MONITORING SUSCEPTIBILTY TO Bt TOXINS IN AUSTRALIAN *HELICOVERPA* SPECIES

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Abstract

The susceptibility to Bt toxins in Australian field populations of *Helicoverpa armigera* and *Helicoverpa punctigera* has been monitored at the Australian Cotton Research Institute since 1993 (Forrester and Bird 1996). This paper reviews the methods and results for the Bt resistance monitoring survey for the 1999/2000 cotton-season and compares them with data from previous seasons. No changes in susceptibility to discriminating doses of Dipel® or MVP2® have been recorded in any of the Australian field populations of *H. armigera* or *H. punctigera* collected from cotton to date. Significant reductions in susceptibility to Dipel® or MVP2® were recorded in field populations of *H. armigera* collected from sweet corn crops in coastal Victoria.

Introduction

Bt (*Bacillus thuringiensis* var. *kurstaki*) is a soil borne bacterium, which produces proteins that are toxic to *Heliothis/Helicoverpa* spp. on ingestion. Bt has been used by the Australian cotton industry for the last 20 years as selective foliar sprays for control of *Helicoverpa* species. Several commercial products are available containing mixed insecticidal proteins (Cry1Aa, Cry1Ab, Cry1Ac, (P1) Cry2A, (P2) Cry2B and a spore (sold as Dipel®, Costar® and BTK®) and more recently a refined foliar spray containing a single protein (Cry1Ac (P1) encapsulated in killed *Pseudomonas fluorescens* cells (sold as MVP2®). The latest development in the use of Bt in cotton is in the form of transgenic cotton varieties (Ingard®) expressing the single protein Cry1Ac (P1) delta endotoxin, which have been commercially available in Australia for the last 4 seasons.

The cotton industry is concerned about the potential for *Helicoverpa* species to develop resistance to Bt proteins in foliar sprays and transgenic cotton varieties, which could threaten the long-term sustainability of profitable cotton production in Australia. In response to this concern, the Cotton Research and Development Corporation has funded research programs to develop resistance monitoring assays and since 1993, to conduct an annual survey to monitor the susceptibility to Bt toxins in Australian field populations of *Helicoverpa* *armigera* and *Helicoverpa punctigera*. The outcomes of this research are part of an ongoing National Registration Authority audit required for the continuing registration of transgenic cotton and are incorporated into the pro-active Bt transgenic cotton Resistance Management strategy.

Materials and Methods

Collections of eggs were made throughout the cotton season from cotton and other host crops by consultants and agronomists in each of the valleys. These were sent to the A.C.R.I. where eggs were transferred onto artificial diet, hatched and 'grown out' to an early third instar (small/medium) stage when they were visually sorted according to species. These larvae were transferred into a 'testing diet' made up from the same artificial diet mixed with a discriminating dose of a foliar Bt product. Larvae were assessed for mortality after 7 days of exposure to the testing diet. The discriminating dose for each product represents an LC99 for each species, that is a dose that should cause mortality of 99% of susceptible larvae (Forrester and Bird 1996). The fact that eggs were tested direct from the field (the P1 generation) is an advantage for the quality of data from the monitoring survey. Any laboratory rearing of the insects could result in a change in frequency of resistance genes and therefore not accurately reflect the situation in the field (Roush and Daly 1991). Because the frequency of genes for resistance to Bt (and other new technologies for control of *Helicoverpa* species) is expected to be low, large sample sizes of insects need to be tested to have a strong probability of picking up any statistically significant changes in their frequency. For example almost 300 insects would need to be tested to have a 95% probability of detecting resistance genes at a frequency of 1% in a population (Roush and Miller 1986).

Discriminating doses of two foliar Bt products are tested. Dipel®, (discriminating dose of 2mg/ml diet) was first tested in 1993 and contains a mixture of Bt toxins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B and a spore). A total of 14295 *H. armigera* larvae and 1496 *H. punctigera larvae* from all regions were tested with Dipel® this season (Table 1), with an average survival of the discriminating dose of 0.7% and 0.2% respectively. MVP2® (discriminating dose of 3mg/ml of diet) was first tested in 1997 and contains a single Bt toxin (Cry1Ac encapsulated in killed cells of *Pseudomonas fluorescens*), the same Bt toxin that is expressed by current single gene varieties of transgenic Ingard® cotton. A total of 11275 *H. armigera* larvae and 1884 *H. punctigera* larvae from all regions were tested with MVP2® this season (Table 2), with an average survival of the discriminating dose of 2.6% and 2.7% respectively.

