DEVELOPMENT OF RESISTANCE ASSAYS FOR BT IN AUSTRALIAN *HELICOVERPA* SPP.

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The diet incorporation technique has been used to determine the variability in the baseline susceptible response to Bt in a number of field populations of both *Helicoverpa armigera* & *Helicoverpa punctigera*. The commercial *Bacillus thuringiensis* subspecies *kurstaki* formulation DiPel 2X (32,000 IU/mg) was incorporated into the standard soyflour/ wheat germ/ agar artificial diet. Newly moulted early third instar larvae were confined on the treated diet in 24 well tissue culture trays for up to 8 days at 25°C. The criterion for assessing mortality was the same as for conventional insecticides: no co-ordinated movement when prodded. LC50s are expressed in mg DiPel 2X per ml of diet.

Twentyone *H. armigera* & 19 *H. punctigera* populations have been tested so far. Average *H. armigera* LC50s varied from 14.3 at day 2 down to 0.08 at day 8. *H. punctigera* were lower at day 2 (4.9) but thereafter were not significantly different to *H. armigera*. Slopes were low (approx 1.2) initially for both species but increased with time to a maximum of 2.9 & 2.8 at day 8 in *H. armigera* & *H. punctigera*, respectively. In order to maximise the precision of the discriminating dose, assessment times with the highest slopes should be used (in this particular case, 8 days for both *H. armigera* & *H. punctigera*). However, for the sake of logistical simplicity, it was decided to settle on 1 week assessment times for both species. The average 7 day LC50s were 0.10 and 0.19 for *H. armigera* & *H. punctigera*, respectively. Between strain variability in the LC50s at 7 days was 0.03-0.20 (6.7 fold) and 0.06-0.35 (5.8 fold) in *H. armigera* & *H. punctigera*, respectively. Evaluation of possible discriminating doses indicated for *H. armigera* & *H. punctigera*, respectively :- 0.5 (97.3 & 86.3% mortality), 1.0 (99.2 & 97.2%

mortality) and 2.0 (99.6 & 99.8% mortality). Although more strains need to be screened, the early indications are that 2.0 mg DiPel 2X per ml of diet for 7 days, is the appropriate discriminating dose for early third instars of both species. This dose, as well as 0.5 & 1.0 mg DiPel 2X per ml of diet, was trialled on field populations of both *H. armigera* & *H. punctigera* in the 1993/94 season (collected as eggs from the field and reared through to early 3rd instars in the lab). For *H. armigera*, mortalities at the three potential discriminating doses of 0.5, 1.0 & 2.0 mg DiPel 2X per ml diet were:- 89.5% (n=1,431), 97.9% (n=1,420) and 98.5% (n=1,354), respectively. Similarly for *H. punctigera*, mortalities at the three potential discriminating doses were:- 83.4% (n=3,806), 96.3% (n=3,754) and 99.1% (n=3,809), respectively. These agreed well with the predicted mortalities and for future screening, 2.0 mg DiPel 2X will be used as the discriminating dose for both species. Further studies are aimed at developing resistance assays for *Bacillus thuringiensis* subspecies *aizawai* and the individual crystal proteins from Bt.

