CONVENTIONAL INSECTICIDE AND BT TRANSGENIC RESISTANCE MANAGEMENT IN AUSTRALIAN COTTON

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The Australian cotton industry has been practising curative conventional insecticide resistance management for thirteen years now. Initially it was targeted at pyrethroid and endosulfan resistance management in *Helicoverpa armigera* but more recently has also included other conventional insecticide groups (such as the organophosphates and carbamates) and acaricides for control of *Tetranychus urticae*. In addition, the imminent commercialisation of transgenic cotton expressing the Cry1Ac insecticidal crystal protein from *Bacillus thuringiensis*, has necessitated the introduction of a preventative Resistance Management Strategy. This paper aims to give a brief history of the Australian conventional Insecticide Resistance Management (IRM) Strategy and an outline of the proposed Resistance Management Strategy for Bt transgenic cotton.

The conventional Insecticide Resistance Management Strategy

The Australian IRM strategy was first implemented in the 1983/84 season and was based on the rotation of unrelated chemical groups on a per generation basis, alongwith with a reliance on ovicide/larvicide mixtures under high pressure situations. The pyrethroid window was targeted in the middle of the season to preserve natural enemies as long as possible and to prevent premature flaring of secondary pests such as mites and whitefly. The duration of the window was set at 42 days, the time needed to complete one *Helicoverpa armigera* (Hübner) generation in the field. This strategy kept resistance frequencies in check for a number of years but by the 1988/89 season, resistance frequencies began to escalate markedly, particularly during the late Stage II / early Stage III period. It was realised that the spraying of the long residual pyrethroids in the last few days of Stage II on crops that were nearing 'cut out' (that is, at a time of reduced growth dilution), was resulting in double selection of overlapping generations. This necessitated a shortening of the pyrethroid window to 35 days in the following seasons and this also had a positive delaying effect on pyrethroid resistance.

Early additions to the conventional Insecticide Resistance Management Strategy

Over this period, there were also a number of problems encountered which resulted in additional recommendations to the key strategy guidelines eg. the recognition of the importance of:-

i) overwintering diapause pupae carrying over highly resistant populations from the end of one season to the start of the next. The lack of cultivation of these diapausing pupae in the winter of 1986 because of depressed cotton prices, highlighted the important role of this life stage in the ecology of resistance in *H. armigera* and resulted in the adoption of the "pupae busters" campaign. This campaign promotes the timely and effective cultivation of cotton residues as soon as possible after picking, as a non-chemical control measure to reduce resistance selection pressure.

ii) the impact of adult selection and repellency of surviving moths. As the damaging stage of *Helicoverpa* spp. is the larva, traditionally, little thought has been devoted to the impact of insecticides on the non-damaging adult stage. However, when insecticides are applied to a cotton crop, they act on all stages of the pest present in the crop and this includes the adult. It was found that the moth can express resistance to the synthetic pyrethroids but that it is still highly irritated by the pyrethroid deposit, resulting in the repellency of these resistant moths onto neighbouring unsprayed crops or down lower into the untreated portion of the canopy.

iii) the need to target sprays on smaller larvae, preferably neonates. The functionally recessive nature of pyrethroid resistance in *Helicoverpa armigera* (that is, genetically resistant small larvae can still be killed by normal field rates of pyrethroids alone) has been known for about a decade and has been exploited with the recommendation to spray pyrethroids at egg hatch. This has proved successful, particularly as resistance levels have increased and the mixture approach (discussed later) has become less effective and more expensive. However, under protracted and/or heavy pest pressure, this approach is less acceptable as it results in more frequent spraying (sometimes down to 5-6 day spray intervals).

