Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

# REPORTS

## *Part 1 - Summary Details*

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

**CRDC Project Number:** **CSE84C**

**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:** Insect pest resistance and the role of induced responses to damage in Australian cottons

**Project Commencement Date:** 1/7/99 **Project Completion Date:** 30/6/2003

**Research Program:** 3 Crop Protection

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## *Part 3.3 – Final Reports*

### 1. Outline the background to the project.

*Host Plant Resistance of Cotton genotypes*. Australian cotton is attacked by a diversity of insect pests. While transgenic Bt cottons will increasingly provide control of *Helicoverpa*, conventional host plant resistance remains a valuable component for tolerance to other pests and to “support” the transgenic technology by enhancing background levels of resistance. This proposal sought to continue research on host plant resistance from the previous project (CSE59C Evaluation of Pest Resistance in Australian Cottons) through: (i) continued field and laboratory studies of a range of cotton genotypes; (ii) increased emphasis on sucking pest resistance, particularly aphids and mirids; and (iii) research on the biochemical changes in cotton associated with induced defences, compensation ability and the efficacy of transgenic cottons.

Previous research has provided valuable information on the relative tolerance of cotton germplasm to a range of pests. Genotypes studied have included many combinations of biochemical and morphological traits. This work provided an opportunity for evaluating the performance of a range of cotton germplasm including the current commercial varieties under unsprayed conditions at several sites. Our research on host plant resistance has been progressively integrated with our research on the evaluation of Bt transgenic material, with the same sites and similar techniques being employed. Based on progress in CSE59C Dr. Greg Constable has produced a range of breeding lines with combinations of HPR characters (high gossypol, superokra leaf, nectariless, glabrous and frego) and Bt genes. These lines are in the early stages of evaluation and required further selection and assessment through this project to determine whether any useful synergies are apparent between the HPR characters and the Bt transgenes.

Our main focus to date has been to identify useful plant traits which impart resistance to *Helicoverpa*. It is likely that Bt and other transgenes will provide the main source of improvement in *Helicoverpa* tolerance in the future. Likewise with some of the sucking pests there are prospects of useful transgenes but these are not yet close to field evaluation. In CSE59C we demonstrated considerable value in some morphological characters for aphid and whitefly resistance (Fitt et al 1996). With the emergence of pesticide resistance problems in aphids we believe there is value in continuing to pursue sources of natural resistance to these pests. We proposed to use techniques established by Dr. Lewis Wilson to induce outbreaks of aphids and so evaluate the resistance of diverse germplasm against high and predictable levels of aphids, in contrast to the ad hoc approach we have followed previously. At the same time we proposed to maintain a watching brief on whitefly populations in our replicated sites and continue to collect data on their reaction to different genotypes, including new lines just available for assessment (eg. Gohar 87). Elsewhere mirid resistance has been associated with exceptionally hirsute cottons, with increased levels of terpenoids and with the nectariless trait (Benedict et al 1983). Because hairy cottons are not commercially acceptable in Australia we have focussed on the nectariless trait for mirid tolerance and have some nectariless/ Bt combinations ready for evaluation against mirids. Our assessments drew on information on the oviposition behaviour and development of green mirids in cotton.

*Efficacy of Transgenic Cottons*. As part of the Cotton CRC funded project “Evaluation and Management of Transgenic Cottons” we (i) evaluated the efficacy of different Bt genes and their interaction with varietal background, (ii) quantified variation in efficacy across commercial Bt crops and sought to correlate a range of agronomic factors with that variation, (iii) experimentally assessed the impact of some agronomic factors (sowing date, irrigation treatments) on efficacy. Work on the efficacy of new genes and interactions with variety and environment continued with some support from this grant.

*Biochemistry of Cotton Resistance to Pests***.** A final component of the project was to measure the induced biochemical changes in cotton plants following damage caused by active insect feeding or by artificial means and to assess the suitability of damaged plants for subsequent feeding by *Helicoverpa*. In many plants damage from herbivores or infection by pathogens results in an induced response which leads to increased levels of defensive chemicals at the site of damage and elsewhere in the plant (Karban and Baldwin 1997). This is a natural mechanism which allows plants to avoid some of the metabolic costs of constitutive defences (present all the time, eg. gossypol), by rapidly inducing the defensive compounds only when they are challenged by a disease or herbivore. In cotton the phenomenon of induced defences is well known in resistance to fungal and bacterial pathogens, and to mites. When attacked, the damaged tissues induce the production of a mobile chemical signal which activates the defence systems throughout the plant. In cotton, Dr. Gary Felton and co-workers (UC Davis and University of Arkansas) have documented changes in oxidative (eg. peroxidase (POD) and polyphenol oxidase) and antinutritive compounds in cotton tissues after feeding damage by *Helicoverpa zea* (Bi et al 1997a). Salicylic acid has been identified as the signal in the signal-transduction pathway leading to these biochemical changes (Bi et al 1997b). Recently Hoover et al (1998) have shown that these biochemical changes in the plants reduce the efficacy of baculoviruses applied against *Helicoverpa* larvae. Plants with higher POD activity after damage had lower efficacy of virus against *H. virescens* larvae. This result indicates that efficacy of virus, and perhaps Bt sprays, is only predictable if the chemical composition of the plant is specified and has implications for the predictable use of these tools in IPM.

