TRAVEL REPORT

CRDC Project No.: CSE106C

Title: **Travel: Dr Ray Akhurst to travel to International Conference on *Bacillus thuringiensis*, Iguassu, Brazil**

Organisation: **CSIRO Entomology**

Project start: **1 July 2002**

The VIII International Colloquium on Invertebrate Pathology and Microbial Control and the VIth International *Bacillus thuringiensis* Conference were held at Iguassu Falls, Brazil, August 18-23, 2002.

Of the 347 registrants at the Colloquium, >100 were from Latin America, primarily from Brazil and Mexico. There was an unusually strong contingent from the UK, indicative of an upswing in invertebrate pathology activity there. The only Australian registrants were Dudley Pinnock (Microbial Products, Adelaide), Cheryl Beard (CSIRO Entomology) and me. The Colloquium had the usual full program of symposia, contributed paper, poster sessions and workshops, including one on *Ethics, Legal and Regulatory Concerns of Transgenic Plants*. The plenary session was devoted to baculoviruses, presumably because of the interest of the Chairman of the Organising Committee because there appeared to be no new developments flagged in any of the four presentations. As there were three concurrent sessions, my attention was given largely to the bacterial presentations. The highlights of the conference were the report of resistance to Bt in a natural populations of a second species, the large interaction between plant variety and Bt toxins, and the absence of non-target effects of Bt.

I presented two contributed papers and chaired a Contributed Papers session. At the Business Meeting during the conference, I took over the role as secretary of the Society for Invertebrate Pathology, having previously been elected by postal ballot.

Bacillus thuringiensis

Mode of Action

The role of lipid rafts in binding of the Cry toxins was discussed by the Bravo (Mexico) and Gill (USA) groups. Lipid rafts are elements of the cell membrane that are rich in cholesterol and glycolipid. They are known to be important for signal transduction. Working with *Manduca sexta* and *Heliothis virescens*, they have shown that aminopeptidases (APNs) that have been linked to Bt toxicity occur mainly in lipid rafts. It appears that APNs and cadherin-like proteins may migrate into the lipid rafts in response to the presence of Cry1 toxins. This model shows how APNs and cadherin-like proteins may both be involved in Cry1 binding.

Raffi Aroian (USA) uses the nematode *Caenorhabditis elegans* as a model system for investigating resistance mechanisms. He reported that four *bre* genes associated with resistance to the Cry5B Bt toxin by *C. elegans* are glycotransferases. He proposed that *bre2 —bre5* operate in sequence to build up glycosylation chains. He also reported the identification of 12 mutants resistant to Cry6A. These *brx* mutations occurred at a much lower frequency than the *bre* mutation (5.10^{-5} and 3.10^{-4}, respectively). The *brx* mutatnts were not cross-resistant to Cry5B.

A new model for the action of Cyt toxins was proposed (Butko, USA). This model suggests that the Cyt proteins act as detergents rather than by forming pores. Several methodologies (FTIR, proton/deutron exchange, and differential scanning calorimetry) provided support for this model.

Characterising Bt Genes/Toxins
The rate of discovery (= reporting) of new Bt toxin genes is not slowing (Crickmore, UK). Over the past 15 years the rate has been approximately eight new genes per year.

An analysis of the megaplasmid (*pBtoxis*) of *B. thuringiensis israelensis* (Bti), which has now been completely sequenced, was presented (Berry, UK). There is little overall conservation between *pBtoxis* and *pTOX1*, the toxin gene-bearing megaplasmid of *B. anthracis*, and >30% of that conservation is due to transposon sequences. There is some conservation at the origin of replication that might indicate an evolutionary link between these plasmids. *pBtoxis* carries four *cry* and two *cyt* genes that are complete and several toxin gene fragments. Surprisingly, the plasmid carries a complete set of germination genes.

Maxygen (USA) has used gene evolution to increase the toxicity of Cry1Ca for *Spodoptera exigua*. DNA shuffling with several genes produced a three-fold improvement in toxicity in the first two rounds. A key element in the process is the high throughput bioassay system used.

Synergism
There are some very significant interactions between Bt toxins and plants. Quintanilla-Perez *et al.* (Mexico) compared the performance of a sprayable Bt formulation on eight varieties of corn. Although no differences in attractiveness for oviposition or palatability were detected, there was a 370-fold variation in LC_{50} on different varieties.

Berry (UK) noted that the toxicity of Bti is greater than the sum of the toxicities of its Cry and Cyt toxins. It is not clear if the synergism is only due to interactions between these toxins or if virulence factors also play a role.

Wirth (USA) found that Cyt1A alters the binding pattern of the binary toxin of *Bacillus sphaericus* in *Culex quinquefasciatus*. In *C. quinquefasciatus* the binary toxin binds predominantly in the gastric caecum and posterior midgut whereas Cyt1A binds throughout the midgut. The binary toxin does not bind the midgut of *B. sphaericus*-resistant *C. quinquefasciatus* or *Aedes aegypti*, for which it is only weakly toxic. However, in the presence of Cyt1A, the binary toxin binds throughout the midgut of both species and becomes highly toxic. The binary toxin, when combined with Cyt1A, moves quickly into the cells of resistant *C. quinquefasciatus* although in the absence of Cyt1A it binds to the

microvilli of susceptible larvae. This observation is consistent with the detergent mode of action for Cyt1A proposed by Butko.

Synergism between *B. sphaericus* and two dipteran-toxic strains of Bt (*morrisoni* and *jegathesan*) was reported (Park, USA). Addition of *B. sphaericus* to *Bt jegathesan*, which is not notably toxic for *Culex* spp., increased the toxicity for *C. quinquefasciatus* by 15-fold.

