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Cotton Research and Development Corporation

FINAL REPORT

"Use of Bt for the management of Heliothis in cotton
- Development of resistance assays for Bt"

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Abstract

A diet incorporation technique was developed to establish baseline susceptible bioassay data for populations of *Helicoverpa armigera* and *Helicoverpa punctigera* from throughout Australia for *Bacillus thuringiensis* var. *kurstaki* (DiPel 2X®, Delfin®), *Bacillus thuringiensis* var. *aizawaii* (Xentari®) and a *Bacillus thuringiensis* var. *kurstaki* / *Bacillus thuringiensis* var. *aizawaii* transconjugate strain (Agree®). Discriminating doses were evaluated and implemented (DiPel 2X® only) from the 1993/94 season for larvae reared from field collected eggs. The observed survival data for the last two seasons match closely the expected survival data, indicating no resistance to conventional Bt at this stage.

The two Australian *Helicoverpa* spp. (*H. armigera* and *H. punctigera*) are similar in their response to conventional Bt as the American *Helicoverpa zea*, with all three of these *Helicoverpa* spp. about 10x more tolerant to conventional Bt than the relatively more susceptible *Helothis virescens*. This finding has important commercial implications for control of late season *Helicoverpa* populations on maturing transgenic plants expressing decreasing levels of Bt toxins.

Helicoverpa larvae stressed with low sub-lethal doses of Bt are not more physiologically susceptible to conventional insecticides. Any predisposition to greater mortality to conventional insecticides in Bt stressed larvae is probably through smaller, starved larvae simply requiring lower doses of conventional insecticides to achieve similar mortality levels.

Smaller instars (neonates to early fourths) are killed by similar concentrations of toxin in the diet but mortality occurs a lot quicker in neonates. Larger larvae (fifth and sixth instars) require slightly higher concentrations of toxin (up to about 5x that to kill neonates) but nowhere near the concentrations that would be anticipated from the differences in larval size.

AIMS

i) To review current testing techniques and then develop a simple, cheap and sensitive resistance monitoring technique based on a discriminating dose of Bt on larvae reared from field collected eggs.

ii) To commence discriminating dose monitoring for both *Helicoverpa armigera* and *Helicoverpa punctigera* and assess natural variability in base line response.

INTRODUCTION

Bacillus thuringiensis (Bt) is a bacterial pathogen of *Helicoverpa* spp. It is produced by fermentation, formulated as either a liquid or granule and applied as a bio-pesticide through conventional ground equipment or aircraft. It is specific to larvae of the Lepidoptera, safe to beneficials, does not flare mites, aphids or whitefly and is safe to mammals, fish and other non target organisms. It is being increasingly adopted into the insect control programme of the Australian cotton industry both as a resistance management tool and as a means of reducing the industry's reliance on the environmentally liable endosulfan.

The active principle in Bt is a mixture of three insecticidal crystal proteins (Cry IAb, Cry IAc and Cry IIA) and a spore. Two of these three toxins (Cry IAc and Cry IIA) are the insecticidally active protein products being genetically engineered into transgenic cottons which will be released commercially soon in Australia (probably for the October 1997 planting). The potential development of insect resistance to these insecticidally active plants is of major concern to the cotton industry, as this technology is the best hope for the industry to solve its current resistance and environmental problems. In anticipation of this problem, researchers and others are in the process of developing a Resistance Management Strategy for transgenic cotton. A major component of this Strategy will be the implementation of a resistance monitoring programme in order to evaluate the impact of the programme and to assist in early identification of any problems so that appropriate timely measures can be undertaken.

RESULTS & DISCUSSION

1. *Development of Resistance Assays for Bt*

Three techniques for assaying Bts were evaluated. These included diet incorporation and surface comtamination and droplet feeding. The diet incorporation technique proved to be the easiest and most reliable and all further work concentrated on this method. Five commercial Bt products were incorporated into the standard soyflour, wheat germ, agar artificial diet. These five products were:-

i) DiPel 2X® (*Bacillus thuringiensis* var. *kurstaki*) from Abbott (32,000 IU per mg).
ii) Delfin® (*Bacillus thuringiensis* var. *kurstaki*) from Sandoz (53,000 SU per mg).
iii) Xentari® (*Bacillus thuringiensis* var. *aizawaii*) from Abbott (10.3% lepidopteran active toxins).
iv) Agree® (*Bacillus thuringiensis* var. *kurstaki* / *Bacillus thuringiensis* var. *aizawaii*) transconjugate strain from Ciba-Geigy.
v) MVP® (Cry IAc toxin encapsulated in dead *Pseudomonas fluorescens* cells). The work done with this product is only preliminary at this stage and results are not reported here.

Newly moulted early third instar larvae were confined on the treated diet in 24 well tissue culture trays and mortality assessed at 2-8 days at 25°C. Some work was also done on neonate larvae (see below) for comparison with USA data. However, the field screening programme concentrated on developing assays for early third instar larvae, because it is not possible to identify (non-destructively) larval *Helicoverpa* spp. until the late second instar. Bioassay proceedures were optimised eg. sonication time (two minutes), treated diet storage life (one week at 2°C), assessment time (seven days ideal for both species, LC_{50}s stabilised and slopes maximised by this time, see attached figure). The criterion for assessing mortality was the same as for conventional insecticides: no coordinated movement when prodded. Some work was initiated on weight gain inhibition assays but the results are only preliminary and are not reported here. LC_{50}s were expressed in mg product per ml of diet. The consistency of the Bt diet incorporation assay technique was evaluated by testing a number of colonies of both *Helicoverpa* spp. on repeated generations. The results indicated good repeatability (maximum of 4-6 fold variation) which compares well with the consistency evaluations for topically applied conventional insecticides such as synthetic pyrethroids and endosulfan (2-3 fold).

The four Bt products were tested on a range of field populations collected from a variety of hosts throughout Australia (see attached tables). Most were collected from the field as larvae and assayed in the following F_1 laboratory generation (occasionally on the F_2). The average 7 day LC_{50}s for the four Bt products were remarkably similar for both species (except for DiPel 2X® where *H. punctigera* was just slightly harder to kill than *H. armigera*). These average LC_{50} values will serve as the baseline susceptible response for determining any future resistance development to conventional Bts. The range in baseline response indicates tolerance factors of around 2.0 to 2.5x for all products (see attached table), so resistance factors (RFs) should be at least 3x (LC_{50} of the suspected resistant strain ÷ the average susceptible LC_{50}) before any resistance problem is suspected. Examination of the attached tables indicates 2.0 mg/ml diet as the ideal discriminating dose for all four products on both species.

2. *Resistance monitoring*

The DiPel 2X® discriminating dose indicated above has been used for the past two seasons on both *Helicoverpa* spp. collected as eggs from the field and reared in the laboratory to early third instar larvae. It is planned to add the Xentari® discriminating dose from the 1995/96 season onwards. In the first season (1993/94), the optimum discriminating dose had not yet been determined, so, in order not to lose a season's potential data, three potential discriminating doses were evaluated (0.5, 1.0 and 2.0 mg DiPel 2X®/ ml diet). However, by the second season (1994/95), the 2.0 mg dose had been confirmed as the optimum discriminating dose for both *Helicoverpa* spp. and only this dose was used from that season onwards. In the 1993/94 season, only cotton was sampled and only in three areas; the Namoi/Gwydir river valleys of northern NSW, the St. George Irrigation Area of southern Queensland and the Emerald Irrigation Area of central Queensland. However, in the following season (1994/95), sampling was extended to a range of crops over a variety of sites, including intensively sprayed tomato crops around Bundaberg and Childers in central Queensland (areas with extensive use of conventional Bts). The recorded survivals at the discriminating dose were no different from the expected survival for both *Helicoverpa* spp. in both seasons, indicating no resistance to conventional Bt at this stage (see attached tables).

3. *Relative susceptibility to Bt of Australian and USA bollworms*

It has been shown in the USA that *Heliothis virescens* is inherently more susceptible to Bts than the closely related *Helicoverpa zea* (see attached tables). It was decided to assess whether there was any such difference with our two species and to compare their susceptibility to the two American species. This is a commercially important question for transgenic cottons expressing Bt toxins as it has been shown that the end of season toxic protein levels are not high enough in the plant to kill *Helicoverpa zea* (though apparently still high enough to kill the relatively more susceptible *Heliothis virescens* species). The American data was generated with diet incorporated DiPel® (16,000 International Units / mg) on neonate larvae, so extra assays were set up using neonates of both *H. armigera* and *H. punctigera*. DiPel® (16,000 IU / mg) is unavailable in Australia, [only DiPel 2X® (32,000 IU / mg)], so LC50s were adjusted accordingly (see attached tables). The data indicated that the two Australian species have a similar response to Bt as the more tolerant American *Helicoverpa zea* species, rather than the more susceptible *Heliothis virescens* species. This is not surprising, given the also poor control of late season populations of both *Helicoverpa* species in Australia on maturing transgenic cottons.