All the US work has involved a couple of varieties of cotton (DP50 and Acala SJ2). Work described in Sadras and Fitt (1997) shows significant variation in the recovery capacity of a range of cotton genotypes which correlated with our estimates of pest resistance of those genotypes in the field. The results suggested that genetic variation in recovery capacity (a measure of compensatory ability) could be an important component in variation in resistance to insect pests. This relationship could have potential for developing new robust varieties for future IPM systems where plants will be required to suffer and tolerate increased levels of damage from some pests. Being able to select genotypes which show rapid and strong defensive responses will be an advantage. We proposed to explore the level of genotypic variation in induced biochemical changes which may be subjected to selection by breeding. In this work we would characterise the induced responses of current commercial varieties, breeding lines and a broad range of other germplasm initially in glasshouse experiments. The same germplasm would be evaluated in unsprayed/ sprayed comparisons in the field as well. So far as we are aware there have been no such comparisons of cotton genotypes elsewhere in the world. Most of the analytical equipment for this work is available at ACRI or in CSIRO Canberra. We highlight at this point that such preliminary work could form the basis for a full proposal to involve a phytochemist at ACRI to research this and other issues.

We also proposed to collaborate with Dr. Andy Richards (CSIRO) and Dr. Jonathon Holloway (NSW Agriculture) to investigate the impact of induced defences on the efficacy of *Helicoverpa* viral sprays, however both scientists left during the course of the project and this area of work never commenced.

References

Bi J.L, Murphy J.B., Felton G.W. (1997a) Antinutritive and oxidative compounds as mechanisms of induced resistance in cotton to *Helicoverpa zea*. J.Chem.Ecol. 23:97-117.

Bi J.L, Murphy J.B., Felton G.W. (1997b) Does salicylic acid act as a signal to cotton for induced resistance to *Helicoverpa zea*. J. Chem. Ecol. 23: 1805-1818.

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Hoover, K., Stout, M.J., Alanz, S.A., Hammock B.D., Duffey S.S. (1998) Influence of induced plant defences in cotton and tomato on the efficacy of baculoviruses on noctuid larvae. J. Chem Ecol. 24: 253-271.

Karban, R. and Baldwin I.T. (1997). Induced Responses to Herbivory (Interspecific Interactions). University of Chicago Press.

Sadras, V.O. and Fitt G.P. (1997) Resistance to herbivory in cotton lines: quantification of recovery capacity after damage. Field Crops Research 52: 127-134.

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

Original aims of the project are listed below:

(i) To continue evaluation of the insect resistance of a range of cotton genotypes, including lines derived from crosses of promising HPR varieties with high yielding commercial varieties, including combinations with transgenic Bt lines

(ii) To evaluate levels of aphid and mirid resistance in a broad range of genotypes under conditions where high densities of these pests will be induced

(iii) To quantify biochemical changes in cotton genotypes associated with efficacy of transgenic cotton and the compensatory ability of different genotypes

(iv) To identify the potential for induced defensive response to be used in selective breeding or to interfere with the efficacy of IPM tools such as virus sprays.

In each year we addressed specific objectives relevant to these aims.

Aim (1) was largely achieved with some progress made on the large scale evaluation of mirid tolerant breeding lines and much progress with evaluation of various transgenic cottons expressing Bt proteins.

Over the course of the project the significance of Aim (ii) – HPR for sucking pests was diminished as it became clear that more specialised techniques were required and such work was proposed to be pursued by Dr. Lewis Wilson and Dr Geoff Baker.

At the same time the relative importance of work on the efficacy of transgenic Bt cottons (Aim iii) increased. Funds provided in CSE84C complemented those provided in the Cotton CRC project 2.1.1 and some of the objectives are common.