<u>Later additions to the conventional Insecticide Resistance Management Strategy</u>

Despite the widescale adoption of all these practices, resistance frequencies still continued to climb, albeit slowly. Research on pyrethroid resistance mechanisms indicated the possibility of using partial resistance breaking pyrethroids (eg. bifenthrin) or monooxygenase synergists such as piperonyl butoxide with the latter introduced commercially in the 1990/91 season. Once again, this was a successful delaying tactic but by the 1992/93 season there were clear indications of a declining effectiveness of piperonyl butoxide due to the development of alternative metabolic resistance mechanisms. Research on new synergists was commenced (eg. propargite) and alternative larvicide mixing partners were sought. The most successful of these has proven to be *Bacillus thuringiensis* (Bt) which is now used extensively in mixtures with both endosulfan and pyrethroids when resistant *H. armigera* are present. However, the main problem with this approach has been the inability to determine when resistant *H. armigera* are present in significant enough numbers to warrant the more expensive mixture approach. This problem has now been solved with the recent commercialisation of a monoclonal antibody test to determine the species of *Helicoverpa* present, either the fully susceptible *H. punctigera* or the multiresistant *H. armigera*. The more expensive mixtures can now be precisely targeted on resistant *H. armigera* populations resulting in lower costs and less spray failures. This new technology has resulted in a relaxation of the previously tightly controlled 35 day pyrethroid window which was extended firstly to 50 days in the 1993/94 season and ultimately deregulated completely by the 1994/95 season. The basis of the strategy for pyrethroids and endosulfan is now technological rather than biological as previously and relies on the precise targeting of appropriate mixtures. The concept of a window strategy based on rotation of unrelated chemical groups is still being used for those products where resistance levels are still relatively low (i.e. profenofos and thiodicarb).

<u>Incorporation of acaricides into the Resistance Management Strategy</u>

The strategy has also recently expanded to now take into account resistance management for acaricides against *Tetranychus urticae*, some of which interact with *Helicoverpa* control measures eg. bifenthrin for direct control of both *Helicoverpa* and mites and propargite, an acaricide with synergistic properties for pyrethroids on *Helicoverpa*. Acaricide use in Australian cotton is increasing and the generally decreasing effectiveness of the organophosphates has put increasing selection pressure on the alternatives, in particular propargite. The general approach has been to ground spray the contact acaricides first eg. dicofol and then to use propargite (with its vapour action) when aerial application is required. The pressure on propargite will be relieved somewhat with the registration of alternative acaricides such as abamectin, chlorfenapyr and diafenthiuron.

Expanded scope of the IRM program - higher early season thresholds

The increasing resistance problems over the past few years have resulted in increased use of insecticides (particularly as mixtures of larvicides) with a concomitant increase in the costs of insect control and hence decreased profitability, all classic signs of a deteriorating resistance situation. In an effort to decrease selection pressure, a major campaign was introduced in the 1995/96 season to reduce early season insecticide use by setting a pre-flowering early season threshold of 2 larvae per metre, instead of the generally used threshold of 1 larva per metre. This was designed particularly to reduce selection pressure on endosulfan and thiodicarb and was generally well accepted, although many growers and consultants were concerned that the threshold was too high and that they would be unable to control the larger larvae. This particular strategy recommendation has been in place for the past season only so it is too early yet to determine its impact.

Impact of environmental problems

Environmental problems have also acted to constrain the choice of some products in the IRM strategy. For example, the acute fish toxicity of endosulfan and profenofos, the mercaptan odour drift problem of profenofos and the residue problems of endosulfan and chlorfluazuron, have all acted to limit the use of these products (in fact chlorfluazuron was voluntarily withdrawn from use by the Australian cotton industry in the 1995/96 season) and hence put increasing selection pressure onto the few remaining alternatives.

Future developments & the Bt cotton Resistance Management Strategy

The strategy is also destined to change in the future with the introduction of new synthetic insecticides (eg. chlorfenapyr) and acaricides (as above), new biological insecticides (eg. spinosad and new Bt products and viruses, both conventional and genetically engineered), new pests (such as the recently introduced silverleaf whitefly, B type *Bemisia tabaci*) and transgenic cottons. Already, the resistance potential of this last mentioned technology has been recognised and a preventative IRM strategy has been devised and is in the process of implementation. The Strategy outlined below was endorsed by the ACGRA (Australian Cotton Growers' Research Association) TIMS (Transgenic and Insecticide Management Strategy) Committee in early April 1996 and was passed onto the NRA (National Registration Authority) for consideration by that body as part of the requirement for the registration of the Bt transgenic cotton technology.

