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January, August & Final Reports

Part 1 - Summary Project Details — REPORTS

Please use your TAB key to complete parts1,2,4 & 5

CRDC Project Number: **CSE91C**

January Report:	☐	Due 29-Jan-01
August Report:	☐	Due 03-Aug-01
Final Report:	☑	Due within 3 months of project completion
Project Title:		Travel to Vth International Conference on *Bacillus thuringiensis* – Guanajuato, Mexico

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The Vth International Conference on *B. thuringiensis* was well attended, with almost all the leading Bt researchers participating. In addition to four symposia on various aspects of Bt research and contributed paper and poster sessions, the Bt conference included a workshop "Public Concerns about Transgenic Plants" that ran for three sessions. The Bt conference ran in parallel with at least two concurrent sessions on other aspects invertebrate pathology. Interesting issues addressed at the Meeting were the relationship between *B. thuringiensis* and the human pathogens, *B. cereus* and *B. anthracis*, public concern issues relating to Bt and transgenic plants, and developments in the identification of functional binding sites for Cry1 proteins.

Workshop on Public Concerns about Bt Plants

Transgenic maize provided the focal point for this workshop, although other crops were considered. Mexico is not generally opposed to the use of transgenic crops but is sensitive about transgenic corn. Balthazar *et al.* (Pioneer) discussed the problem of gene flow from corn to teosinte in Mexico, the centre of origin of maize-related crops. They reported that corn maize pollen was short-lived, with most being dead within 1h and all within 2h. The larger size of maize pollen in comparison to that of teosinte affects its ability to fertilise the latter; teosinte males fertilised 50-60% maize females but maize males fertilised only 0.001% teosinte. They estimated that a 200m isolation zone was sufficient to avoid gene flow.

Bartsch and Mucher (Aachen University of Technology) pointed out that genes flow from 12 of the 13 most important crops to wild relatives somewhere in their cultivation area (peanut is the exception).

Papers on safety of Bt crops were presented by Sue McIntosh (Aventis) on behalf of the major companies involved and Bruce Chassy (University of Illinois). A list of 15 publications relating to feed performance and safety trials was provided (Appendix). Both speakers presented details of the huge amounts of maize that an animal would have to eat to reach even the No Observable Effect Level (e.g. a 75kg person would have to eat Bt 300,000 kg corn per day). In discussing allergenicity issues, it was noted that feed containing allergenic factors did not make animal products allergenic and that no Bt protein or DNA was found in animals fed on transgenic products in several studies. Some indirect benefits were noted for transgenic crops: Bt corn reduces the risk of mycotoxins, an hypoallergenic rice was created by eliminating the allergenic protein, and increased productivity results in reduced water usage per unit of product. However, out of session Hellmich acknowledged that milkweed grows in the crop after canopy closure. He commented that the significance of this is unknown and that further studies are required to establish the importance of cornfields to monarchs and the distribution of milkweed.

Richard Hellmich (USDA) presented an update on the monarch butterfly/transgenic maize issue. In field experiments he was unable to detect transgenic pollen beyond 150m from the crop and found most of it within 30m. About 30% of the pollen gets onto milkweed but is quickly washed off by rain. In choice feeding experiments, monarch larvae avoided leaves with excessive pollen but preferred some pollen to none. With Event 176, the highest expressing construct (5000 ng/g), mortality was noted at 2m from the crop and some effect on growth rate at 4m; with other events (100-250 ng/g) there was no detectable effect 2m from the crop.

An allozyme study was conducted in France to assess gene flow between the crop and alternate host plants (refugia). It showed that the European corn borer population on corn

was different from that on mugwort and hops, suggesting that the latter could not be used as refuge crops.

New Toxins

Several papers showed that enhanced efficacy for various pests can be achieved by domain swapping. Sivasubramanian (Monsanto) reported that the toxicity to *Heliothis virescens* obtained of hybrids of Cry1Ac and Cry1F was enhanced or reduced depending on the exchange site. A hybrid of Cry3/Cry1I/Cry1B was toxic to Colorado potato beetle; domain I of Cry3 was important for toxicity and a mosaic of Cry1I and Cry1B was more toxic than either alone with Cry3.

The large protein toxins of *Photorhabdus* and *Xenorhabdus* were discussed by Tom Meade (Dow AgroSciences). There are four large toxins (A & B are ca 860kDa, each with two major peptides). B is a single gene product; A, C and D are produced from 3, 3 and 2 genes, respectively. Toxin A expressed in tobacco exists in processed and unprocessed forms. Although maize expressing Toxin A was protected against corn rootworm (Coleoptera), the plants were not as good as the uninfested controls when a high challenge was applied. The question of abnormalities was avoided. In response to a question on non-target issues, Meade noted that the Toxin A plants did not appear to restrict homopteran pests.