Aim (iv) was delayed in commencement due to problems with methodology, delays in a visit by Dr. Gary Feldman (an expert in the field of induced plant responses) – in fact he never visited ACRI. Nonetheless considerable progress has been made with some germplasm to assess biochemical changes after artificial and real damage from *Helicoverpa.* We believe this area of research warrants ongoing attention.

## 3. Detail the methodology and justify the methodology used.

*Conventional Host Plant Resistance – Field Studies*

We used well established protocols for field experiments involving unsprayed and sprayed treatments which allow damage by different categories of insects to be defined. Experimental designs were randomised complete blocks with 4 replications. Plot sizes for most work were 15 metres x 4 or 8 rows. Field plots were managed with standard agronomic inputs, irrigated as required and harvested using CSIRO's single row plot picker. Subsamples of all entries were taken for gin outturn and quality assessment.

Insect sampling was conducted weekly using a range of techniques. Visual samples of whole metres of row were used routinely for *Helicoverpa* spp, and most other pests and beneficial species. Suction sampling and beat sheets have also been used on occasions for specific purposes.

*Biochemical Assays.*

Well defined protocols are available for assays of the many primary and secondary compounds analysed in different plant samples. All the expertise and equipment required was available at ACRI. Plant samples were collected direct from field grown plants and snap frozen in liquid nitrogen before being roughly ground and then freeze dried. They were then milled to a fine powder and stored frozen for later analysis. Analytical techniques included:

Nitrogen – Kjeldahl; Sugars – spectrophotometric assay; Condensed and Soluble Tannins - spectrophotometric assay; Bt proteins – Quantitative ELISA; Protease inhibitors – techniques still being developed.

*Induced responses to insect damage.*

A repeatable protocol for damage and bioassay was finalised during the project. This allowed us to characterise the capacity of plants to show induced responses to damage. This was first developed with a couple of varieties (MHR 11 and Siokra V15) where responses in the damaged tissues have been shown. The protocol involves either artificial or real (Helicoverpa feeding) damage to plants with samples at certain times after damage for use in bioassays or biochemical tests.

*Field Evaluation of Transgenic Cottons*. Our research on conventional host plant resistance has been progressively integrated with research on the evaluation of Bt transgenic material, with the same sites and similar techniques being employed.

We developed and validated a simple bioassay for studies of Bt cotton efficacy which has been used throughout the project, together with Elisa techniques used to quantify Bt protein content of different plant tissues. These techniques, combined with field counts of *Helicoverpa* life stages will be used to assess field efficacy.

A considerable amount of time has been devoted to finalising the quantitative ELISA technique to measure Bt concentrations. This is based on work done by Dr. Helen Holt, but with several enhancements made by Cheryl Mares. The Elisa differs from the commercially available EnviroLOGIX kit in that it used a highly efficient extraction procedure to remove proteins from the plant substrate, quantifies total protein and Bt protein and allows Bt concentration to be expressed as % total protein or % dry weight. The technique is applicable to freeze dried samples and likely to be more reliable than the commercial kit (Holt, Mares and Akhurst 2002).

An example of the range of germplasm included in the research is given in the following table which shows entries for studies in 2002/03 at PBI, Narrabri.

| Variety | Conventional | CryIAc only | CryIAc + RR | Cry 2Ab only | CryIAc + Cry 2Ab |
|---|---|---|---|---|---|
| Sicala V2 | X | | | X | |
| Sicala 40 | X | X | | | X |
| Sicala 80 | X | | | | X |
| Sicot 189 | X | | | | |
| Siokra V16 | X | X | X | | X |
| Siokra V17 | X | | | | X |
| Sicot 289 | | X | | | X |
| Siokra S221 | | X | | | |
| Sicala V3 | | X | X | | |
| | | | | | |
| Opal | X | | | | |
| NuOpal | | X | | | |
| NuPearl | | X | | | |
| NuTopaz | | X | | | |

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

*Field Evaluation of Conventional and Transgenic Lines*.

Over the course of the project unsprayed and sprayed field experiments were established at ACRI, the University of Sydney Plant Breeding Institute, Narrabri (PBI), Biloela Research Station, Queensland, CSD Wee Waa and a commercial property at Byee.

Each year between 50 and 100 genotypes were included with most present at all sites and with sprayed and unsprayed treatments. Genotypes included all current commercial lines, a number of standards (e.g. glandless DP16) which have been used for some time and a range of other lines with traits which are expected to impact aphids and mites in particular. Field trials included some new breeding lines which combined conventional traits (high gossypol, high tannin) with the Cry IAc gene.