4. *Effect of sub-lethal Bt stress on Bioassay of Conventional Insecticides*

Larvae of one *H. punctigera* and three *H. armigera* strains were stressed by feeding early 3rd instars on artificial diet containing 0.015 mg DiPel 2X (32,000 IU/mg) per ml of diet. The percentage of larvae reaching testing size (that is early 4th instars weighing 30-40 mg) by 10 days from the early 3rd instar (weighing < 5 mg) was 65-75% & 90% for *H.a* & *H.p* respectively, compared to 99% for the control diet. In addition, those larvae that did survive were about twice as slow to develop from the early 3rd instar to testing size (2.9 & 5.7 days for control and Bt diet, respectively). When larvae reached testing size, they were placed on fresh untreated diet and bioassayed with either fenvalerate, endosulfan or profenofos representing the three major classes of conventional insecticides used against *Helicoverpa* spp. in Australia.

The one *H.p* strain tested was fully susceptible to all 3 insecticides and there were no significant differences in LC50s for any of the insecticides for larvae fed on Bt or untreated diet. Of the 3 *H.a* strains tested, 2 were moderately resistant to pyrethroids with low resistance to endosulfan and no resistance to profenofos while the third had moderate resistance to both pyrethroids and endosulfan and low resistance to profenofos. For fenvalerate, the LC50s of the Bt stressed larvae were the same as for larvae on the control diet for one strain, 2.2x higher for another & 2.9x lower for the third. For endosulfan on the two low endosulfan resistance strains, the LC50s of the Bt stressed larvae were 2.5x higher for one and 1.6x lower for the other. For endosulfan on the moderately endosulfan resistant strain, the LC50 for the Bt stressed larvae was 2.4x lower. For profenofos, the LC50s of the Bt stressed larvae were the same for 2 strains and 1.4x higher for the third.

In most cases, the LC50s of Bt stressed larvae were the same as those for larvae fed on normal untreated diet (6/12 possibilities). In the other cases, there were 3 instances where Bt stressed larvae were more susceptible and another 3 where they were more tolerant.These relatively minor and inconsistent differences are well within the expected range of variability for bioassay of segregating heterogenous populations with mixed resistance mechanisms (note the lack of variability in the fully susceptible *H.p* strain and for the relatively resistance naive profenofos on *H.a*), see attached table.

These results would seem to indicate that when the effect of Bt inhibition on weight gain is allowed for, that Bt stressed larvae are not more physiologically susceptible to conventional insecticides. Any predisposition to greater mortality to conventional insecticides in Bt stressed larvae is probably through smaller, starved larvae simply requiring lower doses of conventional insecticides to achieve similar mortality levels.

5. *Effect of larval size on bioassay response to Bt in* **Helicoverpa punctigera**

The effect of larval size on the efficacy of Bt using the diet incorporation technique, was tested on the full range of instars of one strain of *Helicoverpa punctigera*. Weights ranged from 11, 57, 265, 931 to 3,000 times that of neonates for second, third, fourth, fifth & sixth instars, respectively. However, LC50s at day 8 ranged only from 1.1, 1.7, 2.1, 5.4 to 4.1 times that of neonates for second to sixth instars, respectively. Earlier assessment times did indicate larger differences from neonates but still nowhere near that of the differences in weights. For example, the greatest variation was recorded at day 2 with 7.7, 7.0, 13.1, 33.5 to 13.8 fold differences in LC50s relative to neonates for second to sixth instars, respectively.

This indicates that the smaller instars (neonates to early fourths) are killed by similar concentrations of toxin in the diet but that mortality occurs a lot quicker in neonates. Larger larvae (fifth and sixth instars) require slightly higher concentrations of toxin (up to about 5x that to kill neonates) but nowhere near the concentrations that would have been anticipated from the differences in larval size.

Future work arising from this project

i) Continue discriminating dose screens for *Bacillus thuringiensis* var. *kurstaki* and *Bacillus thuringiensis* var. *aizawaii* on field populations of both *Helicoverpa* spp.

ii) Develop a weight gain inhibition assay

iii) Develop individual insecticidal crystal protein (Cry IAc and Cry IIA) and transgenic plant discriminating dose assays

iv) Investigate sub-lethal effects of conventional Bt and transgenic plants on *Helicoverpa* spp. biology

v) Establish baselines for both conventional Bt and the two insecticidal crystal proteins above, in the non-heliothine lepidopteran pests of cotton

Publications arising from this work

A). Contributions to technical conferences

FORRESTER, N.W. & FORSELL, L. (1993). Development of resistance assays for *Bacillus thuringiensis* in Australian *Helicoverpa* spp. Second Australian Bt Workshop, Canberra, ACT.

FORRESTER, N.W. & FORSELL, L. (1993). Effect of larval size on bioassay response to *Bacillus thuringiensis* in *Helicoverpa punctigera*. Second Australian Bt Workshop, Canberra, ACT.

FORRESTER, N.W. (1994). Resistance management options for conventional *Bacillus thuringiensis* and transgenic plants in Australian summer field crops. OECD Workshop: Ecological implications of transgenic crops containing *Bacillus thuringiensis* toxin genes. Queenstown, New Zealand.

FORRESTER, N.W. & FORSELL, L. (1994). Development of resistance assays for *Bacillus thuringiensis* in Australian *Helicoverpa* spp. World Cotton Research Conference-1, Brisbane, Australia.

FORRESTER, N.W., BIRD, L. J. & FORSELL, L. (1994). Effect of sub-lethal Bt stress on bioassay of conventional insecticides in Australian *Helicoverpa* spp. World Cotton Research Conference-1, Brisbane, Australia.

FORRESTER, N.W. & FORSELL, L. (1994). Development of resistance assays for *Bacillus thuringiensis* in Australian *Helicoverpa* spp. 25th Scientific Conference of the Australian Entomological Society, Adelaide, SA.

FORRESTER, N.W. & FORSELL, L. (1994). Development of resistance assays for *Bacillus thuringiensis* in Australian *Helicoverpa* spp. 7th Australian Cotton Conference, Broadbeach, Qld.

FORRESTER, N.W., BIRD, L. J. & FORSELL, L. (1994). Effect of sub-lethal Bt stress on bioassay of conventional insecticides in Australian *Helicoverpa* spp. 7th Australian Cotton Conference, Broadbeach, Qld.

FORRESTER, N.W. & FORSELL, L. (1994). Development of discriminating dose assays for *Helicoverpa* spp. in Australia. The Pacific Rim Conference on Biotechnology of Bt and its Impact to the Environment. Taipei, Taiwan.

FORRESTER, N.W., FITT, G. P. & ROUSH, R. (1995). Resistance management options for transgenic cottons. 26th Scientific Conference of the Australian Entomological Society. Tamworth, NSW, Australia.

FORRESTER, N.W. & FORSELL, L. (1995). Development of discriminating dose assays for conventional Bts in Australian *Helicoverpa* spp. Australian Society for Microbiology Scientific Meeting and Exhibition. National Convention Centre, Canberra, ACT, Australia.

B). Advisory articles

FORRESTER, N.W. (1992). The future for Bt in cotton pest management. *The Australian Cotton Grower* 13(2) : 66-70.

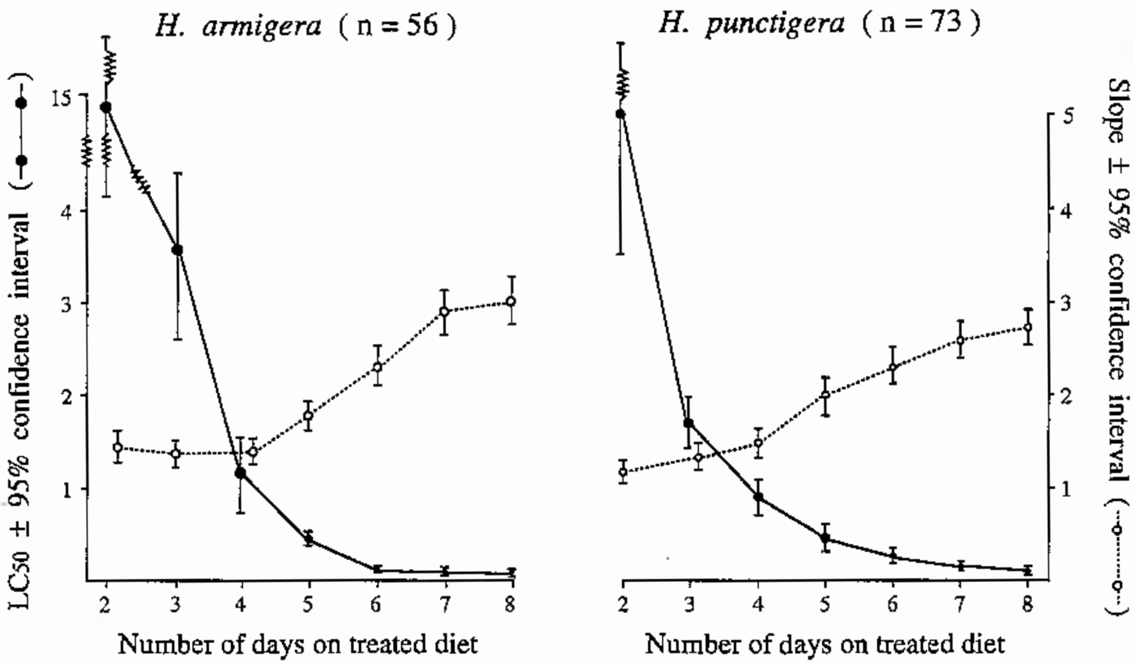
C). Refereed research publications

FORRESTER, N.W. (1994) Use of *Bt* in integrated control, especially on cotton pests. *Agriculture Ecosystems and Environment* **49**, 77-83.

