranted.

STAFF

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Associate Professor Peter Gregg, University of New England

Mr Richard Tennant, Laboratory Assistant, University of New England

Mr Dan Alter, Senior Professional Officer, University of New England

Dr Chris Moore, Principal Chemist, Queensland Department of Primary Industries, Brisbane

Professor Jia-wei Du, Shanghai Institute of Entomology, China

PROGRESS

This project continues the work of an earlier one in the CRC for Sustainable Cotton Production. It aims to develop attractants based on plant volatile chemicals for adult *Helicoverpa* spp., especially females. We have screened a total of 40 plants, thirty six single chemicals and 28 chemical blends in an olfactometer, which tests their attractiveness to *H. armigera* females. We have also conducted a total of twenty-one field trapping trials testing 32 types of attractant lures.

Of the blends tested in the olfactometer, 20 were found to be very attractive. We investigated the effects of systematically adding complexity to chemical blends, either within groups of leaf volatiles or floral volatiles, or by combining the two. Two of these blends were highly attractive in the olfactometer.

We analysed the hairpencils of male moths to identify chemicals in male pheromones that might modify close-range attractiveness of female moths to our volatile blends. Initial field trials using blends combined with the fatty acids and their alcohol equivalents found in the hairpencils did not yield significant increase in trap catches. An electric grid trap was designed to improve trap catches in the field. This type of trap caught about five times more than the AgriSense® canister traps using pheromone lures. We have not yet tested it using plant volatile blends.



We designed an innovative field wind tunnel and used it in a total of 17 trials involving twenty seven wind tunnel/nights to test various types of formulations, and to characterise moth responses around lures using night-vision glasses. On nights when the weather was suitable, many moths were observed to approach the lure. Characteristic plume-following and hovering flight behaviour indicated they were responding to the volatiles. Many moths made repeated approaches to the lures, hovered then backed off. Behavioural observations suggest that there are specific close-range stimuli (chemical or visual) that are missing from the way we present lures in field wind tunnels and probably traps. Work on analysis of substances in male hairpencils and role of visual cues (e.g. artificial flowers) will be continued.

Feeding experiments under laboratory and field conditions were conducted to determine if moths would ingest a formulation containing the volatiles, a feeding stimulant and a toxicant, and to see if ingestion of the formulation would kill moths. A dye, Brilliant Green, was added so that moths could be dissected to determine whether they had ingested the material. Two types of formulations were used: water/oil mixtures and 'sloppy' Sirene[®]. Insecticides used were either 1% carbaryl or 1% methomyl. Small droplets (50-100µL) of the formulations were placed in a 1L container with the moths. Kill rates with the formulation in 1% carbaryl were between 69 and 94%. With 1% methomyl the kill rate was 100%. 'Sloppy' Sirene[®] formulations did not separate and retained liquidity overnight, making them more suitable than the water/oil mixtures that have previously been used for such products.

A provisional patent application, 'Attractants for moths', (Patent No. PR4797) was lodged with the Australian Patent Office in May 2001. Expressions of interest in supporting this work have been received from a number of semiochemical and pesticide companies.

Project Number: 2.2.4 AC

Bioremediation Enzyme for Endosulfan Sulfate

Milestones for this project are:

- Year 1 Isolation of endosulfan sulfate degrading microorganism.
- Year 2 Characterisation of endosulfan sulfate degrading enzyme.
- Year 3 Cloning and expression of the gene encoding the endosulfan sulfate degrading enzyme and field evaluation of this enzyme.

STAFF

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Ms Kahli Weir, Technical Officer, CSIRO Entomology, Canberra

Dr Anthony Zera, University of Nebraska

PROGRESS

Endosulfan is a cheap and efficacious insecticide for the cotton industry but its ongoing availability is threatened by off-site residue problems. Applying endosulfan gives rise to three toxic residues. These are the alpha and beta isomers of the applied endosulfan, and endosulfan sulfate, which is a toxic breakdown product of alpha-endosulfan in particular. The sulfate is both more water soluble and more persistent in the environment than the parent endosulfan compound. It is produced in contaminated soil, and then washed off in irrigation or rainwater. It can survive in the soil/water for several months and so there is an ongoing threat of contaminated water leaving the farm. Of the three compounds, endosulfan sulfate is the major long-term contaminant of downstream water (albeit the parent isomers predominate in the first few days after application) and it is the major component of the residues found in contaminated beef.

**Project Number:
2.2.5 AC**

The aim of this project is to isolate an enzyme capable of single step detoxification of endosulfan sulfate and to evaluate its efficacy in cleaning up contaminated run-off water. The goal of this project for 2000-2001 was to isolate a bacterium capable of degrading endosulfate to a non-toxic compound. This goal was achieved and the isolated bacterium is now being investigated as a source of a gene enzyme system capable of detoxifying endosulfan sulfate.

Molecular Diagnosis of *Fusarium* Wilt of Cotton in Australia

AIMS AND MILESTONES

Fusarium wilt of cotton, (caused by *Fusarium oxysporum* f.sp. *vasinfectum*) (*Fov*), has emerged as a major threat to cotton production since it was first recorded in Australia in 1993. We are proposing to develop a DNA-based diagnostic test for *Fusarium* wilt that will allow more rapid and accurate disease diagnosis and better disease management. This diagnostic test will enable the rapid detection and identification of *Fov* from infected plants, infested soil, and cottonseed. It would also be a useful research tool to monitor the distribution and spread of *Fusarium* wilt throughout the cotton production areas of Australia, and to evaluate the effectiveness of different agronomic practices (e.g. crop rotation) on the survival of *Fov* in affected fields.

