Development of discriminating dose assays for *Bacillus thuringiensis* subspecies *kurstaki* in Australian *Helicoverpa* spp.

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(Forrester, 1994). Annual useage in cotton has levelled off at around 230,000 litres which represents just under one spray over the whole cotton area. In addition to this relatively low useage, Bt is rarely used on its own but is used in mixtures with synthetic insecticides such as thiodicarb, endosulfan and synthetic pyrethroids (Forrester, 1994). Clearly, the current low useage of Bt in cotton and the widespread use of Bt mixtures, present a low resistance risk for Bt at the moment. However, Bt useage may increase in cotton or it may be registered on other crops which are alternate hosts for *H. armigera* in particular, thus increasing the overall selection pressure for resistance. In addition, the development and adoption of transgenic plants, particularly transgenic cotton expressing the insecticidal crystal proteins (ICPs) from Bt, will also potentially increase the selection pressure for resistance to Bt and its ICPs (Forrester, in press). Consequently, it was decided to :- 1) determine the variability in the baseline susceptible response to *Bacillus thuringiensis* subspecies *kurstaki* in both *H. armigera* and *H. punctigera* (the two key lepidopteran pests of Australian summer field crops), before the widescale use of Bt and/or transgenic plants; and 2) develop a simple discriminating dose screening technique for detection and monitoring of potential resistance to Bt and/or transgenic cottons.

Materials & Methods

The *Bacillus thuringiensis* subspecies *kurstaki* used in these experiments was DiPel 2X (labelled 32,000 IU / mg) from Abbott Laboratories, North Chicago, USA. Each 500g can of DiPel 2X used was calibrated against the international reference standard strain of *Bacillus thuringiensis* subspecies *kurstaki* (18,700 IU / mg) supplied by Dr. Brian Melin, Abbott Laboratories, North Chicago, USA. A range of solutions of DiPel 2X powder were made up in distilled water, sonicated for two minutes and incorporated into the standard soyflour/wheat germ/ agar artificial diet for *Helicoverpa* spp. (Appendix 1 in Forrester *et al.*, 1993) to give a range of concentrations expressed as mg DiPel 2X / ml diet. About 0.5-1.0 ml of diet was then poured into each 3.5 ml (1.6 cm diam.) well of a 24 well tissue culture tray (ICN Biomedicals Australasia, Seven Hills, NSW), modified for entomological use by removal of the small aeration lugs to allow better sealing. The Bt diet was used on the day of preparation (or very occasionally the day after).

Numerous field strains of both *Helicoverpa* spp. were collected as either eggs, larvae or pupae from a range of hosts across Australia from 1993 to 1994, reared through to the F1 generation and tested on Bt incorporated diet as newly moulted early third instar larvae weighing approximately 2.3 and 4.5 mg for *H. punctigera* and *H. armigera*, respectively. Eleven Bt concentrations and a control were used for each assay ranging from 16.0 to 0.015

armigera and *H. punctigera*, respectively (unpublished data). This is also quite satisfactory but again is slightly more variable than would be expected with topical testing of conventional insecticides (1.5 to 3.0 fold, Appendix 2 in Forrester *et al.*, 1993).

Discriminating doses on field material

The LC50 data from Figure 1 suggested that 6 to 8 days would be a suitable assessment time for both species. However, the data on slopes suggested that eight days would be the optimum assessment time for a discriminating dose test (that is, it gave the highest slope). However, logistically, a seven day assessment time (that is one week) is much easier to integrate into a normal five day working week. For this reason, and the fact that the seven and eight day LC50s and slopes were so similar, it was decided to adopt a seven day assessment time for both species for all subsequent discriminating dose screens on field collected material.

The predicted mortalities for the three highest doses (0.5, 1.0 and 2.0 mg DiPel 2X / ml diet), averaged across the 34 *H. armigera* strains, were 96.8, 99.5 and 99.8%, respectively while the corresponding data for the 23 *H. punctigera* strains were 85.0, 95.6 and 99.1%, respectively (Table 2). Clearly the 2.0 mg dose would be the most suitable dose for both species. The 1.0 mg dose would have probably been satisfactory for *H. armigera* but the data indicated that it was a bit low for *H. punctigera* and logistically it is much easier to manage a resistance detection/monitoring programme employing the same discriminating dose for both species, particularly for a diet incorporation feeding assay. Consequently, the 2.0 mg dose was used to screen the 1993/94 field material but the two lower doses were also trialled just in case the laboratory calibrations were not indicative of the field situation.