The owners of the technology (Monsanto) intend to make compliance with this strategy compulsory by incorporating the strategy requirements into their licence agreement with growers. The options described below are not necessarily the preferred options for the future and the strategy will be reviewed annually by the industry's TIMS Committee.

There are four basic parts to the Bt cotton strategy:-

1. Refuges

Each grower will be required to grow a refuge crop to produce Bt susceptible *Helicoverpa* moths that will mate with any resistant survivors from Bt cotton crops and thus maintain Bt cotton resistance at low levels. For each 100 hectares of transgenic Bt cotton planted, a grower is required to plant a minimum of one of the following:-

i) an area of conventional irrigated cotton of 10 hectares which will be untreated for any reason with products to control *Helicoverpa*. If the Bt cotton crop is dryland, then the refugia crop may also be dryland.

ii) an area of conventional irrigated cotton of 50 hectares which can be sprayed with insecticides for *Helicoverpa* or other pests BUT not with conventional foliar applied Bt (that is *Bacillus thuringiensis* var. *kurstaki*) products. If the Bt cotton crop is dryland, then the refugia crop may also be dryland.

iii) an area of irrigated sorghum or corn of 20 hectares which will be untreated for any reason with products that control *Helicoverpa* and managed to flower from January 15 to February 28.

General conditions for all refuges are:-

a) Refuge crops are to be planted and managed so that the refuge is attractive to *Helicoverpa* during the growing period of the Bt cotton crop.

b) All refuges are to be planted within the farm unit growing the Bt cotton crop, preferably on one side of, or adjacent to, the Bt cotton field.

2. Planting window

All Bt cotton crops (irrigated and dryland) to be planted into moisture or watered up by the 15th of November.

3. Pupae destruction

Each grower will be required to undertake *Helicoverpa* pupae destruction as soon as possible after harvest in Bt cotton fields, according to the industry guidelines. Sprayed refuge crops should also be similarly cultivated to manage resistance to conventional insecticides. Unsprayed refuge crops should preferably be left uncultivated.

4. Heliothis spray thresholds

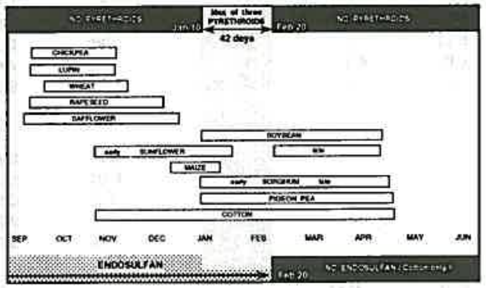
The Bt cotton should be carefully monitored throughout the season for sucking pests and *Helicoverpa*. For preventative resistance management of *Helicoverpa*, late season larval populations must be controlled with an effective insecticide if a threshold of 3 small larvae (second instar) or 1 medium larva (third/fourth instar) per metre of row, continue to survive over two consecutive checks. Specifically exclude all VS (very small = neonates / first instar) larvae from the spray threshold.

Summary and conclusions

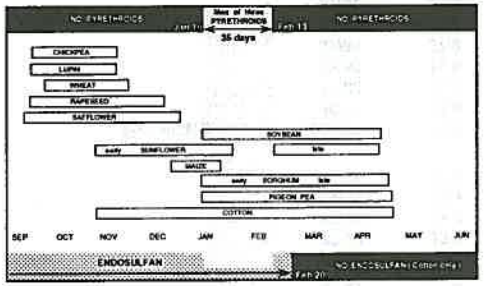
In summary, the obvious question that should be asked is : "Has the Australian IRM strategy been a success?" And undoubtedly the answer is yes, as it has clearly bought time (a valuable twelve years or so) to allow the introduction of new technologies such as novel insecticide groups and synergists, the monoclonal antibody based species test kit and most importantly, genetically engineered transgenic cottons. Without this breathing space, the Australian cotton industry would not have been able to survive and prosper during this difficult period and may have declined as in other countries afflicted with the same problem at the same time. Also, the successful experience with this conventional IRM strategy has given the local industry confidence that it has the capacity and capability to design and implement an IRM strategy for the forthcoming environmentally desirable Bt transgenic cotton technology.

