Cotton ***Research and Development Corporation***

Project Title	:	Second Canberra Bt Meeting
Project Number	:	CSE35C
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A final report prepared for the Cotton Research and Development Corporation

SUMMARY

Bacillus thuringiensis (Bt) is the only technology currently available for tackling the resistance and environmental problems confronting the cotton industry. It is imperative that the industry has a strong knowledge base from which it can make its judgements about Bt usage to avoid the problems of resistance and inappropriate treatments.

The Second Canberra *Bacillus thuringiensis* Meeting was held in the Manning Clark lecture theatre complex at the Australian National University from 21st to 23rd September 1993. It was attended by 120 registrants, including 29 from 10 other countries in Asia, Europe, New Zealand and North America. The registrants were drawn from research institutions, universities, commercial interests, regulatory bodies and R&D Corporations.

The Meeting was well supported by R&D Corporations (CRDC, RIRDC), AIDAB, The British Council and 11 Bt production and distribution companies. CRDC funds were used to bring Dr F. Gould, North Carolina State University, Raleigh to participate in the conference as one of the key speakers. Dr Gould's research focusses on the interactions between Bt and *Heliothis virescens*; it encompasses behavioural, genetic and resistance management studies.

The Second Canberra *Bacillus thuringiensis* Meeting was convened to build on the successes of the 1991 Bt Workshop in Canberra and of the 1992 Bt Management Workshop funded by CRDC in identifying the important factors in Bt usage. The opening address by Professor B.A. Federici, University of California Riverside, provided a global view of knowledge of *Bacillus thuringiensis* (Bt) and its various uses and opportunities for expansion of those uses. The 19 invited papers, 13 contributed papers and 13 posters explored details of the mode of action of Bt toxins, resistance to Bt toxins, novel toxins, production and application technologies, use of Bt genes in transgenic organisms, and conventional spray usage of Bt. Bt in transgenic plants, the social and environmental impact of Bt, and regulation of Bt products were the subject of three 2 hour forums.

The presentations and discussions at the Second Canberra *Bacillus thuringiensis* Meeting strongly supported the view that Bt is a major resource for the control of many economically significant pests. Moreover, new developments are expanding the opportunities and efficacy of Bt formulations and transgenic organisms expressing Bt toxin genes. Several opportunities for the Australian cotton industry were suggested, together with some warnings.

Transgenic cotton expressing Bt provides some significant advantages for controlling heliothis. However, the use of the transgenic cottons

needs to be implemented carefully with due consideration being given to the potential for heliothis species to develop high levels of resistance. It is probable that, like the CryIA(b)-cotton tested in the Australian 1992/93 season, the CryIA(c)-cotton suffers a decline in the availability Bt toxin in older plants. Depending on the severity of this decline, the transgenic plants will facilitate the onset of resistance or at least increase the resistance level because of the ability of resistant individuals to survive on old transgenic cotton. It is clearly important that some means of measuring the availability of toxin in cotton at all growth stages and under the full range of growing conditions be obtained.

The very high levels of resistance (perhaps 15000-fold) detected in *H. virescens* are obviously a matter of concern. It is clearly important to proceed on the understanding that resistance to Bt will develop in *H. armigera* and to take all reasonable steps to minimise that development. This involves continued support for CRDC projects investigating strategies for deploying transgenic plants, using conventional Bt sprays, and developing the sensitive tests for monitoring resistance.

The opportunities identified from the Meeting arise from the discovery of new Bt toxins and the potential for synergism between toxins and between toxins and other molecules. It is imperative that an alternative to the CryIA(c) toxin be found so that the industry will have the means with which to manage resistance when it develops. One approach is to determine the efficacy of these novel toxins for Bt and the potential for synergistic interactions with Bt toxins. The use of non-Bt control strategies (e.g. viruses) is another approach.

ABSTRACT

The Second Canberra *Bacillus thuringiensis* Meeting was held in the Manning Clark lecture theatre complex at the Australian National University from 21st to 23rd September 1993. The Meeting was organised by the CSIRO Division of Entomology with financial support from R&D Corporations (CRDC, RIRDC), AIDAB, The British Council and 11 Bt production and distribution companies. It was attended by 120 registrants, including 29 from 10 other countries in Asia, Europe, New Zealand and North America. The registrants were drawn from research institutions, universities, commercial interests, regulatory bodies, and R&D Corporations.

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Introduction

Bt is the only technology currently available for tackling the resistance and environmental problems confronting the cotton industry. It is imperative that the industry has a strong knowledge base from which it can make its judgements about Bt usage to avoid the problems of resistance and inappropriate treatments.