Because of the abundance of the *H. punctigera* species, particularly in the early and mid season, large numbers of this species were able to be screened for each of the three potential discriminating doses (approximately 3,800 larvae for each dose) and the actual field results agreed remarkably well with the predicted laboratory mortalities (Table 2). However, because of the lower numbers of the *H. armigera* species and the need to allocate some of these larvae to the conventional insecticide resistance monitoring programme, fewer *H. armigera* were able to be screened (only approximately 1,400 larvae for each dose). The actual field data also agreed fairly well with the predicted laboratory results but were generally slightly lower than expected, particularly at the lower doses (Table 2). The significance of this slight departure from the expected is unclear at the moment with only data for one year to go by, but the good agreement between the predicted and actual levels for the *H. punctigera* data and the proclivity for the *H. armigera* species to develop resistance (Forrester *et al.*, 1993), give cause for close scrutiny of the situation in the future. On the other hand, the slight differences may be simply due to

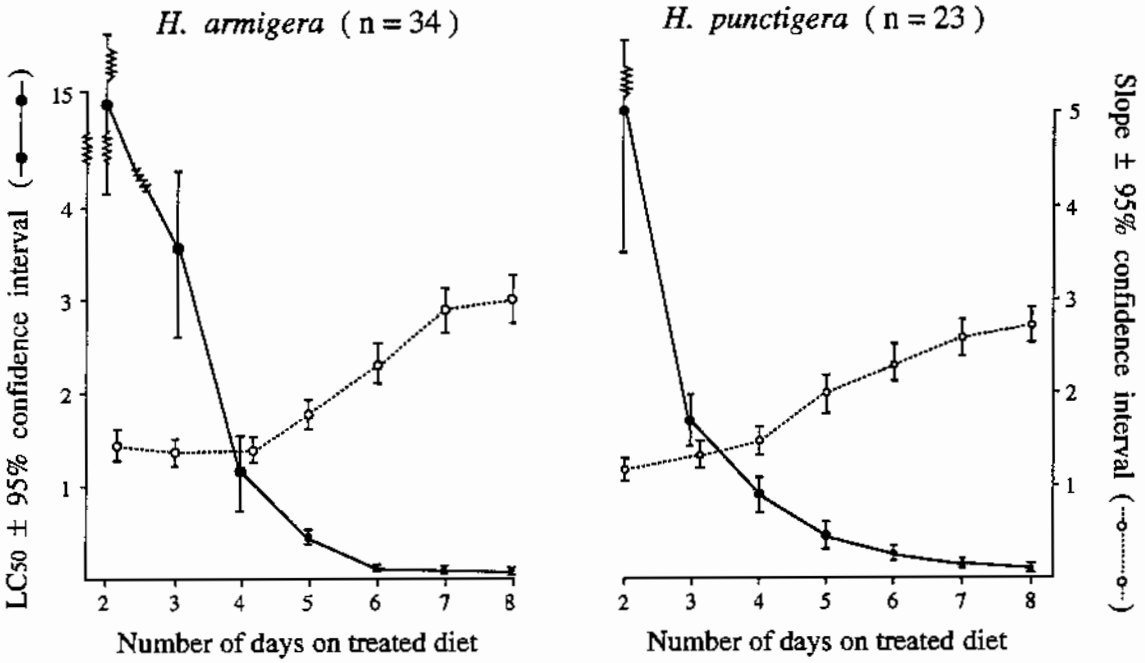

Fig. 1. Mean LC50 (mg DiPel 2X / ml diet) and slope (both ± 95% confidence intervals) for 34 *H. armigera* and 23 *H. punctigera* strains, collected across Australia from 1993 to 1994 and tested in the F1 as early third instars held on diet incorporated Bt for 2-8 days.

Table 2. Mean mortalities at three potential discriminating doses of diet incorporated Bt (0.5, 1.0 and 2.0 mg DiPel 2X / ml diet for 7 days). Predicted - mean mortality and 99% confidence intervals for 34 *H. armigera* and 23 *H. punctigera* strains, collected across Australia from 1993 to 1994 and tested in the F1 as early third instars. Actual - whole season pooled mortality of *H. armigera* and *H. punctigera* reared from eggs collected across the Australian cotton belt in the 1993/94 season and tested as early third instars in the same generation (n= total number of larvae screened for each discriminating dose).

Species	Mean Mortality	% mortality at 7 days		
		(mg DiPel 2X / ml of diet)		
		0.5	1.0	2.0
H. armigera	Predicted	96.8	99.5	99.8
	(99% confidence interval)	(94.7 - 98.9)	(98.8 - 100)	(99.1 - 100)
	Actual	89.5 n= 1,431	97.9 n= 1,420	98.5 n= 1,354
H. punctigera	Predicted	85.0	95.6	99.1
	(99% confidence interval)	(78.0 - 92.0)	(92.2 - 99.0)	(97.5 - 100)
	Actual	83.4 n= 3,806	96.3 n= 3,754	99.1 n= 3,809