Toxins similar to the large toxins of *Photorhabdus* and *Xenorhabdus* are apparently also produced by *Serratia entomophila*, a pathogen of New Zealand grass grub. The virulence encoding region of the large plasmid that confers amber disease has been completely sequenced. Three ORFs have significant homology to the *Photorhabdus* toxins A, B and C.

Mode of Action/Resistance to Bt

The role of aminopeptidases (APNs) as Cry1A-binding proteins was addressed. Linkage mapping showed that the APNs of *Plutella xylostella* and *H. virescens* did not map to the same linkage groups as Cry1A resistance (David Heckel, University of Melbourne). This was consistent with data presented by several other researchers that indicated that the loss of binding by APNs is most likely due to a change in glycosylation rather than a change in the primary structure of the protein. Using soybean agglutinin, Jurat-Fuentes *et al.* (University of Georgia) showed that N-acetyl-galactosamine (GalNAc) was less in Cry1-resistant *H. virescens* than in susceptible. Adang *et al.* (University of Georgia) showed that resistance in *H. virescens* strain YHD2 (RR>22,000; no Cry1A binding) is correlated with altered glycosylation patterns. Pearce *et al.* (University of Cambridge) presented a new model for Cry1A binding in which domain III locates an APN as a docking protein and then domain II binds to another site. They propose that domain II might bind to a carbohydrate that is attached to many surface proteins of the gut epithelium. Adang *et al.* showed that GalNAc is not present on all Cry1A-binding proteins. Caroll *et al.* (University of Cambridge) cleaved the 120kDa Cry1A-binding APN of *M. sexta* and showed that the peptides purified by affinity to Cry1Ac lacked glucose or galactose and have significant amounts of fucose. The presence of fucosylated N-linked oligosaccharides was demonstrated immunologically.

The role of the peritrophic membrane was raised in two papers. Heckel found that an AFLP marker for Cry1A resistance had significant homology to peritrophin from *D. melanogaster*. Rees *et al.* (University of Cambridge) noted that species tolerant of Cry1Ac tended to have higher molecular weight proteins in the peritrophic membrane than susceptible species. The

susceptible *P. brassicae* contains only one. In *M. sexta* Cry1Ac was cleaved to a ca 40kDA protein on the peritrophic membrane.

The involvement of proteinases in resistance has now been demonstrated in several strains of *Plodia interpunctella* and two of *H. virescens*. In the Forcada strain of *H. virescens* an additional proteinase was detected in resistant insects whereas in the YHD2 strain there was an additional proteinase and chymotrypsin was apparently upregulated. Both Oppert *et al.* (USDA) and Grove *et al.* (Pennsylvania State University) reported differences in trypsin:chymotrypsin ratios between resistant and susceptible species. However, no proteinase patterns absolutely correlated with sensitivity to Bt toxin.

Both current models of toxin insertion into epithelial membrane were challenged. Alzate and Dean (Ohio State University) using electron paramagnetic resonance spectroscopy and proteinase K digestion concluded that the whole toxin protein inserts into the membrane. Another study, using Fourier transformation infrared absorbtion spectroscopy showed that the β-sheet becomes highly disorganised in lipid (Schwartz *et al.*, NRC, Canada). These data were interpreted to indicate that the toxin becomes completely embedded in the cell membrane rather than having domains II and III remaining on the surface as proposed by the umbrella and penknife models.

Ecology

There was some interest in the persistence of Bt in soils but little evidence was presented. A Swiss project being established to examine the dynamics of spores and horizontal transfer of genes after 12 years of Bti application was described. Streett (USDA) proposes to use stunting assays to track the persistence of Cry1A from Bollgard cotton. He showed that he could detect 300ng MVP II per ml diet, which does not seem to be very sensitive. He was unable to detect Cry1Ac in cotton fields six months post-harvest in 1999-2000.

Posters presented:

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APPENDIX

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DNA Detection in Milk, Meat and Eggs

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Part 4 – Final Report Plain English Summary

You must submit a half to one page Plain English Summary of your research proposal that is not commercial in confidence, and that can be published on the World Wide Web. An electronic copy of the Plain English Summary must also be forwarded by e-mail (angela@crdc.org.au).

Dr Ray Akhurst attended the Vth International Conference on *Bacillus thuringiensis*, in Guanajuato, Mexico to monitor new developments in research on insecticidal proteins for transgenic plants. A major feature of this conference was a symposium on *Public Concerns about Bt Plants*. The symposium provided data on feed performance and safety trials, impacts on non-target species, gene flow from transgenic crops to wild relatives, and use of refugia for resistance management. Reading lists of animal feeding studies and detection of recombinant DNA were provided. Other issues covered at the conference included new information on mechanisms involved in resistance to Bt toxins, the discovery of new insecticidal proteins, and the persistence of Bt and its toxins in soil.