Historical summary of the on-going development of the Australian Summer Crops Resistance Management Strategy since its inception in the 1983/84 season

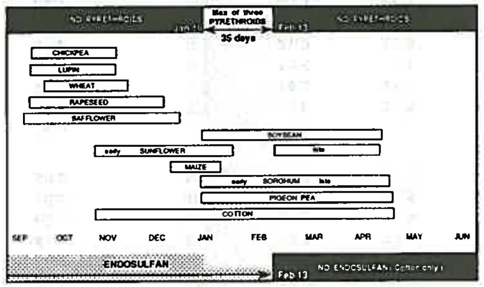
1983/84 to 1988/89 (six seasons)
SUMMER CROP RESISTANCE MANAGEMENT STRATEGY



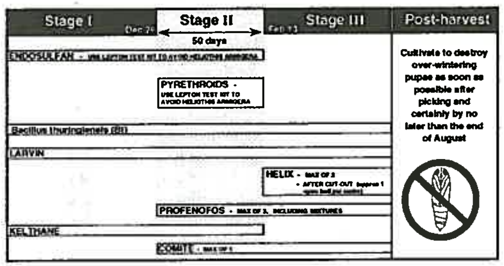
1989/90 SUMMER CROP
RESISTANCE MANAGEMENT STRATEGY



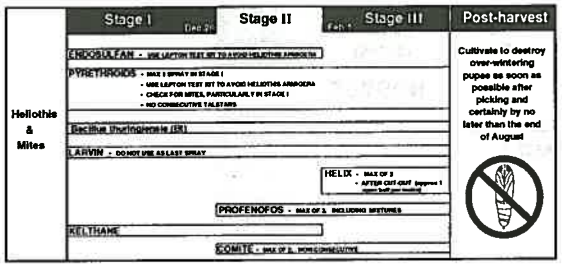
1990/91 to 1992/93 (three seasons)
SUMMER CROP RESISTANCE MANAGEMENT STRATEGY



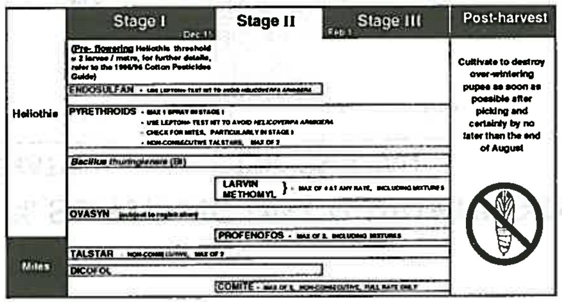
COTTON RESISTANCE MANAGEMENT STRATEGY for 1993/94 season



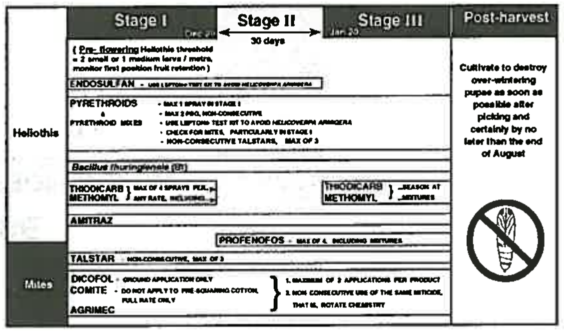
COTTON RESISTANCE MANAGEMENT STRATEGY for 1994/95 season