FORRESTER, N.W. (1994). Resistance management options for conventional *Bacillus thuringiensis* and transgenic plants in Australian summer field crops. *in* Proceedings of the OECD Workshop: Ecological implications of transgenic crops containing *Bacillus thuringiensis* toxin genes. *Biocontrol Science & Technology* **4**, 549-553.

Budget Summary ($)

	CRDC funds	NSW Agriculture funds
1992/93	57,310	78,000
1993/94	67,926	78,000
1994/95	71,392	78,000



Mean LC_{50} (mg DiPel 2X / ml diet) and slope (both ± 95% confidence intervals) for 56 *H. armigera* and 73 *H. punctigera* strains, collected across Australia from 1993 to 1995 and tested in the F_1 as early third instars held on diet incorporated Bt for 2-8 days.

Bioassay of early third instar *Helicoverpa* spp. held for 7 days on diet incorporated *Bacillus thuringiensis* var. *kurstaki* (DiPel® or Delfin®), *Bacillus thuringiensis* var. *aizawaii* (Xentari®) or a transconjugate of *Bacillus thuringiensis* var. *kurstaki* and *Bacillus thuringiensis* var. *aizawaii* (Agree®).

Helicoverpa species and *Bacillus thuringiensis* product tested	No. of strains tested	LC50 (mg / ml diet)		Tolerance Factor* for the most tolerant susceptible strain	Slope	% Mortality at the 2.0 mg / ml diet discriminating dose
		Average	Range			
Heliothis armigera						
DiPel 2X®	56	0.11	(0.03 - 0.20)	1.8x	2.9	99.7
Delfin®	35	0.10	(0.03 - 0.24)	2.4x	3.6	99.7
Xentari®	42	0.13	(0.03 - 0.33)	2.5x	2.8	99.8
Agree®	33	0.13	(0.04 - 0.28)	2.2x	3.4	100
Heliothis punctigera						
DiPel 2X®	73	0.20	(0.06 - 0.35)	1.8x	2.3	99.3
Delfin®	50	0.10	(0.03 - 0.21)	2.1x	2.7	99.9
Xentari®	62	0.13	(0.05 - 0.26)	2.0x	2.1	99.6
Agree®	47	0.15	(0.07 - 0.36)	2.4x	2.5	99.8

* Highest recorded susceptible LC50 ÷ the average susceptible LC50

Bioassay of Agree and calibration of discriminating doses on 33 strains of *Helicoverpa armigera* collected as eggs or larvae on a range of hosts from 1994 to 1995 and tested in the F1 as early third instars on diet incorporated Bt.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Byee QLD*	chickpea	0.04	0.029	0.047	1.9	100	100	100
Mareeba QLD*	tobacco	0.07	0.042	0.116	3.2	100	100	100
Narrabri NSW	sunflower	0.07	0.054	0.096	2.7	100	100	100
Bundaberg QLD* '94	tomato	0.07	0.055	0.101	2.7	97.9	100	100
Edgeroi NSW	sorghum	0.07	0.062	0.083	3.5	100	100	100
St George QLD*	cotton	0.07	0.062	0.089	2.4	95.7	95.8	100
Wee Waa NSW	maize	0.09	0.066	0.131	2.9	97.9	100	100
Bundaberg QLD* '95	tomato	0.09	0.073	0.102	2.7	100	100	100
Kingaroy QLD	sorghum	0.09	0.079	0.110	2.9	97.9	100	100
Wee Waa NSW*	sorghum	0.10	0.076	0.146	2.8	97.9	100	100
Devonport TAS '95*	maize	0.10	0.090	0.113	5.5	100	100	100
Come-by-Chance NSW	sorghum	0.10	0.090	0.115	5.0	100	100	100
Kingaroy QLD	navy bean	0.10	0.076	0.139	2.2	93.8	100	100
Katherine NT	sorghum	0.11	0.059	0.218	4.0	100	100	100
Devonport TAS ?	maize	0.11	0.093	0.126	3.4	100	100	100
Bourke NSW*	cotton	0.11	0.099	0.132	3.4	100	100	100
Delungra NSW	sunflower	0.11	0.102	0.127	6.0	100	100	100
St George QLD '95	mung bean	0.11	0.087	0.140	3.4	95.6	100	100
St George QLD '95	mung bean	0.11	0.084	0.139	2.6	95.8	97.9	100
Emerald QLD	cotton	0.12	0.044	0.309	4.2	100	100	100
Gunnedah NSW	sorghum	0.12	0.087	0.171	3.2	100	100	100
Cassilis NSW*	sorghum	0.13	0.064	0.279	3.7	100	100	100
Boggabri NSW*	maize	0.13	0.097	0.185	3.0	95.8	95.7	100
Narrabri NSW*	cotton	0.15	0.079	0.292	3.1	91.7	100	100
Dirranbandi QLD	cotton	0.15	0.130	0.172	3.7	95.7	100	100
Bourke NSW	maize	0.16	0.144	0.186	4.6	97.8	100	100
Delungra NSW	sorghum	0.16	0.133	0.183	2.9	87.2	97.9	100
Cryon NSW	sorghum	0.17	0.060	0.500	3.6	97.9	97.9	100
Dalby QLD*	chickpea	0.17	0.149	0.205	3.0	85.4	100	100
Bellata NSW	sorghum	0.23	0.201	0.257	4.7	93.8	100	100
St George QLD*	navy bean	0.24	0.195	0.307	3.1	89.4	91.7	100
Spring Ridge NSW	sorghum	0.26	0.165	0.408	2.6	85.1	95.8	100
Emerald Hill NSW	sunflower	0.28	0.244	0.323	3.6	79.2	97.9	100
Average		0.13			3.4			

* mortality also assessed at 2-8 days for these colonies.

Bioassay of Agree and calibration of discriminating doses on 47 strains of *Helicoverpa punctigera* collected as eggs or larvae on a range of hosts from 1994 to 1995 and tested in the F_1 as early third instars on diet incorporated Bt.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Coonabarabran NSW	lucerne	0.07	0.053	0.080	2.1	97.9	100	100
Warren NSW*	lucerne	0.08	0.069	0.102	2.1	93.8	100	100
Dubbo NSW*	linseed	0.08	0.073	0.953	4.1	100	100	100
Gurley NSW	marshmallow	0.09	0.064	0.132	1.8	100	97.9	100
Bourke NSW	crownbeard	0.09	0.069	0.110	1.6	91.7	97.9	100
Bundaberg QLD* '94	tomato	0.09	0.075	0.106	2.6	97.9	100	100
Edgeroi NSW*	lucerne	0.10	0.072	0.148	2.4	95.8	100	100
Goondiwindi QLD	cotton	0.10	0.084	0.119	2.6	95.6	100	100
Moree NSW*	chickpea	0.10	0.085	0.117	2.9	100	100	100
Channel Country QLD*	wildflowers	0.10	0.082	0.115	2.7	95.7	100	100
Boort VIC*	tomato	0.10	0.073	0.143	2.7	100	100	100
Cassilis NSW	lucerne	0.11	0.076	0.150	2.5	97.9	100	100
Macquarie Marshes NSW	malvastrum	0.11	0.074	0.158	2.0	100	100	97.9
Bundaberg QLD* '95	tomato	0.11	0.094	0.129	2.9	97.9	100	100
Rochester VIC*	lucerne	0.11	0.092	0.142	2.0	97.0	100	100
Narrabri NSW	cotton	0.11	0.093	0.136	2.2	93.8	97.9	100
Manjimup WA	moths at nectar	0.12	0.098	0.136	2.7	93.8	100	100
Manilla NSW*	lucerne	0.12	0.083	0.163	2.4	100	100	100
Kununurra WA '95*	cotton	0.12	0.098	0.137	2.7	95.8	100	100
Delungra NSW	sunflower	0.13	0.092	0.172	2.5	95.8	100	100
Cowra NSW*	lucerne	0.13	0.092	0.186	2.8	100	100	100
Forbes NSW*	lucerne	0.13	0.096	0.172	1.9	93.6	95.7	100
Mareeba QLD	tobacco	0.13	0.095	0.189	2.6	91.7	100	97.9
Kununurra WA '95	cotton	0.13	0.110	0.158	2.3	95.8	97.9	100
Wee Waa NSW*	faber bean	0.13	0.079	0.220	2.8	100	100	100
Emerald Hill NSW	sunflower	0.13	0.107	0.146	3.1	100	100	100
Wellington NSW*	lucerne	0.14	0.089	0.214	1.7	92.5	100	100
Moree NSW	chenopodium	0.14	0.121	0.169	2.7	97.7	100	100
Byee QLD*	chickpea	0.15	0.126	0.168	3.6	100	100	100
Brewarrina NSW	malvastrum	0.15	0.106	0.215	2.2	100	100	97.9
Dubbo NSW*	lucerne	0.15	0.109	0.206	3.4	94.3	100	100
Narrabri NSW*	caltrop	0.15	0.129	0.178	2.8	97.9	100	100
Come-by-Chance NSW	chenopodium	0.16	0.134	0.195	2.1	89.6	91.7	100
St George QLD '95	cotton	0.17	0.114	0.240	2.2	89.4	97.9	97.9
Esperance WA	lupins	0.17	0.115	0.255	2.3	91.7	97.8	100
Bourke NSW	cotton	0.18	0.134	0.231	2.3	79.2	100	100
Spring Ridge NSW	cotton	0.18	0.127	0.247	2.3	93.8	97.9	100
Trangie NSW	tomato	0.19	0.149	0.233	1.6	74.4	89.4	97.8
St George QLD '95*	cotton	0.20	0.111	0.367	3.0	93.8	100	100
Devonport TAS	pea	0.20	0.138	0.288	2.2	87.2	100	100
Bellata NSW	sunflower	0.22	0.158	0.313	2.4	91.7	91.7	100
Hopetoun WA	lupins	0.23	0.131	0.398	1.8	77.1	100	100
Coolabah NSW	malvastrum	0.23	0.194	0.270	2.8	80.9	100	100
Merah North NSW*	faber bean	0.27	0.199	0.357	1.9	66.7	85.4	100
Waroo via Forbes NSW	lucerne	0.31	0.191	0.516	2.5	62.5	97.8	100
Albany WA*	linseed	0.32	0.175	0.581	1.9	65.5	100	100
Breeza NSW	lucerne	0.36	0.238	0.544	3.2	57.4	100	100
Average		0.15			2.5	91.6	98.7	99.8

* mortality also assessed at 2-8 days for these colonies.