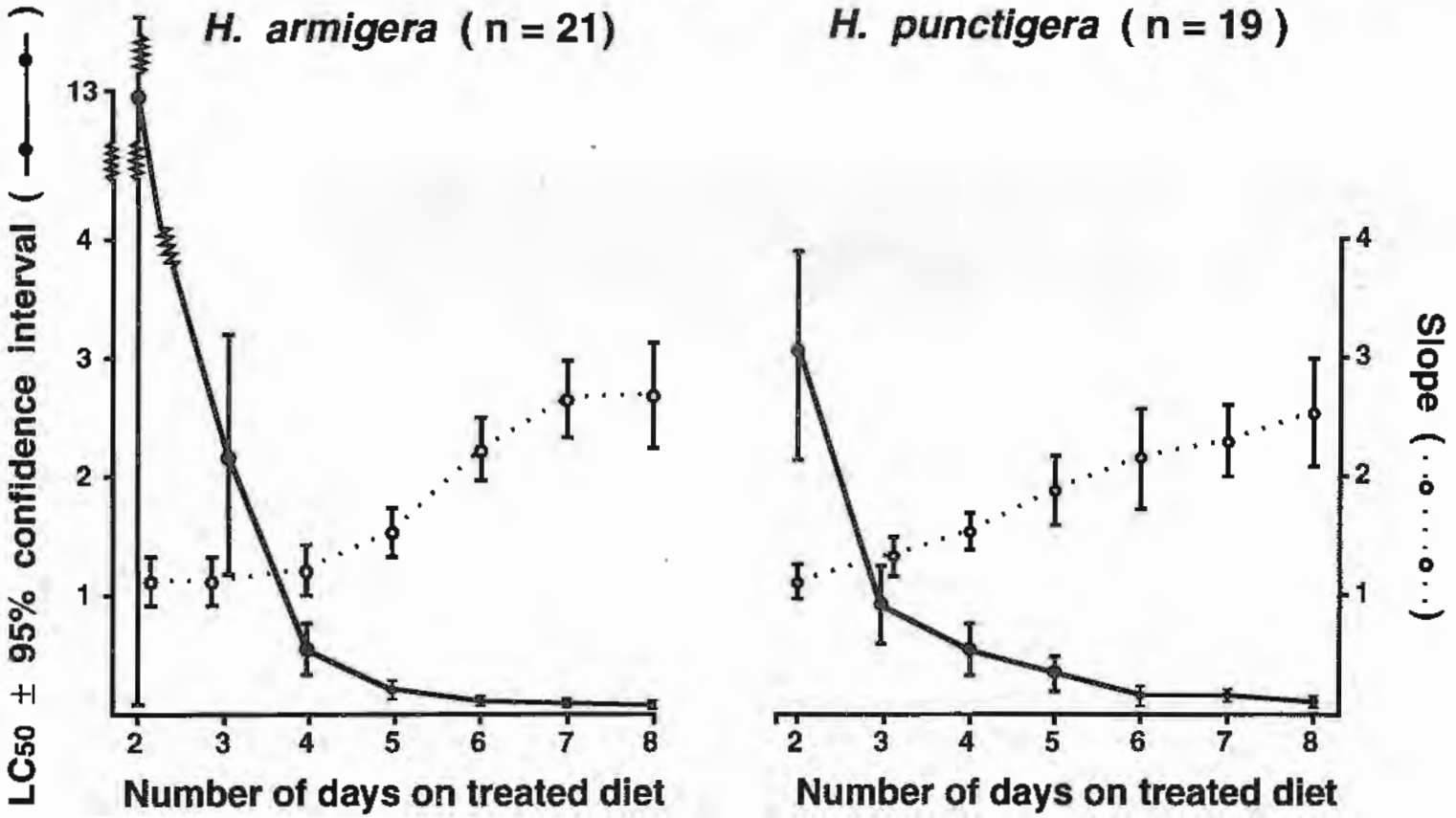
A comparison of the relative Bt susceptibility of the Australian and USA bollworm species indicate that our two species (*Helicoverpa armigera* and *Helicoverpa punctigera*) are similar in their level of susceptibility to the USA *Helicoverpa zea* species. However, the USA *Heliothis virescens* species is about 10x more susceptible to Bt and thus would be expected to be more easily controlled by transgenic cottons. Conversely, our bollworm species (and the USA *H. zea*) would be expected to be more difficult to control with transgenic cottons than *Heliothis virescens*, the key target species in the USA.

Calibration of three possible discriminating doses on *Helicoverpa armigera* and *Helicoverpa punctigera*

1993/94 season all areas

		% kill at 7 days (mg DiPel 2X / ml of diet) 0.5	1.0	2.0
H. armigera	- predicted	97.3	99.2	99.6
	- actual	89.5 n= 1,431	97.9 n= 1,420	98.5 n= 1,354
H. punctigera	- predicted	86.3	97.2	99.8
	- actual	83.4 n= 3,806	96.3 n= 3,754	99.1 n= 3,809

Average LC50 and slope for bioassay of early third instar *Helicoverpa* spp. on diet incorporated Bt



Relative Bt susceptibility of Australian and USA bollworms (USA data from Stone & Sims 1993)

	neonate LC_{50} (µg DiPel (16,000 IU / mg) per ml of diet) at 7 days	
H. armigera	average = 218	range (93 - 300)
H. punctigera	average = 180	range (126 - 260)
H. zea	average = 131	range (25 - 394)
H. virescens	average = 17	range (9 - 34)