At Biloela, CSD and Byee we conducted the first large scale evaluation during 2001/02 of a new, potentially mirid tolerant germplasm from CSIRO breeders. Dr. Greg Constable developed a series of breeding lines of Siokra V16 which had combinations of the Cry IAc and the nectariless trait. Field trials involved Siokra V16 (conventional), Siokra V16i (Ingard) and Siokra V16iN (Ingard, nectariless). We used these lines in order to identify any influence of the Bt and nectariless traits on the abundance of *Helicoverpa*, mirids and various predator species. A Poster outlining the results was presented at the 2002 Australian Cotton Conference.

Mirids were relatively abundant at all sites, but particularly so at Byee, where high numbers were present through January and February during flowering and boll filling. There was no obvious influence of the nectariless trait on mirid numbers at any site. Nectariless and Bt genes also had little impact on predator densities. Yields from each site clearly showed the benefit of the CryIAc Bt gene in the unsprayed situation, but at only one site was there a significant interaction of the Bt and nectariless characters. This was at Byee where there was a significant yield increase in the Bt/nectariless combination, compared to Bt alone. While it was encouraging that the nectariless trait appeared to give a yield effect at the site where mirids were most abundant (Byee) further evaluation is needed to confirm any consistent advantage. No further evaluations of this material was possible due to lack of water at Qld sites.

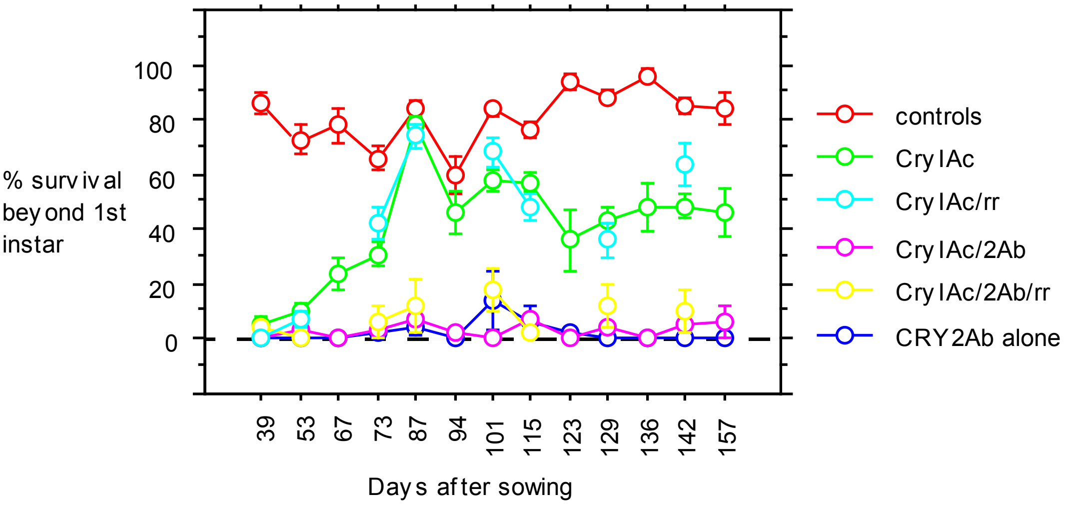
*Efficacy and biochemistry of Transgenic Bt cottons*

One aspect of our work has been to evaluate the field efficacy of a number of transgenic varieties expressing the CryIAc and Cry2Ab genes from *Bacillus thuringiensis*. One variety, Sicala V2, was also available with only Cry 2Ab, while a wide range of varieties expressing Cry IAc was included.

Results using laboratory bioassay of node 4 leaves have consistently shown a seasonal decline in efficacy of the CryIAc varieties but high and consistent efficacy of the Cry IAc/Cry2Ab combinations (Bollgard II) and of Cry 2Ab alone (Figure 1). The combination of the Roundup Ready gene with either of the Cry genes had no influence on efficacy. The two-gene combination showed bioassay survival of 10% or less throughout the growing season. We have shown previously that such efficacy would result in no survival under field conditions.

These results from Sicala V2 expressing only Cry 2Ab are significant in that they suggest that most of the performance of Bollgard II plants relies on that protein. While from an efficacy point of view the two gene plants offer considerable advantages in further reducing pesticide use, there will be a need for ongoing vigilance in resistance management, as Bollgard II clearly does not provide the season-long two gene strategy which would provide maximal protection against resistance. Nonetheless the two gene plants have considerably less resistance risk than INGARD which relies on one gene (CryIAc).