COTTON RESISTANCE MANAGEMENT STRATEGY for 1995/96 season



COTTON RESISTANCE MANAGEMENT STRATEGY for 1996/97 season

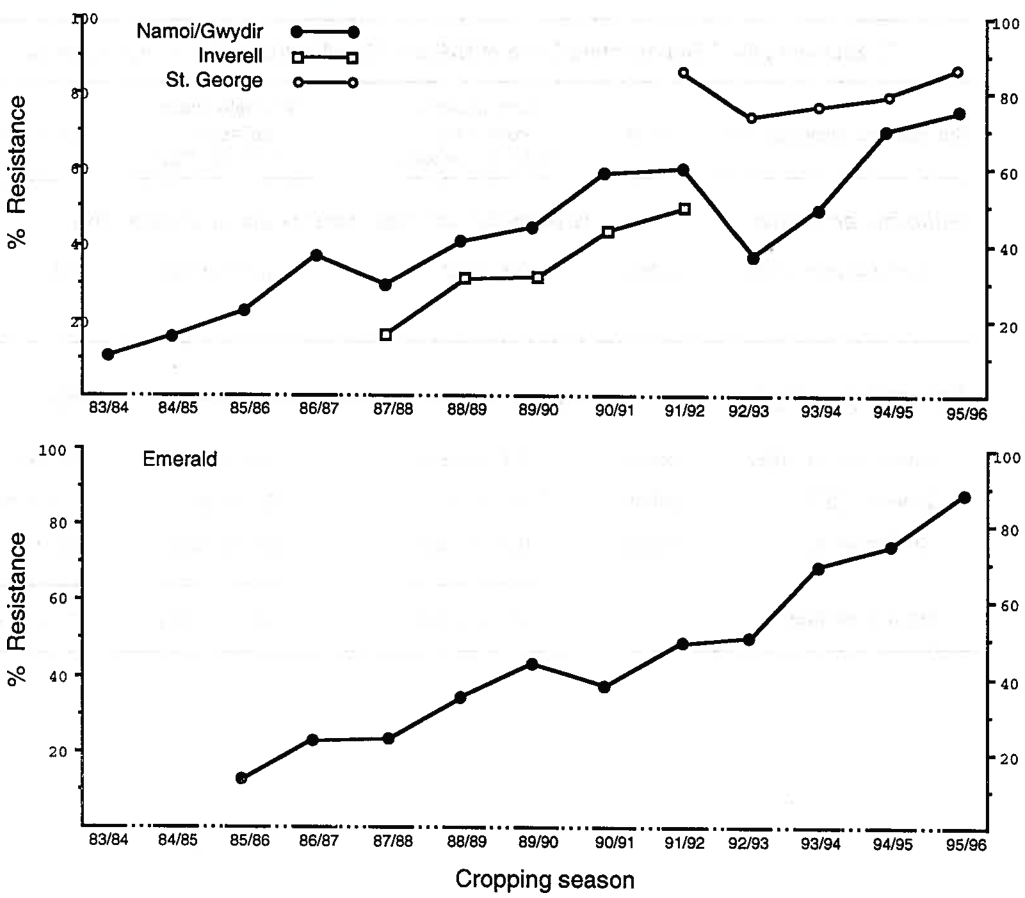


% SURVIVING DISCRIMINATING DOSE

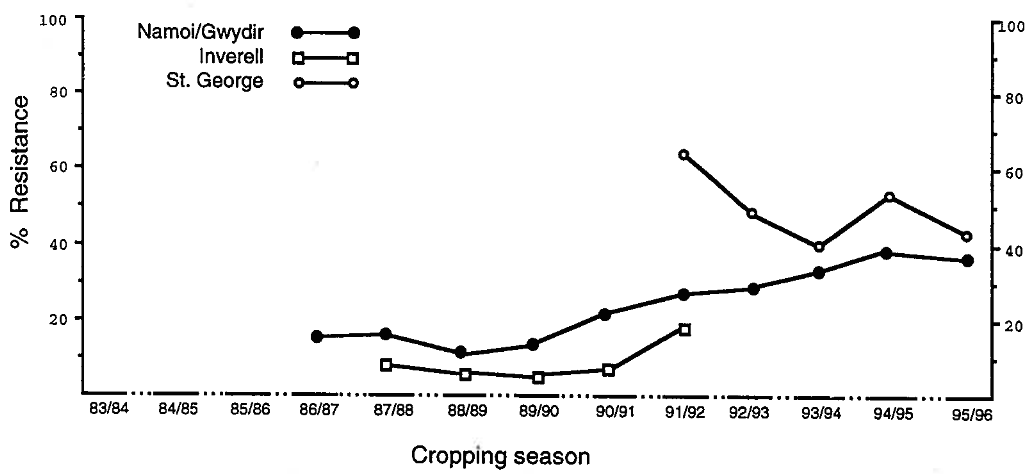
STUDY AREA	SEASON	FENVALERATE I	FENVALERATE II	FENVALERATE III	ENDOSULFAN I	ENDOSULFAN II	ENDOSULFAN III	PROFENOFOS I	PROFENOFOS II	PROFENOFOS III
Namoi/Gwydir	1983/84	9.3	9.5	14.6	-	-	-	-	-	-
	84/85	7.5	12.9	27.9	-	-	-	-	-	-
	85/86	7.8	13.0	44.5	-	-	-	-	-	-
	86/87	32.2	36.7	42.9	7.1	16.7	20.1	-	-	-
	87/88	19.8	30.1	38.4	7.3	17.6	23.0	-	-	-
	88/89	19.6	42.4	60.7	8.8	13.2	10.6	-	-	-
	89/90	24.7	45.3	62.5	9.2	14.8	15.9	-	-	-
	90/91	55.7	61.1	61.5	12.2	22.7	31.3	-	-	-
	91/92	46.0	64.1	68.4	9.2	32.4	40.6	-	-	-
	92/93	33.9	34.0	41.9	24.7	35.2	27.7	-	0.9	0.2
	93/94	31.3	50.3	66.1	30.5	32.2	32.6	-	0.7	2.0
	94/95	63.5	71.6	74.6	39.1	38.6	40.6	2.2	1.6	1.6
	95/96	79.0	78.0	80.0	45.8	37.2	33.3	0.9	6.6	4.0
Emerald	1985/86	6.8	17.1	14.4	-	-	-	-	-	-
	86/87	8.8	26.5	29.8	7.7	20.6	17.3	-	-	-
	87/88	15.9	27.1	27.0	9.5	14.3	13.7	-	-	-
	88/89	19.8	38.7	44.3	8.1	13.6	7.1	-	-	-
	89/90	27.9	44.6	54.6	3.1	21.0	20.9	-	-	-