Bioassay of Delfin and calibration of discriminating doses on 35 strains of *Helicoverpa armigera* collected as eggs or larvae on a range of hosts from 1994 to 1995 and tested in the F1 as early third instars on Bt incorporated diet.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Byee QLD*	chickpea	0.03	0.023	0.034	3.3	100	100	100
Moree NSW	sorghum	0.04	0.022	0.084	2.0	100	100	100
Moree NSW	sunflower	0.04	0.026	0.053	2.1	100	100	100
Bundaberg QLD* '94	tomato	0.04	0.037	0.053	2.6	97.9	100	100
Kununurra WA*	cotton	0.04	0.034	0.046	3.6	100	100	100
Kingaroy QLD	sorghum	0.05	0.040	0.058	2.4	100	100	100
St George QLD	mung bean	0.06	0.042	0.079	3.3	100	100	100
Bundaberg QLD* '95	tomato	0.06	0.048	0.064	3.8	100	100	100
Kingsthorpe QLD	maize	0.07	0.062	0.082	3.8	100	100	100
Wee Waa NSW	maize	0.07	0.048	0.091	3.8	100	100	100
Bourke NSW*	cotton	0.07	0.059	0.076	4.7	100	100	100
Narrabri NSW	cotton	0.07	0.061	0.078	4.8	100	100	100
Wee Waa NSW	sorghum	0.07	0.062	0.082	3.6	100	100	100
Narrabri NSW	sunflower	0.08	0.055	0.122	3.1	100	100	100
Katherine NT	sorghum	0.08	0.054	0.121	2.8	93.6	100	100
Mareeba QLD*	tobacco	0.08	0.073	0.091	5.8	100	100	100
Cryon NSW*	sorghum	0.09	0.062	0.129	3.3	100	100	100
Delungra NSW	sunflower	0.09	0.063	0.129	4.1	100	100	100
Dirranbandi QLD	cotton	0.09	0.068	0.129	3.3	97.9	100	100
Come-by-Chance NSW	sorghum	0.09	0.081	0.102	5.4	100	100	100
Gindie QLD	sorghum	0.10	0.086	0.120	2.7	95.6	100	100
St George QLD*	cotton	0.10	0.080	0.125	4.3	97.9	100	100
Devonport TAS*	maize	0.10	0.090	0.113	7.6	100	100	100
Boggabri NSW*	maize	0.11	0.081	0.156	3.6	100	100	100
Mullaley NSW	sorghum	0.11	0.100	0.125	5.6	100	100	100
Gunnedah NSW	sorghum	0.12	0.084	0.165	3.0	87.5	100	100
Boggabri NSW	sunflower	0.12	0.086	0.177	3.4	95.0	100	100
Delungra NSW	sorghum	0.13	0.108	0.149	2.8	100	100	100
St George QLD*	navy beans	0.15	0.097	0.226	2.2	82.2	97.9	95.8
Spring Ridge NSW	sunflower	0.15	0.053	0.442	3.7	100	100	100
Spring Ridge NSW	sorghum	0.16	0.104	0.245	3.2	93.5	100	100
Edgeroi NSW*	sorghum	0.17	0.129	0.233	2.8	85.1	100	100
Bellata NSW*	sorghum	0.19	0.091	0.386	3.6	97.9	100	97.9
Spring Ridge NSW*	cotton	0.22	0.165	0.296	2.8	85.4	95.8	100
Emerald Hill NSW	sunflower	0.24	0.179	0.312	2.5	83.3	100	95.2
Average		0.10			3.6			99.7

* mortality also assessed at 2-8 days for these colonies.

Bioassay of Delfin and calibration of discriminating doses on 50 strains of *Helicoverpa punctigera* collected as eggs or larvae on a range of hosts from 1994 to 1995 and tested in the F1-2 as early third instars on diet incorporated Bt.

Collection data		LC_{50} (@ day 7)	95% Conf. interval		Slope	% Mortality at mg/ml diet		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Warren NSW*	lucerne	0.03	0.023	0.039	1.8	100	100	100
Lakeland Downs QLD*†	lab lab	0.05	0.044	0.060	3.0	100	100	100
Hopetoun WA	lupins	0.06	0.035	0.090	2.2	100	100	100
Dubbo NSW*	linseed	0.06	0.050	0.068	3.1	100	100	100
Edgeroi NSW*	lucerne	0.06	0.051	0.071	2.7	100	100	100
Moree NSW*	chickpea	0.06	0.052	0.072	2.8	100	100	100
Bundaberg QLD* '94	tomato	0.06	0.055	0.076	2.7	100	100	100
Pallamallawa NSW*	chickpea	0.06	0.047	0.083	2.7	100	100	100
Spring Ridge NSW	field pea	0.07	0.051	0.091	2.5	100	97.9	100
Dubbo NSW*	lucerne	0.07	0.047	0.013	2.4	100	100	100
Merah North NSW*	faber bean	0.07	0.047	0.091	3.0	100	100	100
Wee Waa NSW*	faber bean	0.07	0.052	0.096	3.9	100	100	100
Manilla NSW*	lucerne	0.07	0.048	0.100	2.4	100	100	100
Bundaberg QLD* '95	tomato	0.07	0.062	0.081	3.9	100	100	100
Narrabri NSW	caltrop	0.07	0.057	0.084	2.1	91.7	100	100
Esperance WA	lupins	0.07	0.060	0.083	2.8	100	100	100
Moree NSW	chenopodium	0.08	0.045	0.131	2.0	100	100	100
Coonabarabran NSW	lucerne	0.08	0.048	0.132	2.0	100	100	100
Spring Ridge NSW	cotton	0.08	0.057	0.104	2.3	100	100	100
Manjimup WA	moths at nectar	0.08	0.058	0.097	3.3	95.8	100	100
Murgon QLD	lucerne	0.08	0.059	0.119	2.5	94.4	97.9	100
Byee QLD*	chickpea	0.08	0.071	0.093	4.1	100	100	100
Narrabri NSW*	lucerne	0.09	0.050	0.176	2.7	100	100	100
Mareeba QLD	tobacco	0.09	0.058	0.134	2.6	97.9	100	100
Narrabri NSW	cotton	0.09	0.067	0.118	2.4	97.9	100	100
Boort VIC	tomato	0.09	0.072	0.107	2.4	95.0	100	100
Albany WA*	lucerne	0.09	0.050	0.153	2.3	100	100	100
Devonport TAS	pea	0.09	0.076	0.111	3.3	100	100	100
Channel Country QLD*	wildflowers	0.09	0.071	0.116	3.4	100	100	100
Wellington NSW	lucerne	0.10	0.070	0.137	2.3	100	100	100
Trangie NSW	tomato	0.10	0.082	0.112	3.0	97.9	100	100
Coolabah NSW	malvastrum	0.10	0.086	0.118	2.9	100	100	100
Brewarrina NSW	malvastrum	0.11	0.070	0.169	1.9	100	100	100
Cassilis NSW	lucerne	0.11	0.081	0.146	2.5	100	100	100
Kununurra WA*	cotton	0.11	0.097	0.131	3.2	93.6	100	100
Urrbrae SA	daisy	0.11	0.091	0.135	2.1	91.7	97.9	100
Bourke NSW	crownbeard	0.12	0.078	0.195	2.2	95.8	97.9	100
St George QLD	cotton	0.12	0.082	0.175	3.3	100	100	100
Warren NSW '95	cotton	0.12	0.098	0.148	2.3	95.8	100	100
Attunga NSW*	lucerne	0.12	0.100	0.137	3.0	97.9	100	100
Gurley NSW*	marshmallow	0.12	0.101	0.140	2.8	100	97.9	100
Warren NSW '95	cotton	0.12	0.107	0.144	3.2	97.9	100	100
Macquarie Marshes NSW	malvastrum	0.13	0.090	0.188	2.3	95.8	100	100
Cowra NSW*	canola/lucerne	0.15	0.073	0.307	3.1	100	100	100
Forbes NSW	lucerne	0.15	0.108	0.205	2.0	95.8	95.7	97.9
Rochester VIC	lucerne	0.15	0.111	0.196	2.1	91.7	100	100
Condobolin NSW	lucerne	0.15	0.111	0.207	3.0	93.6	100	100
Bourke NSW	cotton	0.16	0.140	0.184	4.1	95.8	100	100
Bellata NSW	sunflower	0.17	0.138	0.201	2.2	83.3	95.8	100
Come-by-Chance NSW	chenpodium	0.21	0.183	0.245	3.9	97.9	97.9	100
Average		0.10			2.7	97.9	99.6	99.9

† indicates colony tested in the F2 generation, all other colonies tested in the F1.
* mortality also assessed at 2-8 days for these colonies.