Although Bt is a highly efficacious insecticidal agent, it is complex and must be utilised correctly. Bt is actually a complex of many serotypes of the bacterium *Bacillus thuringiensis* producing a multiplicity of toxins. The target specificity (e.g. for Lepidoptera or Coleoptera or Diptera) varies between toxin classes (Cry I - VI, cyt, exotoxin). There is also considerable variation in efficacy for any one insect species within toxin classes, with CryIA(c) being ten times as toxic for *H. armigera* as CryIA(b). Non-toxin synergists are essential for either expression or activity of some of these toxins. The efficacy of the Bt toxins may be severely reduced by environmental factors such as UV radiation, alkaline pH and even the insect's diet. Consequently the appropriate toxin or mix of toxins and synergists for the target insect and the factors that affect their efficacy must be understood if the full benefit of Bt is to be realised.

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Objectives

The Second Canberra Bt Meeting was convened to build on the successes of the 1991 Bt Workshop in Canberra and of the 1992 Bt Management Workshop funded by CRDC in identifying the important factors in Bt usage. The understanding of Bt and its toxins is expanding rapidly and constantly presenting new opportunities and revealing new problems. The rapid rate at which developments are

occurring and the very slow rate of publication of those developments mean that we have to create opportunities for discussion with the leading international figures in research and development of Bt. The Second Canberra Bt Meeting created an excellent opportunity.

It was not feasible for Dr Gould to visit the Cotton Research Unit at Myall Vale as planned because of his commitments and the meeting of the Cotton Bt Working Group in Canberra. However, arrangements were made for Dr Gould to meet with Dr Fitt and Dr Forrester in Canberra.

Results and Discussion

The opening address by Professor B.A. Federici, University of California Riverside, provided a global view of knowledge of *Bacillus thuringiensis* (Bt) and its various uses and opportunities for expansion of those uses. The 19 invited papers, 13 contributed papers and 13 posters explored details of the mode of action of Bt toxins, resistance to Bt toxins, novel toxins, production and application technologies, use of Bt genes in transgenic organisms, and conventional spray usage of Bt. Bt in transgenic plants, the social and environmental impact of Bt, and regulation of Bt products were the subject of three 2 hour forums.

In his paper "Insect Resistance to Bt toxins: Can it be delayed" Dr Gould presented data supporting the view that resistance to toxins is inevitable in heliothis if Bt usage is high. He also reported that the level of resistance to Bt toxins by *Heliothis virescens* is not only 1000-fold but may be of the order of 15000-fold. In subsequent discussions he revealed that although resistant neonates died when placed on young transgenic cotton expressing the CryIA(c) toxin, they survived when placed on older transgenic cotton that killed susceptibles.

The mode of action of Bt toxins and the mechanisms of resistance were examined. These papers suggested that the mechanisms of resistance postulated for diamondback moth and indian meal moth may not be the only mechanisms operating in these pests. These papers not also suggested some alternative mechanisms for resistance but also possibilities for enhancing the effectiveness of Bt toxins through synergism.

The synergistic interactions between different cry toxins and between Cry toxins and other components of Bt (e.g. spores, cell membrane proteins) were discussed by Professor Federici. Synergism has been best demonstrated with the dipteran-active CryIV toxins and CytA. However, there is growing evidence that the efficacy of Bt toxins for Lepidoptera can be enhanced by synergism with other molecules.

Dr Chilcott presented data on the efficacy of some of the newer Cry toxins for a range of insects, though not *H. armigera* or *H. punctigera*. His data showed significant differences between *H. virescens* and *H. zea* in absolute values of LC_{50} and relative efficacy of various Cry toxins.

Several contributors discussed ways of improving the efficacy of Bt applications. In some cases this is achieved by transgenic technologies, with Bt genes being expressed in plants (e.g. cotton, eucalypts, clover) or bacterial (non-Bt) hosts to deliver the Bt-toxin to the feeding zone of the pests and/or to provide protection from UV and alkaline degradation. An alternative approach has been to create novel combinations of Bt toxins in a Bt host by conjugal transfer of plasmids. This method has been utilised to create a Bt that is active for both Lepidoptera and Coleoptera.

The use of Bt in Australia as conventional formulations was also discussed in several presentations. These contributors examined current usage and examined some possible strategies for Bt usage for controlling pests in cotton, vegetables, and forests, on sheep and for mosquito control in waterways. The integration of low dosage Bt usage with natural enemies was shown to have potential for achieving effective control of some pests.

The spray technology required to deliver Bt formulations was analysed in two papers and a poster. The physical properties of the formulation were shown to be very important in ensuring that suitable coverage and minimal splash loss are obtained.