	90/91	24.7	52.2	34.5	10.1	37.1	16.0	-	-	-
	91/92	32.1	52.7	62.1	7.9	50.5	47.3	-	-	-
	92/93	40.2	50.6	59.1	25.9	51.6	59.5	-	-	1.1
	93/94	66.0	61.8	79.8	50.0	48.7	52.3	-	-	-
	94/95	56.0	74.0	92.8	38.8	53.1	42.5	3.2	4.2	1.2
	95/96	-	89.2	-	-	-	-	-	14.9	15.4
Inverell	1987/88	10.2	20.4	19.0	11.3	10.5	5.8			
	88/89	21.9	28.9	41.7	9.4	4.8	5.4			
	89/90	22.1	32.7	38.2	4.0	5.2	7.1			
	90/91	47.8	34.6	45.1	3.4	8.5	10.8			
	91/92	37.8	55.3	55.5	24.2	17.3	14.0			
	92/93	-	-	-	-	-	-			
St. George	1991/92	-	80.2	90.9	-	60.9	67.4	-	-	-
	92/93	63.7	76.0	80.4	29.9	59.7	57.5	-	1.4	3.7
	93/94	-	71.2	81.8	-	45.4	34.7	-	-	11.4
	94/95	72.3	76.0	87.2	48.5	55.4	53.7	-	4.5	6.0
	95/96	83.1	88.8	-	-	43.1	-	-	-	3.2

Average pyrethroid, profenofos (Curacron®) and endosulfan resistance levels in *Heliothis armigera* for each Stage(I, II & III) of the Resistance Management Strategy, for 4 study areas (the Namoi and Gwydir valleys of northern NSW, the Emerald Irrigation Area of central Queensland, the St. George Irrigation Area of southern Queensland and a sample of the unsprayed refugia area centred on Inverell in northern NSW).