Bioassay of DiPel 2X and calibration of discriminating doses on 56 strains of *Helicoverpa armigera* collected as eggs, larvae o pupae on a range of hosts from 1993 to 1995 and tested in the F_{1-2} as early third instars on diet incorporated Bt.

Collection data		LC50 @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Narrabri NSW '93*	cotton	0.03	0.015	0.040	1.7	100	100	100
Mareeba QLD '95	tobacco	0.03	0.017	0.046	1.6	97.7	100	100
Lab colony (pyrethroid resistant)		0.05	0.032	0.064	1.9	100	100	100
Boggabri NSW*	cotton	0.05	0.035	0.058	2.3	100	100	100
Byee QLD	chickpea	0.05	0.031	0.083	1.6	97.6	97.5	95
Wee Waa NSW ?*†	cotton	0.05	0.041	0.062	2.6	100	100	100
Lab colony (susceptible)		0.04	0.038	0.050	4.0	100	100	100
Bundaberg QLD '94*	tomato	0.06	0.038	0.100	1.8	100	100	100
St George QLD '93*	cotton	0.06	0.049	0.070	2.9	100	100	100
St George QLD '93*	cotton	0.06	0.047	0.075	2.2	97.6	100	100
Bongeen QLD*	cotton	0.06	0.050	0.071	2.4	100	100	100
Wee Waa NSW	maize	0.06	0.052	0.072	2.9	100	100	100
Wee Waa NSW*	faba bean	0.07	0.057	0.081	2.5	100	100	100
Mareeba QLD '93*	tobacco	0.07	0.056	0.075	3.8	100	100	100
Camballin WA*	cotton	0.07	0.059	0.080	3.5	100	100	100
Emerald QLD*	cotton	0.07	0.060	0.083	3.1	100	100	100
[illegible]oktown QLD*	lab lab	0.08	0.069	0.090	4.2	100	100	100
Bundaberg QLD '95	tomato	0.08	0.058	0.123	2.1	97.9	100	100
Katherine NT	sorghum	0.08	0.069	0.101	2.1	95.7	95.8	100
Kununurra WA '95*	cotton	0.09	0.084	0.107	5.1	100	100	100
Merah North NSW*	cotton	0.09	0.077	0.109	2.7	100	100	100
Wee Waa NSW*	potato	0.09	0.080	0.106	3.5	97.9	100	100
Narrabri NSW '93*	cotton	0.09	0.080	0.109	3.2	100	100	100
Lab colony (endosulfan resistant) F3		0.10	0.079	0.115	2.4	100	100	100
Mareeba QLD '94	tobacco	0.10	0.072	0.148	3.1	100	100	100
Bourke NSW	cotton	0.10	0.071	0.148	2.3	95.8	100	100
Narrabri NSW ?*	cotton	0.10	0.081	0.111	3.1	97.7	100	100
Kununurra WA '93*	cotton	0.10	0.081	0.113	2.9	100	100	100
Chinchilla/Miles QLD*	cotton	0.10	0.084	0.115	2.9	97.9	100	100
Warra QLD*†	cotton	0.11	0.091	0.124	3.3	100	100	100
Wee Waa NSW '93*	cotton	0.11	0.094	0.133	2.5	95.8	100	100
Moree NSW*†	cotton	0.11	0.098	0.135	2.8	93.8	100	100
Dolgelly NSW*†	cotton	0.12	0.096	0.138	2.4	97.9	100	100
Wee Waa NSW	sorghum	0.12	0.102	0.144	2.5	93.8	100	100
Wee Waa NSW*	mung bean	0.13	0.104	0.150	2.4	86.0	100	100
Narrabri NSW '94*	cotton	0.13	0.111	0.146	4.1	100	100	100
Devonport TAS	maize	0.13	0.115	0.148	4.5	100	100	100
Cassilis NSW	sorghum	0.14	0.095	0.218	3.2	100	100	100
Boggabri NSW*†	cotton	0.14	0.116	0.168	2.2	83.3	100	100
Bundaberg QLD '94*	tomato	0.14	0.118	0.163	2.8	97.9	100	100
[illegible]es QLD*	cotton	0.14	0.120	0.167	2.7	91.7	100	100
Goondiwindi QLD*	cotton	0.14	0.122	0.160	4.2	95.8	100	100
Lakeland Downs QLD*	lab lab	0.14	0.124	0.167	3.4	93.8	100	100
Darlington Point NSW*	sweet corn	0.15	0.130	0.173	3.6	100	100	100
St George QLD '93*†	cotton	0.15	0.124	0.171	2.9	91.7	97.9	100
Boort VIC*†	maize	0.16	0.134	0.186	2.8	97.9	97.9	100
Nevertire NSW*	cotton	0.16	0.141	0.188	3.6	93.8	100	100
Narrabri NSW '95*	cotton	0.17	0.147	0.195	3.6	97.9	100	100
Spring Ridge NSW	cotton	0.18	0.124	0.274	3.2	100	100	100
Rockhampton QLD*	sorghum	0.18	0.148	0.217	2.1	87.5	95.8	91.7
Boggabri NSW*	maize	0.18	0.154	0.219	2.4	85.1	100	97.9
Bellata NSW	sorghum	0.19	0.166	0.222	3.5	97.9	100	100
Edgeroi NSW	sorghum	0.19	0.168	0.223	3.6	93.6	97.9	100
Come-by-Chance NSW	sorghum	0.19	0.171	0.218	4.9	97.9	100	100
Cryon NSW	sorghum	0.20	0.113	0.364	2.2	93.8	97.9	100
Narrabri NSW*	adzuki bean	0.20	0.167	0.239	2.3	89.6	91.7	100
Average		0.11			2.9	97.0	99.5	99.7

† indicates colony tested in the F2 generation, all other colonies tested in the F1 or long term lab colonies.
* mortality also assessed at 2-8 days for these colonies.

