Protecting an Investment - Managing Resistance Development to Transgenic Cotton by *Helicoverpa armigera*

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INTRODUCTION

The introduction of transgenic cotton expressing the Cry1Ac insecticidal crystal protein from *Bacillus thuringiensis* (Bt) is expected to provide the basis for pest management in cotton in the near future and to reduce the usage of pesticides in the industry. However the potential for over-reliance on Bt-cotton to result in the development of resistance to Bt by *Helicoverpa armigera* has been identified (Edge, 1994). Recognising that this resistance would undermine the basis for pest management the cotton industry has devoted resources to addressing the problem.

Experience with resistance to synthetic chemical insecticides has shown that there are options for managing resistance. One of the major options being examined is the provision of alternative means of control that will complement the Bt cotton. Among these alternatives is the identification of new insecticidal crystal proteins from Bt. Over the past four years an additional 35 Bt insecticidal crystal proteins have been recorded, demonstrating that the diversity of these insecticidal toxins is much greater than previously recognised. We have taken advantage of new molecular technologies to search for new insecticidal crystal proteins that can be used to complement Cry1Ac in a resistance management program.

Not only is it essential to identify the options for managing resistance to Cry1Ac but to recognise that these options will only be effective if implemented before the problem becomes too great for management. A second facet of our research is to

develop a sensitive test for resistance genes in field populations so that the development of resistance can be detected at a very early stage allowing implementation of appropriate management options in a timely manner.

ALTERNATIVE INSECTICIDAL CRYSTAL PROTEINS

Currently Available Insecticidal Crystal Proteins

The first step in identifying alternative insecticidal crystal proteins for *H. armigera* control was to test the efficacy of those that were available. We obtained 11 insecticidal crystal proteins, either as purified protein or a cloned genes, for testing against Australian populations of *H. armigera* and *H. punctigera* and information on three others (Table 1). This showed that only Cry2Aa, which was approximately equal in toxicity for *H. armigera* with Cry1Ac, and Cry2Ab could seriously be considered as alternatives to Cry1Ac.

In addition to toxicity, an important consideration in identifying complementary insecticidal crystal proteins is the interaction with Cry1Ac should the two genes be pyramided into cotton. Our tests for interaction involving bioassays with various proportions of Cry1Ac and Cry2Aa showed that these insecticidal crystal proteins were not complementary but antagonistic. All mixtures of the toxins were 2-3 times less toxic than either toxin, indicating that the strategy of putting both the *cry1Ac* and *cry2Aa* genes into the same plant would not be sustainable. Moreover, unless Cry2Ab is very different from Cry2Aa, none of the known insecticidal crystal proteins is suitable for inclusion in a pyramiding strategy.

Novel Insecticidal Crystal Proteins

On the premise that Australia is a biologically unique continent we expected that some Bt strains isolated here might be significantly different from those isolated in other parts of the world. We collected 780 environmental samples

Table 1. Toxicity of *Bacillus thuringiensis* insecticidal crystal proteins for *H. armigera* and *H. punctigera*

ICP	LC_{50} (ng cm^{-2})[1]	
	H. armigera	*H. punctigera*
Cry1Aa	>2400[2]	nt
Cry1Ab	692	355
Cry1Ac	115	108
Cry1B	>4000	>4000
Cry1C	>4000	>4000
Cry1D	>1500[3]	nt
Cry1E	>4000	nt
Cry1F[4]	>4000	286
Cry1I	>1500	nt
Cry2Aa	149	52
Cry2Ab	421	412
Cry2Ac	1678	6205
Cry3	>1500	nt
Cry4	>1500	nt
Cry9A	>5000	944
Cry9C	>1500[3]	nt

1 Bioassays with neonates; results after 7d
2 Padidam (1992)
3 Data provided by Plant Genetic Systems
4 Tested as a Cell-Cap product; results recorded after 12d

from all states and territories from which we isolated 4732 Bt strains. These were examined by light microscopy to identify those producing proteinaceous crystals and the 902 strains that did so were analysed by polymerase chain reaction (PCR) technology to determine which insecticidal crystal protein genes they carried. Although most of these strains were found to carry genes that were already known, 66 strains had no previously known insecticidal crystal protein genes.

(Ferré *et al.*, 1991). We have identified this as the most likely mechanism for resistance to Cry1Ac by *H. armigera* and directed our research accordingly. However we have also maintained a watching brief on the other two potential mechanisms mentioned above.

To date we have investigated the mode of action of Cry1Ac on *H. armigera* and shown that Cry1Ac recognises two binding sites in this species (Akhurst *et al.*, 1995). There is a difference in binding affinity for these two sites, suggesting that one may be significantly more important for toxicity than the other. We have also shown that Cry1Ac binds to the peritrophic membrane of *H. armigera*, suggesting that this could provide an alternative resistance mechanism for this species.

Using an affinity binding technique we have purified the two binding proteins from *H. armigera*. One is an aminopeptidase N which has previously been shown to be the sole binding site for Cry1Ac in *Manduca sexta*. We have obtained N-terminal sequence data for both proteins and will use the data to clone the genes when suitable funding is obtained. The natural variation in these genes in field population will be investigated by PCR techniques and these data, combined with information from international groups working on the functional domains of Cry1Ac, will be used to design molecular probes for variation indicative of resistance.

CONCLUSION

The future of the Australian cotton industry will be determined by the sustainability of transgenic cotton as a means of counteracting its major pests. This project is contributing to that future by providing essential components of a resistance management strategy. To date we have shown that, although none of

the available Bt insecticidal crystal proteins are suitable, we have options for new insecticidal crystal proteins that can be used for complementing Cry1Ac in transgenic plants. We are also well advanced in developing a technology that will give early warning of resistance problems so that the appropriate management strategy can be adopted.

REFERENCES

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