Resistance

Until this Colloquium, resistance to Bt toxins had only been detected in natural populations of *Plutella xylostella*, the diamondback moth. A study in a very large glasshouse complex in Canada led to the discovery of resistance in *Trichoplusia ni*. Under these conditions, *T.* ni has 10 overlapping generations per year. Bt sprays were applied at intervals as low as 3d. The situation was investigated when growers reported control failures. Resistance was found to increase over the growing season. It declined in the absence of sprays and was associated with low pupal weight and possibly delay in larval development.

Considerable genetic variability in resistance was demonstrated from the analysis of laboratory selection of a Philippines population of *P. xylostella* (Ferr , Spain). The field population was resistant to Cry1Ab (RR=236) but not Cry1Aa, Cry1Ac or Cry1B. After selection another three patterns of resistance were detected. The resistance allele for one gene was recessive and for another was dominant. It was concluded that the 130 insects used to initiate the colony must have carried at least four different resistance genes.

Moar (USA) reported co-dominant resistance in *H. zea* following lab selection. However, as the resistance ratio was <20, it is not certain whether this population is developing single gene resistance or has become multi-gene tolerant. There are currently 144 groups (64000ha) using community refuges for resistance management of Bollgard crops in the US. Moar suggested that this strategy would become more difficult with higher uptake of Bollgard. He reported that alternative crops, such as *Pawlonia* spp., are under investigation for refugia. The contribution of weeds and other crops to refuge populations is also being evaluated; Monsanto has used this argument to press for small refuge size in India. The proposal from the Heliothis Cry1Ac Resistance Monitoring and Mitigation Committee is the use of unsprayed, sentinel plots of Bollgard and conventional cotton. Monitoring involves larval collections, with putative resistance defined as ‡2/100 *H. virescens* larvae surviving to 2nd instar on Bollgard and ‡15/100 *H. zea* larvae surviving to 3rd instar on Bollgard. Resistance will be confirmed by determining the LC_{50} for five independent collections from the suspect population.

Resistance by *Culex pipiens* to the binary toxin of *B. sphaericus* (RR >7000) was associated with loss of the binding site. The target molecule for the binary toxin is an α-glucosidase. Investigation of the resistant strain showed that the α-glucosidase was produced but not located on the microvilli. The mutant molecule lacks the C-terminal sequence that is required for GPI anchor attachment and so cannot be suitably located for toxin activity (Darboux, France).

Safety/Environmental Impact

A worst case scenario test failed to identify any deleterious effects of Bt on a collembolan (Brownbridge, USA). Lab tests with XenTari, DiPel and MVP applied at twice the recommended rate had no effect on the survival, fecundity, or longevity of *Folsomia candida* exposed to the Bt formulations for four weeks and maintained in toxin-free conditions

thereafter. In contrast, neem affected survival and egg production and the effects continued after the insects were transferred away from the toxin.

Monsanto reported the results of trials in Arizona and Mississippi using Bollgard I and II and the parental lines, with and without insecticide applications. The experiments involved 3000 plants per plot with four replicates per treatment. 32000 insects (18 Orders, 100 Families) were identified to Family. No differences in predator abundance could be detected between transgenic and conventional cotton under the same insecticide regime.

Bacterial Virulence Factors

One of the symposia and several contributed presentations addressed the issue of bacterial virulence factors. The papers covered research on Bt, *B. cereus*, *Photorhabdus*, *Xenorhabdus*, and *Serratia entomophila*.

Signature tagged mutagenesis was used to identify virulence factors in Bt after injection into *M. sexta*. The virulence genes were regulated by *fur*, which is known to control iron uptake and storage. Harvie *et al.* (UK) proposed that the change in iron status signals that the bacterium has entered a new environment and the appropriate change in iron status initiates the expression of virulence genes. They noted that all the environmental regulators they had identified occur in both Bt and *B. cereus* and therefore do not explain their different host specificities.

The discovery of a new class of insecticidal proteins in *Photorhabdus* was reported (Darborn *et al.*, 2002, PNAS 99, 10742-10747). The *mcf* (makes caterpillars floppy) gene was identified from the screening of a cosmid library injected into an insect host. The 175 kDA protein has no significant homology to anything in the databases, although it does have a BH3 domain, which is involved in triggering apoptosis by other bacterial species. The protein is apoptotic and is presumably an anti-immune protein as it enables *E. coli* to avoid clearance from haemolymph. The gene occurs in different positions on the chromosome of the W14 and K122 strains of *Photorhabdus*.

For many years it appeared that New Zealand strains of *Serratia entomophila* and *S. proteamaculans* were the only strains capable of causing amber disease of scarab larvae. These bacteria are used as a bioinsecticide for the treatment of New Zealand grass grub, *Costelytra* zealandica in New Zealand. They cannot be used in other countries because they are highly specific for this one species of scarab. At this colloquium there were reports of an *S. entomophila*-like bacterium causing amber disease in *Phyllophaga blanchardi* in Mexico and isolation of pathogenic *S. proteamaculans*-like bacteria in Europe.

Bowen (USA) reported the isolation of *Photorhabdus asymbiotica* from a nematode in the US. *P. asymbiotica* has previously only been found in association with human clinical cases. However, there has to be some doubt about the accuracy of the identification of this new strain, which was based on 16S rDNA sequence analysis. Our experience shows that a *Photorhabdus* isolated from an Australian *Heterorhabditis* sp. also clusters with *P. asymbiotica* in analysis of both 16S rDNA and *gyrB* sequences. However, other indicators of relationship, especially DNA/DNA hybridisation, do not support their close relatedness. The clustering in sequence analysis is an example of group size dependence.