Results and Discussion

Larval survival of discriminating doses of these products so far has ranged from 0-10% with variability between regions, between different collections from the same region and between seasons. For most collections of larvae, this level of survival falls within the natural variability that would be expected for both species. In some cases, for example the mid season collection of *H. punctigera* from Bourke (Table 2), small sample sizes (n = 22) resulted in greater than expected frequencies of survival of MVP2® (10%). However, greater than expected frequencies of survival of MVP2® were recorded in collections of *H. armigera* from Hillside (9.1%) and Orbost (7.3%) in Victoria with substantial sample sizes (2829 and 629 larvae tested, respectively) and these results warrant further investigation. Importantly we have not detected any trend for increased larval survival of either species to either Dipel® or MVP2® in any of the cotton growing regions. If any significant changes in the frequency of survival of the discriminating dose of either of these products are detected in the future, it will spell caution for the use of foliar Bt products in cotton and for the implementation of Ingard®. A thorough Resistance Monitoring program is an essential component of an effective Resistance Management Strategy.

Table 1. 1999-2000 Season Resistance Monitoring of *Helicoverpa armigera* and *Helicoverpa punctigera* larvae, using a discriminating dose of Dipel®.

% Surviving the Discriminating Dose of DiPel "R" (*Bacillus thuringiensis* var. kurstaki)							
Species and collection area	Host	Early Season Collections(Oct-Dec)		Mid-Late Season Collections(Jan- May)		Season total	
Helicoverpa armigera		% R	Number Tested	% R	Number Tested	% R	Number Tested
Lower Namoi Valley, NSW	Cotton	1	(n=230)	0.5	(n=2109)	0.5	(n=2339)
Bundaberg, Qld	Tomatoes	0	(n=77)	4.7	(n=21)	1	(n=98)
Upper Namoi Valley	Cotton	1	(n=176)	0	(n=145)	0.6	(n=321)
Macquarie Valley, NSW	Cotton	0	(n=7)	0	(n=305)	0	(n=312)
Gwydir Valley, NSW	Cotton	0	(n=67)	0.9	(n=654)	0.8	(n=721)
Emerald, Qld	Cotton	0	(n=6)	0.7	(n=2310)	0.7	(n=2316)
D. Downs -Qld	Tomato	0.5	(n=636)	1	(n=293)	0.6	(n=929)
Biloela, Qld	Cotton	0	0	0	(n=39)	0	(n=39)
Macintyre, Qld	Cotton	0	(n=69)	0.7	(n=576)	0.6	(n=645)
St George, Qld	Cotton	0	(n=11)	1.6	(n=552)	1.6	(n=563)
Mungindi, NSW	Cotton	0	0	1.1	(n=174)	1.1	(n=174)
Walgett, NSW	Cotton	0	(n=9)	1	(n=1534)	1	(n=1543)
Bourke, NSW	Cotton,Tomato	0	(n=3)	0	(n=84)	0	(n=87)
Dalby-Qld	Cotton	0	(n=58)	2.8	(n=36)	1	(n=94)
Kununurra-NT	Cotton	0	(n=73)	0	0	0	(n=73)
Dirranbandi-Qld	Cotton	0	0	0	(n=177)	0	(n=177)
Griffith-NSW	Corn	0	0	0.8	(n=599)	0.8	(n=599)
Hillside-Vic	Corn	0	0	2.4	(n=2153)	2.4	(n=2153)
Dalmore-Vic	Corn	0	0	0.3	(n=302)	0.3	(n=302)
Wemen-Vic	Corn	0	0	0	(n=98)	0	(n=98)
Orbost-Vic	Corn	0	0	2.8	(n=712)	2.8	(n=712)
Total of sites		**0.2**	**(n=1422)**	**1.2**	**(n=12873)**	**0.7**	**(n=14295)**
Helicoverpa punctigera	**Host**	**% R**	**Number Tested**	**% R**	**Number Tested**	**% R**	**Number Tested**
Macintyre Valley, Qld	Cotton	0	0	0	(n=3)	0	(n=3)
Lower Namoi Valley,NSW	Cotton	0	(n=10)	0	(n=289)	0	(n=299)

Upper Namoi Valley, NSW	Cotton	0	0	0	(n=11)	0	(n=11)
Mungindi, NSW	Cotton	0	0	0	(n=14)	0	(n=14)
St George, Qld	Cotton	0	(n=3)	0	(n=18)	0	(n=21)
Bundaberg, Qld	Tomatoes	0	(n=1)	0	0	0	(n=1)
Macquarie Valley, NSW	Cotton	0	(n=1)	0	(n=7)	0	(n=8)
Bourke, NSW	Cotton	0	(n=35)	1.5	(n=65)	1.0	(n=100)
Walgett-NSW	Cotton	0	(n=3)	0.5	(n=178)	0.5	(n=181)
Gwydir-NSW	Cotton	0	0	0	(n=24)	0.0	(n=24)
Griffith-NSW	Corn	0	0	2.1	(n=530)	2.1	(n=530)
Dalby-Qld	Cotton	0	(n=1)	0	(n=2)	0.0	(n=3)
D.Downs-Qld	Cotton,Tomato	0	(n=1)	0	(n=11)	0.0	(n=12)
Biloela-Qld	Cotton	0	0	0	(n=3)	0.0	(n=3)
Emerald-Qld	Cotton	0	(n=2)	0	(n=283)	0.0	(n=285)
Kununurra-NT	Cotton	0	(n=1)	0	0	0	(n=1)
Total of all sites		**0**	**(n=58)**	**0.2**	**(n=1438)**	**0.2**	**(n=1496)**

Table 2. 1999-2000 Season Resistance Monitoring of *Helicoverpa armigera* and *Helicoverpa punctigera* larvae, using a discriminating dose of MVP2®.