Bioassay of DiPel 2X and calibration of discriminating doses on 73 strains of *Helicoverpa punctigera* collected as eggs, larvae or pupae on a range of hosts from 1993 to 1995 and tested in the $F_{1\text{-}2}$ as third instars on diet incorporated Bt.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	(mg/ml diet)	lower	upper		0.5	1.0	2.0
Emerald QLD '93*	cotton	0.06	0.044	0.079	1.8	92.9	100	100
Emerald QLD '94*†	cotton	0.07	0.058	0.086	2.9	100	100	100
Wee Waa NSW*	potato	0.09	0.071	0.103	2.2	97.9	100	100
Wee Waa NSW* '94	faba bean	0.11	0.080	0.160	1.9	100	100	100
Bundaberg QLD '95	tomato	0.11	0.091	0.132	2.2	95.7	100	100
Spring Ridge NSW	field pea	0.11	0.093	0.138	2.0	85.1	97.9	100
Warren NSW	cotton	0.12	0.097	0.142	2.1	91.7	100	100
Merah North NSW*	faba bean	0.12	0.097	0.142	2.1	93.8	100	100
Warren NSW*	lucerne	0.13	0.091	0.185	1.7	83.3	100	100
Coonabarabran NSW	lucerne	0.13	0.096	0.186	2.2	100	95.8	97.9
St George QLD '95	cotton	0.13	0.105	0.149	2.4	93.8	100	100
Quandialla NSW	canola	0.13	0.107	0.157	2.2	91.5	100	100
Moree NSW '94*	cotton	0.13	0.108	0.148	3.1	97.9	100	100
Narrabri NSW '93*†	cotton	0.13	0.111	0.158	2.6	100	100	100
Narrabri NSW*	faba bean	0.13	0.113	0.159	2.7	100	100	100
Wellington NSW	lucerne	0.14	0.092	0.228	1.6	82.2	83.3	100
Esperance WA	lupins	0.14	0.112	0.177	1.5	80.9	93.5	100
Boggabri NSW*	chickpea	0.14	0.118	0.163	2.9	97.9	100	100
Byee QLD	chickpea	0.14	0.113	0.166	2.0	91.5	93.6	100
Lakeland Downs QLD*†	lab lab	0.14	0.119	0.160	3.3	100	100	100
Urrbrae SA	daisy	0.15	0.093	0.229	1.8	93.8	100	97.9
Camballin WA*	cotton	0.15	0.128	0.182	2.5	95.8	100	100
Bundaberg QLD '93*	tomato	0.15	0.122	0.173	2.5	66.7	97.8	100
Bourke NSW*	cotton	0.16	0.137	0.187	3.5	95.4	100	100
Wee Waa NSW '95	faber bean	0.16	0.096	0.283	2.3	93.6	100	100
St George QLD '94*	cotton	0.16	0.138	0.197	2.4	93.8	97.9	100
Narrabri NSW	lucerne	0.17	0.103	0.290	1.8	89.6	100	100
Rochester VIC	lucerne	0.17	0.125	0.229	2.2	91.7	100	97.2
Forbes NSW '94*	lucerne	0.17	0.142	0.209	2.2	81.3	95.5	100
Burren Junction NSW*	faba beans	0.17	0.143	0.200	2.7	95.8	100	100
Dubbo NSW	lucerne	0.18	0.099	0.333	2.5	95.0	100	100
St George QLD '95	cotton	0.18	0.130	0.260	2.0	78.9	97.9	100
Narrabri NSW	caltrop	0.18	0.137	0.249	2.4	78.8	100	100
Albany WA	lupins	0.19	0.120	0.287	1.9	83.3	93.8	100
Attunga NSW	lucerne	0.19	0.152	0.229	1.9	81.3	97.4	97.9
Narrabri NSW*	chickpea	0.19	0.162	0.225	2.8	89.6	100	100
Merah North NSW*	cotton	0.19	0.163	0.227	2.8	93.4	95.8	100
Bowen QLD*	cotton	0.19	0.158	0.225	2.5	82.6	100	100
Wee Waa NSW*	lupins	0.20	0.101	0.389	2.6	100	100	100
Edgeroi NSW	lucerne	0.20	0.110	0.348	2.3	91.7	97.8	100
Boggabri NSW	faba beans	0.20	0.117	0.351	2.8	95.8	100	100
Dubbo NSW*	linseed	0.20	0.134	0.297	2.9	89.8	97.9	100
Emerald QLD '94*	cotton	0.20	0.175	0.233	3.6	95.8	100	100
Bundaberg QLD '94*	tomato	0.21	0.132	0.339	2.5	85.4	93.5	100
Hopetoun WA	lupins	0.21	0.140	0.316	1.8	64.6	100	100
Narrabri NSW '93*	cotton	0.21	0.180	0.253	3.0	90.2	97.4	100
Channel Country QLD	wildflowers	0.21	0.174	0.246	2.6	86.4	97.5	100
Brewarrina NSW	malvastrum	0.22	0.123	0.378	1.9	79.2	97.9	100
Walgett NSW*	cotton	0.22	0.182	0.255	2.7	87.5	100	100
Narrabri NSW '94*	cotton	0.22	0.185	0.268	2.2	83.3	95.7	100
Kununurra WA*	cotton	0.22	0.186	0.270	2.1	70.2	95.6	100
Moree NSW '94*	cotton	0.22	0.185	0.260	2.6	82.9	100	100
Manjimup WA	moths at nectar	0.23	0.131	0.419	2.2	90.9	100	100
Waroo via Forbes NSW	lucerne	0.23	0.138	0.394	1.7	75.0	87.5	100
Forbes NSW '94	lucerne	0.24	0.169	0.339	1.7	66.7	86.4	100
Durham Downs QLD*	weeds	0.24	0.199	0.289	2.1	85.4	95.8	97.9
Rochester VIC*	tomato	0.24	0.205	0.289	2.5	87.5	97.8	100
Walgett NSW*	chickpea	0.25	0.216	0.292	3.3	95.8	97.9	100
Boort VIC*	tomato	0.25	0.159	0.408	1.7	53.3	100	93.5
Trangie NSW*	tomato	0.26	0.152	0.439	1.7	71.7	83.3	97.9
Devonport TAS	pea	0.26	0.166	0.410	2.5	76.6	100	100
Moree NSW	chenopodium	0.27	0.182	0.410	1.9	70.8	93.8	95.8
Kununurra WA '95	lab lab	0.27	0.204	0.362	2.2	75.0	93.8	97.9
St George QLD '93*	cotton	0.29	0.240	0.350	2.1	64.5	91.3	95.5
Darling Downs QLD*	cotton	0.30	0.253	0.364	2.3	78.8	87.3	97.9
Narrabri NSW '95	cotton	0.31	0.224	0.434	1.8	61.9	87.5	95.8
Kununurra WA '94*	lab lab	0.32	0.261	0.380	2.1	69.6	77.9	100
Manilla NSW	lucerne	0.34	0.174	0.669	1.4	41.7	100	97.9
Breeza NSW	lucerne	0.34	0.206	0.574	2.2	52.1	95.8	100
Come-by-Chance NSW	chenopodium	0.34	0.247	0.468	2.2	61.4	85.4	100
Simpson Desert NT*	daisy	0.34	0.284	0.407	2.3	62.5	87.5	100
Dolgelly NSW*	cotton	0.35	0.280	0.435	1.9	66.7	86.1	86.1
Goondiwindi QLD*	cotton	0.35	0.303	0.407	3.4	67.9	97.9	100
Average		0.20	0.15	0.27	2.31	84.0	96.5	99.3

† indicates colony tested in the F_2 generation, all other colonies tested in the F_1.
* mortality also assessed at 2-8 days for these colonies.

Bioassay of Dipel 2X bioassays on 19 strains of *Helicoverpa armigera* collected as eggs, larvae or pupae on a range of hosts from 1992 to 1995 and tested as neonates on diet incorporated Bt.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.
Nevertire NSW	cotton	0.03	0.022	0.047	2.9	100	100	10
Narrabri NSW '93	cotton	0.05	0.036	0.059	1.5	100	100	10
Devonport TAS	maize	0.06	0.042	0.081	2.6	100	100	10
Narrabri NSW '94	cotton	0.06	0.047	0.085	3.9	100	100	10
Wee Waa NSW	sorghum	0.06	0.050	0.069	2.8	100	100	10
Gindie QLD	sorghum	0.07	0.058	0.077	4.1	100	100	10
Wee Waa NSW	maize	0.07	0.060	0.085	2.5	100	100	10
Mareeba QLD	tobacco	0.07	0.047	0.091	2.4	100	100	10
St George QLD ?	cotton	0.08	0.050	0.135	3.1	100	100	10
Kununurra WA	cotton	0.08	0.060	0.111	3.1	100	100	10
Bundaberg QLD	tomato	0.08	0.062	0.106	3.0	100	100	10
Byee QLD	chickpea	0.08	0.052	0.138	2.4	100	100	10
Devonport TAS	maize	0.08	0.053	0.110	2.6	100	100	10
Bundaberg QLD '94	tomato	0.08	0.053	0.115	3.7	100	100	10
Lakeland Downs QLD F_2	lab lab	0.08	0.063	0.097	2.0	100	100	10
Cassilis NSW	sorghum	0.09	0.062	0.139	2.8	100	100	10
St George QLD	mung bean	0.10	0.087	0.120	2.9	97.9	100	10
Narrabri NSW F_6* '93	cotton	0.13	0.111	0.153	3.0	93.5	100	10
St George QLD F_5* '94	cotton	0.15	0.126	0.180	2.6	100	100	10

Bioassay of Dipel 2X bioassays on 27 strains of *Helicoverpa punctigera* collected as eggs, larvae or pupae on a range of hosts from 1992 to 1995 and tested as neonates on diet incorporated Bt.

Collection data		LC_{50} @ day 7	95% Conf. interval		Slope	% Mortality @ day 7 (mg/ml diet)		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Wee Waa NSW	faba bean	0.05	0.040	0.065	1.8	100	100	100
Bellata NSW F2	sunflower	0.06	0.047	0.075	1.7	100	100	100
Channel Country QLD	wildflowers	0.06	0.040	0.094	2.9	100	100	100
Narrabri NSW F6* '93	cotton	0.06	0.052	0.078	2.1	100	100	100
Bundaberg QLD ?	tomato	0.07	0.046	0.119	2.0	97.8	100	100
Bellata NSW F2	sunflower	0.07	0.061	0.084	3.3	100	100	100
Walgett NSW	chickpea	0.08	0.554	0.128	1.9	100	100	100
Narrabri NSW F2 '93	cotton	0.08	0.063	0.093	1.9	97.9	100	100
Lakeland Downs QLD F2	lab lab	0.08	0.063	0.091	2.3	100	100	100
Merah North NSW '95	faber bean	0.08	0.070	0.100	2.4	100	100	100
Wee Waa NSW	potato	0.08	0.069	0.101	2.7	100	100	100
Dubbo NSW	linseed	0.08	0.053	0.134	2.2	95.6	100	100
Narrabri NSW	chickpea	0.10	0.088	0.123	3.2	100	100	100
Bundaberg QLD '95	tomato	0.10	0.070	0.140	2.1	100	100	100
Byee QLD	chickpea	0.10	0.068	0.153	2.6	100	100	100
Boggabri NSW	lucerne	0.11	0.066	0.172	2.6	100	100	100
Kununurra QLD '95	cotton	0.11	0.73	0.152	2.5	100	100	100
Boggabri NSW	faba bean	0.11	0.068	0.176	2.5	100	100	100
Emerald QLD	cotton	0.11	0.082	0.145	2.7	100	100	100
Burren Junction NSW	faba bean	0.11	0.091	0.130	2.5	100	100	100
Wee Waa NSW	lupins	0.12	0.074	0.199	1.9	100	100	100
Simpson Desert NT F2	daisy	0.12	0.093	0.162	1.4	100	100	100
Narrabri F17 * '94	cotton	0.13	0.111	0.160	2.3	97.8	100	100
Goondiwindi QLD	chickpea	0.14	0.097	0.192	3.0	97.7	100	100
Boggabri NSW	chickpea	0.16	0.101	0.266	2.5	100	100	100
Kununurra WA '95	cotton	0.16	0.119	0.203	3.2	100	100	100
Merah North NSW '94	faba beans	0.18	0.151	0.231	2.2	100	100	100
Average		0.10			2.4	99.5	100	100

Bioassay of Xentari and calibration of discriminating doses on 42 strains of *Helicoverpa armigera* collected as eggs, larvae or pupae on a range of hosts from 1993 to 1995 and tested in the F_{1-2} as early third instars on diet incorporated Bt.