Discussion in the forum on Evaluation of Transgenic Plants focussed on four issues: changes in the content of Bt toxin in the plants throughout the season, resistance management strategies, stability of transgenic expression, and Regulation. There was general concern that more information was required on changes in Bt toxin content throughout the growing season and under different growth conditions. Since there is difficulty in assessing Bt content in older plants by ELISA, some better technique should be developed.

The nature and size of refugia required for effective resistance management were discussed but there is currently too little data available for firm conclusions to be drawn. The New Zealand policy of restricting the genes available for insertion into transgenic plants to those not available in commercial formulations was discussed. It was generally agreed that this was a very good strategy but more information on mode of action of the various toxin types and their interactions was required before the scheme could be effective.

Genetic instability in plants was a significant issue in the discussion of transgenic perennial such as eucalypts. There was concern that the

controls on sterility and/or toxin production could be lost during the lifetime of the plant leading to undesirable consequences such as spread of Bt toxin genes to the natural population and failure of the high dosage resistance management strategy.

It was generally agreed that there are significant social and environmental issues to be worked through and that it is in the interest of all parties to work together and provide better access to data. The risk that insects that are currently secondary pests (e.g. mirids) might become more serious pests when spraying was reduced with the implementation of transgenic plants was discussed. The risk was recognised but the general consensus was that it was not a good reason for preventing the testing of transgenic plants, or other novel strategies, as an alternative to current practices

The forum on Environmental and Social Issues raised a variety of issues. Concern was expressed that only two seed companies will supply the Australian cotton market and it was suggested that this was a matter of imposing a solution. This argument was countered with a statement that the companies are aware of the industry in a holistic sense and that the Bt strategy is to be incorporated into a broader approach to pest control. Mr Bob Phelps (GenEthics) was asked to explain the position of the Green Movement on Transgenic crops. He suggested that the Greens would like broader community input into decision-making on transgenic organisms. However, this was seen to be undermined by Mr Phelps' earlier recounting of desertification in Brazil arising from the irresponsible application of technology. This led to a discussion of the use of high technology fixes being applied when low technology was equally effective. Serious doubts on the wisdom of insertion of Bt genes into perennial crops, especially eucalypts. The Bt eucalypt project was justified in part on the basis that it provides basic data. It was stressed that an important component of this project was to ensure sterility in the transgenic eucalypts so that the genes would not be transferred native forest trees. One point of concern in this regard was genetic instability in plants that could lead to a loss of the sterility factor or a decline in Bt toxin production to a level where it promoted resistance.

The forum on registration of Bt microbial products in Australia centred on the mechanisms and costs of regulatory assessment. Mr John Sanders (Novo Nordisk) cautioned against making safety and efficacy testing too stringent because it would make it difficult for small Australian producers and, given the relatively small size of the Australian market, make it unattractive for overseas producers to introduce new products.

Conclusions, Recommendations and Application to Industry

The presentations and discussions at the Second Canberra *Bacillus thuringiensis* Meeting strongly supported the view that Bt is a major resource for the control of many economically significant pests. Moreover, new developments are expanding the opportunities and efficacy of Bt formulations and transgenic organisms expressing Bt toxin genes. Several opportunities for the Australian cotton industry were suggested, together with some warnings.

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The differences in the responses of *H. virescens* and *H. zea* to various Bt toxins demonstrates that it is not realistic to extrapolate from data obtained with either of these to the Australian heliothine pests. While published data gained from US trials may be indicative, detailed studies of the Australian pests are essential to obtaining effective control.

Communication of Results

All researchers involved in Bt projects connected with the cotton industry in Australia participated in the meeting. Each registrant was provided with abstracts of all papers presented at the Meeting.

More details of the meeting will be provided by the Proceedings. This publication will consist of papers provided by all the invited speakers and reports on the three discussion forums. Editing has been substantially completed at this time and it is anticipated that the Proceedings will be available in March 1994. All registrants will receive a copy of the Proceedings and additional copies will be available for sale.

APPENDIX

Budget

The CRDC contribution to the Second Canberra *Bacillus thuringiensis* Meeting was to pay the travelling expenses for Dr F. Gould. Actual expenses were less than anticipated because it proved impractical for Dr Gould to visit the Cotton Research Unit at Myall Vale as originally planned.

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Approved Budget	4294
Funds Received to Date	3220
Expenses	
Airfares	2311.29
Accommodation	445.00
Allowances	320.00
Conference Registration	220.00
TOTAL EXPENSES	3296.29
Balance Owing	76.29