% Surviving the Discriminating Dose of MVP2 "R" (CryI Ac delta endotoxin encapsulated in killed *Pseudomonas fluorescens* cells							
Species and collection area	**Host**	**Early Season Collections(Oct-Dec)**		**Mid-Late Season Collections(Jan-May)**		**Season total**	
Helicoverpa armigera	**Host**	**% R**	**Number Tested**	**% R**	**Number Tested**	**% R**	**Number Tested**
Lower Namoi Valley, NSW	Cotton	0	(n=158)	3.3	(n=1624)	3	(n=1782)
Bundaberg, Qld	Tomatoes	0	(n=27)	5	(n=20)	2.1	(n=47)
Upper Namoi Valley,NSW	Cotton	0	(n=24)	4.4	(n=159)	3.8	(n=183)
Macquarie Valley, NSW	Cotton	0	(n=1)	1.6	(n=367)	1.6	(n=368)
Gwydir Valley, NSW	Cotton	0	(n=3)	1.3	(n=376)	1.3	(n=379)
Emerald, Qld	Cotton	0	0	1.4	(n=2268)	1.4	(n=2268)
D. Downs -Qld	Cotton,Tomato	0	(n=31)	0	(n=112)	0	(n=143)
Biloela, Qld	Cotton	0	0	2.3	(n=302)	2.3	(n=302)
Macintyre, Qld	Cotton	0	(n=21)	4.5	(n=22)	2.3	(n=43)
St George, Qld	Cotton	0	(n=13)	3.2	(n=878)	3.1	(n=891)
Mungindi, NSW	Cotton	0	0	3	(n=270)	3	(n=270)
Walgett, NSW	Cotton	0	(n=9)	2.8	(n=252)	2.7	(n=261)
Bourke, NSW	Cotton,Tomato	0	(n=3)	3.1	(n=127)	3	(n=130)
Dalby-Qld	Cotton	0	0	0	(n=17)	0	(n=17)
Kununurra-NT	Cotton	0	0(n=39)	0	0	0	(n=39)
Dirranbandi-Qld	Cotton	0	0	3.3	(n=30)	3.3	(n=30)
Griffith-NSW	Corn	0	0	3	(n=429)	3	(n=429)
Hillside-Vic	Corn	0	0	9.1	(n=2829)	9.1	(n=2829)
Dalmore-Vic	Corn	0	0	1.9	(n=156)	1.9	(n=156)
Wemen-Vic	Corn	0	0	0	(n=31)	0	(n=31)
Orbost-Vic	Corn	0	0	7.3	(n=629)	7.3	(n=629)
Total of sites		**0**	**(n=377)**	**3.8**	**(n=10898)**	**2.6**	**(n=11275)**
Helicoverpa punctigera	**Host**	**% R**	**Number Tested**	**% R**	**Number Tested**	**% R**	**Number Tested**
Macintyre Valley, Qld	Cotton	0	0	0	(n=1)	0	(n=1)
Lower Namoi Valley,NSW	Cotton	0	(n=3)	2.1	(n=657)	2.1	(n=660)
Upper Namoi Valley, NSW	Cotton	0	0	7.7	(n=13)	7.7	(n=13)
Mungindi, NSW	Cotton	0	0	7.7	(n=13)	7.7	(n=13)
St George, Qld	Cotton	0	0	0	(n=12)	0	(n=12)
Bundaberg, Qld	Tomatoes	0	(n=1)	0	0	0	(n=1)
Macquarie Valley, NSW	Cotton	0	(n=2)	0	(n=4)	0	(n=6)
Bourke, NSW	Cotton,tomato	0	(n=10)	13	(n=22)	10.0	(n=32)
Walgett-NSW	Cotton	0	(n=7)	3	(n=433)	3.0	(n=440)
Gwydir-NSW	Cotton	0	0	0	(n=12)	0.0	(n=12)
Griffith-NSW	Corn	0	0	0	(n=30)	0.0	(n=30)
Dalby-Qld	Cotton	0	(n=1)	0	(n=11)	0.0	(n=12)
D.Downs-Qld	Cotton,Tomato	0	0	0	(n=16)	0.0	(n=16)
Biloela-Qld	Cotton	0	0	0	(n=1)	0.0	(n=1)
Emerald-Qld	Cotton	0	0	4.3	(n=164)	4.3	(n=164)
Dirranbandi-Qld	Cotton	0	0	0.7	(n=27)	0.7	(n=27)
Dalmore-Vic	Corn	0	0	5.2	(n=443)	5.2	(n=443)
Kununurra-NT	Cotton		(n=1)	0	0	0	(n=1)
Total of all sites		**0**	**(n=25)**	**2.4**	**(n=1859)**	**2.3**	**(n=1884)**

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