Figure 1. Bioassay survival of neonate *H. armigera* larvae on node 4 leaves from control varieties and those expressing Cry IAc and Cry 2Ab genes (both with and without Roundup Ready (rr) gene). Plants grown at PBI, Narrabri, 2001/2002.




ELISA results for Cry proteins in Bollgard II varieties show the concentration of Cry2Ab (Average 2500 ug/g dry wt) to be almost 100 times higher than Cry 1Ac (35 ug/g dry wt). Cry 2Ab is also present at higher levels in squares than in leaves, opposite to the pattern of Cry IAc which is less concentrated in fruiting structures.

We have now completed ELISA assays of samples from a number of experiments. Using an efficient protein extraction procedure and ELISA we have shown:

- average concentrations of Cry IAc in the 4th leaf from the terminal were about 30ug/g. This is some three times higher than comparable figures from US studies
- considerable variation between 15 varieties (10 expressing CryIAc (2 also with RR) and 5 expressing Cry IAc and Cry 2Ab) ranging from 15 ug/g to 60 ug/g
- seasonal pattern of Cry IAc expression also varied among varieties. In several there was a distinct decline in CryIAc level during the growing season, whilst in others there was not
- variation in CryIAc concentration among plant structures. In most varieties leaves from node 2 and 3 expressed twice the concentration of Cry IAc as leaves at lower nodes or in squares.

We also completed assays on squares of differing sizes and for different components of squares. Squares collected at the same time from three INGARD varieties (Sicot 289I, NuPearl and Sicala V3i) were allocated to five size categories from pinhead (size 1) to candlewick (size 5). A node 4 leaf was also taken from the same plants. Analysis showed that small squares (size 1 and 2) had about the same level of Bt as node 4 leaves, but Bt content declined consistently in larger squares, with size 5 squares having only 40-50% of the Bt concentration in leaves. Squares from one variety were dissected into anthers, bracts, petals, style, calyx and ovary. The ovary had twice the Bt content of the other components and of the whole square.

*Combinations of Bt genes with Conventional traits*

One objective of the HPR project has been to assess the potential for synergies between Bt genes and conventional HPR traits such as high gossypol and high tannin. Progeny from two crosses involving HG660 (high gossypol) and a Bt line were produced by CSIRO breeders for evaluation. For high tannin combinations, a series of breeding lines were produced from crosses involving Ht35 germplasm. In both cases we sought to identify any synergistic interactions between these classes of

secondary compounds and the Cry protein. The high tannin/ Bt combination was of particular interest because of previous results which suggested that tannin in plant tissues may interfere with the activity of Bt proteins and so reduce efficacy of Bt cottons.
Our results with high gossypol/ Bt combinations have shown no consistent synergistic effect of the two traits although high gossypol consistently reduced growth rates of Helicoverpa larvae.

More extensive field trials were completed with 14 high tannin/ Bt lines over three seasons. Biochemical analyses of these lines show a seasonal decline of about 45% in CryIAc concentration (0.0024% in mid Dec to 0.0014% in mid Feb) and a 4-5 fold increase in soluble tannin content (2.5% in mid December to 10% in mid Feb). There was a weak, negative relationship between Bt protein and tannin levels. While no causal relationship could be derived here, Karen Olsen (CSIRO Entomology, Canberra) has previously shown experimentally that tannin is implicated in modifying the activity of Bt protein in INGARD plants. Our tannin analyses to date have measured only soluble tannins. We have now refined a method (in collaboration with Dr. Greg Tanner and Dr. Phil Larkin, CSIRO PI, Canberra) to also measure bound (or condensed) tannins. We have completed assays for insoluble (bound) tannins and have shown they increase to very high levels during plant development reaching 70-80mg/g after flowering and then plateauing (Figure 2). We have shown a stronger negative relationship between bound tannins and Cry IAc concentration (Figure 3).

Figure 2. Seasonal change in content of tannins (soluble + insoluble tannins) in a range of transgenic and conventional cotton varieties.