Fenvalerate resistance - Average of Stages I, II & III



Endosulfan resistance - Average of Stages I, II & III



Pyrethroid and endosulfan resistance (% survival at the discriminating dose; Stage I, II and III resistance levels averaged for each season) in *Helicoverpa armigera* larvae reared from eggs collected from three cotton growing areas (Namoi/Gwydir, St. George and Emerald) and one unsprayed refuge area (Inverell).

1993 / 94 Season - DiPel®

% 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 - DiPel®

% 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)
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)

1995 / 96 Season - DiPel®

% 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	0.2 (n=1,036)	0.2 (n=1,830)	0.2 (n=2,866)
Emerald, QLD	cotton	0 (n=229)		0 (n=229)
Bundaberg, QLD	tomatoes		0 (n=250)	0 (n=250)
Darling Downs, QLD	cotton		0 (n=461)	0 (n=461)
Dawson/Callide QLD	cotton	0 (n=37)	0.5 (n=189)	0.4 (n=226)
Macintyre, NSW/QLD	cotton		0 (n=193)	0 (n=193)
St. George, QLD	cotton	0 (n=58)	0.2 (n=453)	0.2 (n=511)
Lockyer Valley, QLD	tomatoes cotton maize	0 (n=91)	0 (n=263)	0 (n=354)
South Burnett, QLD	navy beans soybeans maize pigeon pea	2.2 (n=45)	0.4 (n=272)	0.6 (n=317)
Mungindi, NSW	cotton	0 (n=75)		0 (n=75)
Total of all sites		0.2 (n=1,571)	0.2 (n=3,911)	0.2 (n=5,482)
Heliothis punctigera	(expected survival range at the 1% error level = 0.1 to 1.3%))			
Namoi/Gwydir, NSW	cotton	0 (n=1,526)	0.5 (n=197)	0.1 (n=1,723)
Emerald, QLD	cotton	0 (n=165)		0 (n=165)
St. George, QLD	cotton	0 (n=483)	0 (n=628)	0 (n=1,111)
Dawson/Callide, QLD	cotton	0 (n=107)		0 (n=107)
Macquarie, NSW	cotton	0 (n=151)		0 (n=151)
Mungindi, NSW	cotton	0 (n=65)		0 (n=65)
Total of all sites		0 (n=2,497)	0.1 (n=825)	0.1 (n=3,322)

1995 / 96 Season - Xentari®

% Surviving the Discriminating Dose of **Xentari**® (*Bacillus thuringiensis* var. *aizawaii*)				
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	0.2 (n=546)	0.1 (n=1,253)	0.1 (n=1,799)
Bundaberg, QLD	tomatoes		0 (n=183)	0 (n=183)
Darling Downs, QLD	cotton		0 (n=199)	0 (n=199)
Lockyer Valley, QLD	tomatoes maize		0 (n=157)	0 (n=157)
Total of all sites		0.2 (n=546)	0.1 (n=1,792)	0.1 (n=2,338)
Heliothis punctigera		(expected survival range at the 1% error level = 0.1 to 1.3%))		
Namoi/Gwydir, NSW	cotton	0 (n=1,297)	0 (n=51)	0 (n=1,348)
St. George, QLD	cotton	0 (n=292)		0 (n=292)
Macquarie, NSW	cotton	0 (n=151)		0 (n=151)
Total of all sites		0 (n=1,740)	0 (n=51)	0 (n=1,791)

All areas combined - DiPel®

% 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)
1995 / 96	0.2 (n=1,571)	0.2 (n=3,911)	0.2 (n=5,482)
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)
1995 / 96	0 (n=2,497)	0.1 (n=825)	0.1 (n=3,322)

All areas combined - Xentari®

% Surviving the Discriminating Dose of **Xentari®** (*Bacillus thuringiensis* var. *aizawaii*)

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%)		
1995 / 96	0.2 (n=546)	0.1 (n=1,792)	0.1 (n=2,338)
Heliothis punctigera	(expected survival range at the 1% error level = 0.1 to 1.3%)		
1995 / 96	0 (n=1,740)	0 (n=51)	0 (n=1,791)