Collection data		LC_{50} (@ day 7)	95% Conf. interval		Slope	% Mortality at mg/ml diet		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Rockhampton QLD*†	sorghum	0.03	0.017	0.042	1.7	100	95.8	100
Wooroolin QLD*	chickpea	0.03	0.018	0.054	1.8	100	100	100
Boggabri NSW '94*	cotton	0.04	0.030	0.054	2.2	97.6	100	100
Byee QLD*	chickpea	0.04	0.024	0.066	2.3	100	100	100
Emerald QLD*	cotton	0.05	0.037	0.055	3.1	100	100	100
Moree NSW	sorghum	0.05	0.042	0.064	2.0	100	100	100
Kununurra WA*	cotton	0.06	0.047	0.064	3.3	100	100	100
Mareeba QLD*	tobacco	0.06	0.053	0.078	2.2	97.9	100	100
Wee Waa NSW*	sorghum	0.07	0.056	0.089	1.7			100
Wee Waa NSW*	potato	0.07	0.063	0.084	3.6	100	100	100
Nevertire NSW*	cotton	0.08	0.064	0.090	3.2	100	100	100
Moree NSW*	cotton	0.08	0.066	0.090	3.0	100	100	100
Lakeland Downs QLD*	lab lab	0.09	0.078	0.112	2.2	95.8	97.9	100
Delungra NSW	sunflower	0.10	0.090	0.120	3.5	100	100	100
Devonport TAS '95	maize	0.11	0.077	0.158	2.9	100	100	100
Bundaberg QLD	tomato	0.11	0.082	0.152	2.6	97.9	100	100
Goondiwindi QLD*	cotton	0.11	0.090	0.128	2.6	100	95.7	100
Dirranbandi QLD	cotton	0.12	0.089	0.158	2.6	97.9	95.8	100
Wee Waa NSW	maize	0.12	0.103	0.139	3.8	100	100	100
Wee Waa NSW*	faba bean	0.12	0.105	0.135	4.8	100	100	100
Katherine NT	sorghum	0.12	0.105	0.145	2.9	100	100	100
Emerald Hill NSW	sunflower	0.13	0.098	0.180	2.3	84.8	100	100
Boort VIC*	maize	0.13	0.104	0.151	2.3	100	100	100
Boggabri NSW '94*†	cotton	0.13	0.109	0.151	2.8	97.9	100	100
Spring Ridge NSW	sunflower	0.13	0.109	0.164	1.8			
Miles QLD*	cotton	0.14	0.118	0.166	2.6	91.5	97.9	100
Devonport TAS '95	maize	0.15	0.092	0.241	3.4	97.9	97.9	100
Dolgelly NSW*†	cotton	0.15	0.123	0.177	2.3	91.7	100	100
Cryon NSW	sorghum	0.16	0.083	0.316	3.4	95.8	100	100
Cassilis NSW	sorghum	0.16	0.093	0.288	1.9	100	100	100
St George QLD	cotton	0.17	0.073	0.386	3.5	91.7	100	100
Mullaley NSW	sorghum	0.17	0.085	0.331	1.7	97.6	100	97.1
Boggabri NSW*	maize	0.17	0.095	0.306	3.2	100	100	100
Edgeroi NSW	sorghum	0.18	0.128	0.250	2.7	100	91.7	97.9
Bellata NSW	sorghum	0.18	0.139	0.244	3.9	100	100	100
Come-by-Chance NSW	sorghum	0.19	0.163	0.215	3.7	95.8	100	100
Narrabri NSW	cotton	0.20	0.176	0.235	3.6	89.6	100	100
Dalby QLD	chickpea	0.21	0.171	0.266	1.7	70.7	95.7	97.9
Boggabri NSW	sunflower	0.21	0.181	0.241	3.6	89.6	100	100
Spring Ridge NSW	sorghum	0.24	0.205	0.281	3.0	85.4	96.2	100
Delungra NSW	sorghum	0.32	0.193	0.547	2.8	89.4	95.8	100
Spring Ridge NSW	cotton	0.33	0.289	0.390	3.8	70.8	93.8	100
Average		0.13			2.8		98.9	99.8

† indicated colony tested in the F_2 generation, all other colonies tested in the F_1 .
* mortality also assessed at 2-8 days for these colonies.

Bioassay of Xentari and calibration of discriminating doses on 62 strains of *Helicoverpa punctigera* collected as eggs or larvae on a range of hosts from 1993 to 1995 and tested in the F1-2 as early third instars on diet incorporated Bt.

Collection data		LC_{50} (@ day 7)	95% Conf. interval		Slope	% Mortality at mg/ml diet		
Site	Host	mg/ml diet	lower	upper		0.5	1.0	2.0
Dirranbandi QLD	cotton	0.05	0.021	0.096	1.1	92.3	95.2	100
Narrabri NSW	caltrop	0.05	0.037	0.062	1.6	100	100	100
St George QLD '95	cotton	0.05	0.039	0.064	1.6		100	97.9
Lakeland Downs QLD*†	lab lab	0.06	0.047	0.066	2.7	100	100	100
Quandialla NSW	canola	0.06	0.048	0.075	1.8		97.9	100
Wee Waa NSW*	faba bean	0.06	0.053	0.078	2.2	97.9	100	100
Warren NSW	lucerne	0.07	0.055	0.089	1.6	89.6	100	100
Edgeroi NSW	sunflower	0.07	0.049	0.102	2.3	100	100	100
Moree NSW '95	cotton	0.08	0.067	0.106	1.8	93.8	97.9	100
Warren NSW '95	cotton	0.09	0.051	0.149	2.0	100	100	100
Trangie NSW	tomato	0.09	0.062	0.143	1.7	97.9	100	100
St George QLD '95	cotton	0.09	0.066	0.112	1.4	97.9	100	100
Delungra NSW	sunflower	0.09	0.075	0.111	2.0	91.7	97.9	100
Mareeba QLD	tobacco	0.09	0.076	0.109	2.4	97.9	100	100
Moree NSW* '94	cotton	0.09	0.078	0.106	3.4	100	100	100
Durham Downs QLD*	weeds	0.09	0.078	0.112	2.2	97.9	100	100
Spring Ridge NSW	cotton	0.10	0.075	0.120	1.6	91.7	91.5	97.9
Boggabri NSW	faba bean	0.10	0.082	0.122	2.0	91.7	100	100
Wee Waa NSW*	lupins	0.10	0.082	0.114	2.7	100	100	100
Coonabarabran NSW*	lucerne	0.10	0.083	0.124	1.9	97.8	97.9	97.9
Wee Waa NSW*	potato	0.11	0.083	0.135	1.5	95.8	97.9	100
Hopetoun WA	lupins	0.11	0.073	0.172	1.9		97.9	100
Esperance WA	lupins	0.11	0.089	0.137	1.9		97.9	100
Goondiwindi QLD	chickpea	0.11	0.090	0.126	2.7	93.8	100	97.9
Macquarie Marshes NSW	malvastrum	0.11	0.093	0.140	1.9	85.4	100	100
Merah North NSW*	faba bean	0.11	0.096	0.137	2.5	95.8	100	100
Edgeroi NSW	lucerne	0.12	0.072	0.202	1.9	95.8	100	100
Manjimup WA	moths at nectar	0.12	0.087	0.169	2.1	95.9	100	100
Narrabri NSW	cotton	0.12	0.094	0.146	1.7	79.1	100	100
Waroo via Forbes NSW	lucerne	0.13	0.089	0.191	2.3	97.8	100	100
Spring Ridge NSW	field pea	0.13	0.081	0.201	1.6		100	100
Bourke NSW	cotton	0.13	0.103	0.163	2.2	91.7	96.6	100
Burren Junction NSW*	faba bean	0.13	0.109	0.156	2.4	97.9	100	100
Devonport TAS	pea	0.13	0.110	0.162	2.1	89.4	97.9	100
Warren NSW '95	cotton	0.14	0.087	0.215	1.6	89.6	100	100
Narrabri NSW*	faba bean	0.14	0.112	0.165	2.1	93.8	100	100
Cassilis NSW	lucerne	0.14	0.116	0.172	2.2	87.5	97.9	100
Warren NSW '95	cotton	0.15	0.100	0.233	1.8			
Manilla NSW	lucerne	0.15	0.115	0.184	1.6	71.4	92.9	100
Forbes NSW	lucerne	0.15	0.171	0.266	1.7	94.4	100	100
Kununurra WA*	cotton	0.16	0.128	0.191	1.9	82.8	95.8	97.9
Urrbrae SA	daisy	0.16	0.093	0.270	2.1	100	97.9	100
Boort VIC	tomato	0.16	0.110	0.240	2.0	83.3	97.9	100
Attunga NSW*	lucerne	0.16	0.117	0.230	2.3	91.7	100	100
Bundaberg QLD*	tomato	0.16	0.136	0.197	2.2	85.4	95.8	100
Kununurra WA* '94	lab lab	0.16	0.138	0.191	2.8	93.5	97.9	100
Narrabri NSW	lucerne	0.17	0.118	0.233	2.0	93.8	97.9	97.9
Albany WA	linseed	0.17	0.139	0.203	2.2	87.2	95.8	97.6
Darling Downs QLD*	cotton	0.17	0.139	0.214	1.6	87.5	89.6	95.8
Coolabah NSW	pigweed	0.17	0.140	0.211	1.9	70.8	100	100
Spring Ridge	sunflower	0.17	0.094	0.145	1.7	89.6	91.7	100
Kunanurra WA* '95	lab lab	0.18	0.116	0.287	1.8	85.4	97.9	100
Narrabri NSW*	chickpea	0.18	0.152	0.211	2.7	85.4	97.9	100
Walgett NSW*	chickpea	0.19	0.158	0.227	2.5	95.7	93.8	100
Cowra NSW	canola/lucerne	0.19	0.156	0.226	2.1	85.4	93.3	100
Bourke NSW	crownbeard	0.20	0.145	0.268	2.3	85.4	100	100
Rochester VIC*	lucerne	0.20	0.172	0.240	2.7	85.4	97.8	100
Dubbo NSW	linseed	0.21	0.145	0.305	2.4	85.4	100	100
Boggabri NSW	lucerne	0.21	0.177	0.240	3.0	85.4	100	100
Boggabri NSW*	chickpea	0.21	0.152	0.281	3.8	97.9	100	100
Dolgelly NSW*	cotton	0.23	0.190	0.288	1.8	74.5	85.4	93.8
Bellata NSW	sunflower	0.26	0.155	0.422	1.7	77.1	89.6	97.9
Average		0.13			2.1	91.1	97.9	99.6