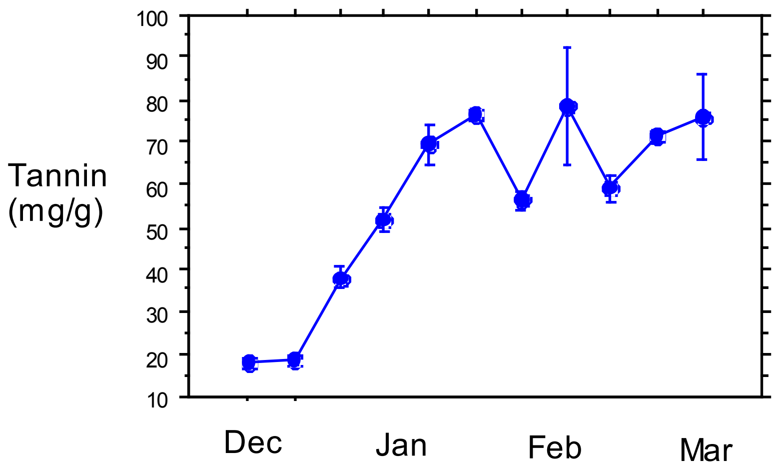
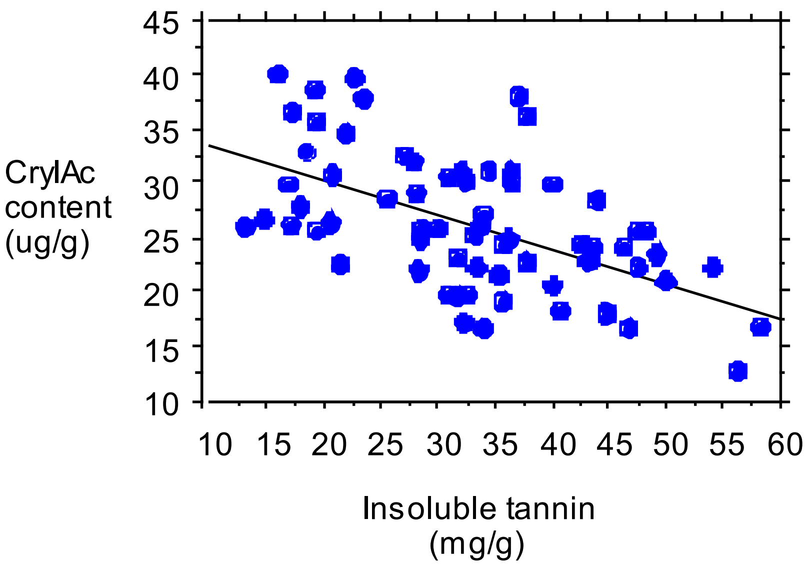


Figure 3. Relationship between content of soluable tannins and CryIAc protein across a suite of cotton breeding lines. Samples involve collections over the growing season.




*Effects of flooding on Bt concentrations.*

One experiment took advantage of floods in the Namoi valley in November 2000. A crop of INGARD cotton (Nucotn 37) which was 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 (submerged), those which had been flooded but not immersed (moderate) and those which had not been flooded at all (dry). All three groups of plants showed declining Bt content over the few weeks after the flood until late December when a foliar fertilizer was applied and Bt levels increased sharply. Tissue analyses showed increases in several nutrients at the same time. 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. This data set also showed a clear relationship between nitrogen content of tissue and Bt levels (Figure 5). A Poster outlining the results was presented at the 2002 Australian Cotton Conference.

Figure 4. Concentration of Bt protein (expressed as % dry wt) in tissues of INGARD plants exposed to varying levels of inundation (November 2000).

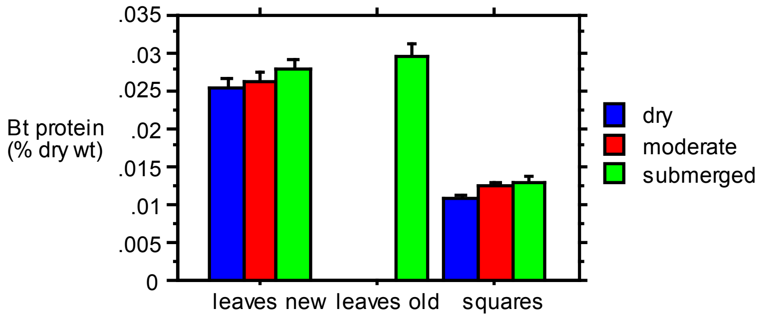
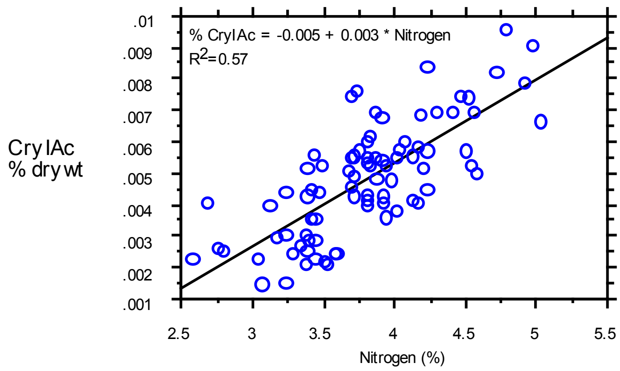


Figure 5. Relationship between nitrogen content and Cry IAc level for all samples collected from INGARD plants exposed to varying levels of inundation.