The milestones for 2000-2001 were to:

1. Establish a more extensive collection of isolates of *Fov* from overseas, especially from the USA (as the most likely source of introduction), Africa and Sudan.
2. Compare Australian genotypes of *Fov* with overseas VCG and races, using various molecular methods, e.g. DNA fingerprinting, sequencing the IGS region of the rDNA and sequencing other genes such as β -tubulin.
3. Characterise the different genotypes distinguished by DNA fingerprinting analysis using vegetative compatibility group (VCG) analysis.
4. Establish the genetic relatedness of *Fov* to other *formae speciales* of *F. oxysporum*.
5. Evaluate the specificity of the available primers by screening them against other *Fov*, fusaria, fungi, microbes and plants.
6. Identify other DNA fragments unique to the two Australian genotypes of *Fov* (if necessary).
7. Develop/refine suitable methods for extracting DNA from infected plants, infected seed and infested soil.
8. Determine the sensitivity of the PCR-based test for detecting *Fov* in infected plant tissue, infected seed and infested soil.

STAFF

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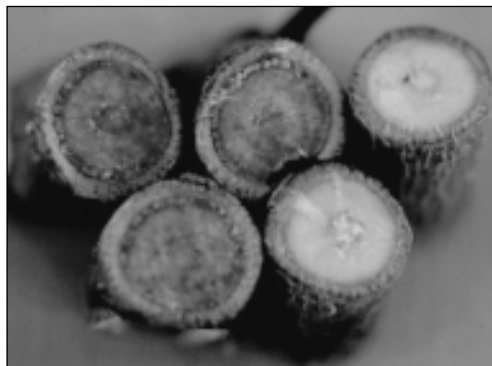
Dr Stephen Allen, Senior Plant Pathologist, Cotton Seed Distributors, Narrabri

PROGRESS

A collection of Australian and overseas isolates of *Fov* that represents all the different races and vegetative compatibility groups (VCGs) that occur has been established, including isolates originating from different geographical regions. This collection of



Fusarium wilt can devastate cotton crops.



isolates of *Fov* is being extensively characterised using a number of different molecular techniques, including DNA amplification fingerprinting (DAF) analysis, restriction enzyme (RE) haplotyping analysis of the intergenic spacer (IGS) region of the ribosomal DNA (rDNA), DNA sequencing of the IGS region of the rDNA, and sequencing of other genes such as the mitochondrial small sub-unit (mtSSU) and α -elongation factor (α -EF) genes. The Australian isolates of *Fov* are being compared to overseas isolates of *Fov*, other *formae speciales* of *F. oxysporum*, non-pathogenic isolates of *F. oxysporum*, and other species of *Fusarium*, to determine the genetic relatedness amongst these groups.

The isolates of *Fov* have been characterised by DAF analysis with ten different arbitrary primers, and the genetic relationships amongst the Australian isolates of *Fov*, and the other groups examined have been determined. The IGS haplotyping analysis using five different restriction enzymes is in progress. The IGS sequencing analysis of selected isolates is mostly complete. DNA sequencing of the mtSSU and α -EF genes has not started yet.

DNA sequence information that is unique to the Australian pathotypes of *Fov* has been identified, and primers that specifically amplify the Australian strains of *Fov* developed. Two sets of PCR primers have been developed that specifically amplify DNA from each of the Australian VCGs 01111 and 01112. Database searches of DNA sequence information published in Genbank have indicated that there are no matches for these primers with any other organism, and laboratory screening of the specificity of these primers is nearly complete. So far, the primers have amplified only DNA from Australian isolates of *Fov* in VCGs 01111 and 01112 and not DNA from the other strains of *Fov*, other *formae speciales* of *F. oxysporum*, other species of fusaria, other fungi and bacteria that have been tested.

The PCR reaction conditions have been optimised for each of the sets of primers (in particular the annealing temperature which affects reaction specificity). Under these optimal conditions the PCR test is working very efficiently with very strong amplification of the specific PCR products being achieved. At present, the PCR tests for each VCG are performed in separate reactions, but we are aiming to combine the two sets of primers into a multiplex reaction that will detect both VCGs in a single reaction. Preliminary results indicate that the multiplex PCR test is able to differentiate Australian VCGs 01111 and 01112 of *Fov* from overseas isolates of *Fov*, and other *formae speciales* of *F. oxysporum*.

The current focus of the laboratory work is to optimise PCR amplification directly from infested soil, infected plant material and contaminated seed. This diagnostic test is being developed in collaboration with C-Quantec Diagnostics and relies on soil DNA extraction methodologies developed by Drs Kathy Ophel-Keller and Alan Mackay from SARDI (whereas the methods for DNA extraction from plant and seed will be developed in our laboratory). Preliminary experiments have shown that the PCR diagnostic test is able to detect *Fov* from soil DNA extracts, extracted using the system developed at SARDI. More thorough testing of soil extracts is currently underway, as are experiments to optimise DNA extraction and PCR amplification directly from infected plant material and contaminated seed.