† indicated colony tested in the F2 generation, all other colonies tested in the F1 .
* mortality also assessed at 2-8 days for these colonies.

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

Helicoverpa / Heliothis species tested	Neonate LC_{50} (µg DiPel* [16,000 IU / mg] per ml of diet) at 7 days		Number of strains tested
	average	range	
H. armigera	160	(60 - 300)	19
H. punctigera	200	(100 - 360)	27
H. zea	137	(25 - 394)	15
H. virescens	17	(9 - 34)	12

* Australian species tested with DiPel 2X® (32,000 IU / mg). The LC50s of the two Australian species were converted to DiPel (16,000 IU / mg) equivalents by doubling the LC50s for DiPel 2X for these two species.

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 56 *H. armigera* and 73 *H. punctigera* strains, collected across Australia from 1993 to 1995 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	97.0	99.5	99.7
	(99% confidence interval)	(95.5 - 98.5)	(99.0 - 100)	(99.2 - 100)
	Actual	89.5 n= 1,431	97.9 n= 1,420	98.5 n= 1,354
H. punctigera	Predicted	84.0	96.5	99.3
	(99% confidence interval)	(79.9 - 88.1)	(94.9 - 98.1)	(98.7 - 99.9)
	Actual	83.4 n= 3,806	96.3 n= 3,754	99.1 n= 3,809

1993 / 94 Season

% Surviving the Discriminating Dose of DiPel® (*Bacillus thuringiensis* var. *kurstaki*)

Species and collection area	Host	Early season collections (October - January)	Mid / late season collections (February - May)	Season total
Heliothis armigera		(expected survival range at the 1% error level = 0 to 0.8%)		
Namoi/Gwydir, NSW	cotton	0.4 (n=226)	1.7 (n=1,128)	1.5 (n=1,354)
Heliothis punctigera		(expected survival range at the 1% error level = 0.1 to 1.3%)		
Namoi/Gwydir, NSW	cotton	0.7 (n=1,514)	1.9 (n=513)	1.0 (n=2,027)
Emerald, QLD	cotton	1.0 (n=206)	0 (n=9)	0.9 (n=215)
St. George, QLD	cotton	0.9 (n=1,068)	0.2 (n=499)	0.7 (n=1,567)
Total of all sites		0.8 (n=2,788)	1.1 (n=1,021)	0.9 (n=3,809)

1994 / 95 Season

% Surviving the Discriminating Dose of DiPel® (*Bacillus thuringiensis* var. *kurstaki*)

Species and collection area	Host	Early season collections (October - January)	Mid / late season collections (February - May)	Season total
Heliothis armigera		(expected survival range at the 1% error level = 0 to 0.8%)		
Namoi/Gwydir, NSW	cotton maize	0.1 (n=697)	0.8 (n=2,638)	0.7 (n=3,335)
Mareeba, QLD	tobacco	0 (n=542)		0 (n=542)
Bundaberg, QLD	tomatoes capsicum	0 (n=102)	1.4 (n=891)	1.2 (n=993)
Bourke, NSW	maize		0 (n=396)	0 (n=396)
Macquarie, NSW	cotton		0 (n=43)	0 (n=43)
Macintyre, NSW/QLD	cotton		0.6 (n=530)	0.6 (n=530)
St. George, QLD	cotton beans sunflowers		0.4 (n=528)	0.4 (n=528)
Childers, QLD	tomatoes		0.4 (n=263)	0.4 (n=263)
Total of all sites		0.1 (n=1,341)	0.8 (n=5,289)	0.6 (n=6,630)

Species and collection area	Host	Early season collections (October - January)	Mid / late season collections (February - May)	Season total
Heliothis punctigera		(expected survival range at the 1% error level = 0.1 to 1.3%))		
Namoi/Gwydir, NSW	cotton	0.6 (n=1,187)	0.3 (n=797)	0.5 (n=1,984)
Manjimup, WA	moths at nectar	0 (n=171)		0 (n=171)
Bundaberg, QLD	tomatoes capsicum	0 (n=32)	0 (n=19)	0 (n=51)
St. George, QLD	cotton sunflowers		1.2 (n=169)	1.2 (n=169)
Macintyre, NSW/QLD	cotton		0 (n=13)	0 (n=13)
Total of all sites		0.5 (n=1,390)	0.4 (n=998)	0.5 (n=2,388)

All areas combined

% Surviving the Discriminating Dose of DiPel® (*Bacillus thuringiensis* var. *kurstaki*)			
Species and collection season	Early season collections (October - January)	Mid / late season collections (February - May)	Season total
Heliothis armigera	(expected survival range at the 1% error level = 0 to 0.8%)		
1993 / 94	0.4 (n=226)	1.7 (n=1,128)	1.5 (n=1,354)
1994 / 95	0.1 (n=1,336)	0.8 (n=5,294)	0.6 (n=6,630)
Heliothis punctigera	(expected survival range at the 1% error level = 0.1 to 1.3%)		
1993 / 94	0.8 (n=2,788)	1.1 (n=1,021)	0.9 (n=3,809)
1994 / 95	0.5 (n=1,390)	0.4 (n=998)	0.5 (n=2,388)

Conventional chemistry testing on Bt stressed larvae

	RHa		Endo R		New MFO		RHp			
	Control	Bt stressed	Control	Bt stressed	Control	Bt stressed	Control	Bt stressed	Control	Bt stressed
Average no. days from early 3rd instar to testing size			2.9 (n=495)	5.8 (n=1034)	3.0 (n=877)	5.6 (n=1057)	2.8 (n=702)	5.8 (n=1208)		
% larvae surviving & reaching testing size by 10 days from the early 3rd instar		73.8 (n=720)	96.5 (n=513)	67.2 (n=1538)	98.4 (n=891)	65.7 (n=1610)	99.2 (n=708)	89.6 (n=1344)		
Fenvalerate										
LD50	0.5097	0.6225	1.6473	0.5757	0.2461	0.5499	0.0115	0.0112		
Lower 95% ci	0.4158	0.4848	1.3620	0.3163	0.2018	0.4250	0.0100	0.0099		
Upper 95% ci	0.6260	0.7872	1.9858	0.8893	0.3009	0.7162	0.0132	0.0128		
Slope	2.2	1.6	2.2	0.9	2.3	1.4	4.0	4.5		
Endosulfan										
LD50	1.0395	2.6094	91.2551	37.8524	2.2409	1.3619	0.6750	0.8889		
Lower 95% ci	0.8453	2.1772	72.6016	29.5713	1.8514	1.1649	0.5817	0.7811		
Upper 95% ci	1.2679	3.1271	115.7272	48.3586	2.7430	1.5974	0.7775	1.0100		
Slope	2.0	2.4	1.5	1.8	2.7	3.4	4.1	4.7		
Profenofos										
LD50	0.1026	0.1459	0.5165	0.4501	0.1143	0.1181	0.0661	0.0728		
Lower 95% ci	0.0901	0.1310	0.4410	0.3858	0.0981	0.1002	0.0585	0.0657		
Upper 95% ci	0.1167	0.1629	0.6022	0.5229	0.1333	0.1389	0.0747	0.0807		
Slope	4.6	6.6	3.4	3.6	4.0	3.2	5.7	7.1		