*Induced Responses to Insect Damage in Cotton Genotypes.*

Several pilot experiments were required to finalise a protocol to examine induced biochemical responses to artificial and real damage for cotton plants. Those studies involved both conventional and transgenic lines. While our focus had been to apply damage treatments and then seek responses in undamaged tissues of the same plants we have seen little consistent response. We have however seen strong and consistent changes in the damaged leaves themselves. In two varieties (MHR11 and Siokra V15) there were dramatic reductions in growth rates of neonate larvae on leaf positions 4 and 5 (from the top of the plant) following moderate damage to those leaves. Larval weight after 5 days was 20 to 60% lower following artificial or real damage on both varieties. Reductions were largest on leaf 5 and smallest on leaf 3.

These protocols were used to examine the response of a wider range of germplasm in experiments completed during June 2002 where real and artificial damage treatments were applied to 12 varieties (Siokra V16, Siokra V16i, Sicala V2, Siokra 1-4, DP16 Glandless, Reba, HT35, HG660, MHR11, DP90, Empire Red and N74 OGF. This was the suite of varieties used previously (Sadras, V.O. and Fitt G.P. (1997) Resistance to herbivory in cotton lines: quantification of recovery capacity after damage. Field Crops Research 52: 127-134). The data showed responses in the form of reduced growth rates of larvae on damaged parts of most varieties, particularly in response to real damage by other feeding larvae, but only on the leaves actually damaged. There was no induced response in the undamaged leaves on damaged plants. There was little effect on survival of any damage treatments. The effect of real damage was most pronounced on glandless cotton and hardly detectable on some lines such as the high tannin HT35. As yet we have been unable to complete biochemical analyses of protease inhibitors or oxidative enzymes in plant samples from the experiments to correlate with observed effects on larvae.

This area of research warrants further effort to understand the nature of changes in response to damage and its potential value in breeding programs.

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

To date we have:

1. Identified natural plant characteristics (morphological and biochemical) which impart tolerance to *Helicoverpa*, whitefly and aphids
2. Characterised the impact of high terpenoid and high tannin germplasm on *Helicoverpa* growth and survival
3. Demonstrated the level of insect tolerance of a wide variety of germplasm (including all commercial varieties) under unsprayed conditions in the field
4. Conducted assessments of combinations of Bt genes with high tannin and high terpenoid lines and assessed the interaction between tannin, Bt protein levels and efficacy

5. Conducted field studies of new breeding lines incorporating HPR characters and commenced field studies of mirid tolerant lines.

A clear take-home message is that the cotton plant itself and it capacity to express varying levels of insect tolerance and to respond to insect pests needs to be an ongoing focus of IPM efforts. The plant provides the template for IPM systems to be constructed upon. Continuing focus of breeders on conventional traits for insect tolerance will add value to the quantum advances possible with transgenic approaches and could provide added stability for future pest management strategies.

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

IPM systems and transgenic Bt cottons have provided considerable economic and environmental benefits to the cotton industry through dramatic reductions in pesticide inputs over the last 5 years (Fitt 2003, 2004; CRDC 2003). The work completed in this project and the complimentary Cotton CRC funded project have supported those changes through improved understanding of the performance of transgenic cottons. Commercial outcomes from our work with conventional HPR will occur over a longer timeframe and be reflected in improved varieties available to Australian growers and ongoing reductions in pesticide dependence if these varieties are used as a component of IPM.

## 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.)

Enhancement of the quantitative ELISA for Bt proteins is a considerable advance supporting ongoing research with Bt cottons.

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

We have refined protocols for studies of induced responses of cotton to damage and identified some useful germplasm for tolerance of *Helicoverpa*.

### c) are changes to the Intellectual Property register required?

No. Most IP in new germplasm remains with CSIRO Plant Industry.

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

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

This project has developed a number of approaches and data sets which provide a platform for future work which will be pursued in a new project (CRC49C - Enhancing the insect tolerance of Australian cottons through conventional and transgenic traits).

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

The outcomes of this work will be progressively written up as scientific papers. Future varieties will be the main mechanisms for outcomes to be disseminated to industry.

### (c) for future research.

There is an ongoing need for research with new transgenics and to evaluate the potential for synergistic interactions conventional and transgenic traits. Ongoing effort with sucking pest tolerant cottons will require reliable techniques to manipulate field populations or to conduct realistic glasshouse infestations.

## 9. List the publications arising from the research project and/or a publication plan.


Fitt, G.P. (2000) An Australian approach to IPM in cotton: integrating new technologies to minimise insecticide dependence. Crop Protection 19: 793-800

Fitt, G.P. (2000) IPM with two-gene cotton. Proceedings Australian Cotton Conference 2000, ACGRA Wee Waa, pp. 175-184.

Fitt G.P., C.L. Mares and G.C. Constable (2002). Enhancing Host Plant Resistance of Australian Cotton Varieties. Australian Cotton Grower Jan/Feb 2002; pp 20-24.

Fitt, G.P. and L.J. Wilson (2002). Non-Target Effects of Bt-Cotton: A Case Study From Australia. In "Biotechnology of *Bacillus thuringiensis* and Its Environmental Impact: Proceedings of the 4th Pacific Rim Conference", Akhurst, R.J., Beard, C.E., Hughes, P.A. (eds). CSIRO, Canberra. Pp. 175-182.

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**10. 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.**

Integrated pest management (IPM) is the cornerstone of sustainable production systems for Australian cotton. Resilient cotton varieties with resistance to key insect pests, whether through conventional means or with transgenics, is the foundation for building sound IPM approaches. While transgenic cottons, like Bollgard II, can provide quantum changes in pest tolerance, conventional HPR is acknowledged to provide only incremental gains. Nonetheless, coordinated use of both approaches can provide progress in dealing with the key pests and some secondary pests as well.

A continued focus on sucking pests will be required as Bollgard II expands into the industry and progressively reduces the immediate threat posed by *Helicoverpa* spp.

## *Part 4 – Final Report Executive Summary*

The cotton plant is highly resilient to insect damage and capable of both tolerating and defending itself against insect pests of various types. While transgenic Bt cottons increasingly provide a valuable management option for *Helicoverpa* spp., conventional host plant resistance (HPR) remains a valuable component for tolerance to other pests and to "support" transgenic technology by enhancing background levels of resistance. Our research on host plant resistance has been progressively integrated with research on the evaluation of Bt transgenic material. Through collaboration with Dr. Greg Constable we now have a range of breeding lines with combinations of HPR characters with Bt genes. Plant breeding is clearly a reliable vehicle for advances in IPM.

This project has built on previous work on host plant resistance where the value of certain morphological and biochemical traits have been identified for key groups of pests, particularly *Helicoverpa* and various sucking pests. In this project we have:

1. finalised field trials of arrange of cotton germplasm and identified some potential combinations of value
2. completed the first field evaluation of a potentially mirid tolerant variety with okra leaf and nectariless traits plus the Cry IAc Bt gene. Where mirids were abundant the nectariless trait showed promise, but further field research is needed.
3. Research potential synergies between Bt genes and conventional traits of high gossypol and high tannin. While we found no interaction of gossypol with Bt, there were consistent negative relationships between tannin and Bt proteins confirming earlier work by Olsen and Daly
4. Documented season-long efficacy of single gene and two gene combinations of Cry IAc and Cry 2Ab. This shown consistent high efficacy of the two gene combination, although this relies heavily on Cry 2Ab.
5. Substantially improved upon the quantitiative ELISA for Bt proteins and applied this to identify patterns in protein expression among varietal backgrounds, stages of development and agronomic treatments.
6. Developed experimental protocols to quantify induced changes in cotton biochemistry in response to *Helicoverpa* feeding and completed the first series of experiments which demonstrated clear induced responses within damaged tissues, but no systemic effect whereby damage to one tissue induces a defensive response in other parts of the plant.

IPM systems and transgenic Bt cottons have provided considerable economic and environmental benefits to the cotton industry through dramatic reductions in pesticide inputs over the last 5 years. The work completed in this project and the complimentary Cotton CRC funded project, have supported those changes through improved understanding of the performance of transgenic cottons. Commercial outcomes from our work with conventional HPR will occur over a longer timeframe and be reflected in improved varieties available to Australian growers and ongoing reductions in pesticide dependence if these varieties are used as a component of IPM.

A clear take-home message form this project is that the cotton plant itself and it capacity to express varying levels of insect tolerance and to respond to insect pests needs to be an ongoing focus of IPM efforts. Ongoing focus of breeders on conventional traits for insect tolerance will add value to the quantum advances possible with transgenic approaches and could provide added stability for future pest management strategies.