Australian Government

Cotton Research and
Development Corporation

FINAL REPORT

Helicoverpa spp. Insecticide Resistance: Monitoring mechanisms and management

CRDC Project No. DAN 193
(incorporating DAN 185)

DAN 193: March 20 2007 – June 30 2008
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Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

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Project Title: Helicoverpa spp. Insecticide Resistance: Monitoring mechanisms and management

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FULL REPORT

1. Background

Resistance is one of the greatest threats to effective pest control in the Australian Cotton Industry, both against insecticides as well as transgenic cotton. For the primary pests of cotton, the cotton bollworm *Helicoverpa armigera* and to a lesser extent *H. punctigera*, this threat could in the worst case result in loss of an important insecticide or loss in effectiveness of one or more Bt genes. It is important therefore that resistance monitoring, and associated mechanism research, is continued to detect the development of resistance and determine the mechanisms involved, in order that appropriate strategies are formulated accounting for this information and implemented before resistance is observed in the field in the form of control failures.

The 2004/05 cotton season saw the introduction of large scale Bollgard II plantings with minor restrictions on the total area that could be grown. Insecticides however have continued to be in demand for use against *Helicoverpa* spp. on conventional cotton, and have been used on Bollgard II crops either targeting other insects such as mirids, using a chemical that also kills *Helicoverpa* spp. (eg endosulfan), or to control *Helicoverpa* spp. under conditions of high insect pressure and/or growing conditions that adversely affect Bt expression. Sprayed conventional cotton (non Bt) is still a popular cropping option as well as an important refuge option for Bollgard II. While Bollgard II may dominate total plantings, conventional cotton plantings represent a significant area that requires insecticidal control and protection against insecticide resistance.

The effects of significant plantings of Bollgard II and planting trends for the future are uncertain for the foreseeable future given such factors as economic constraints (eg low cotton prices and increased Bollgard II fee) and resistance issues associated with Bt toxins. Findings of Dr Robin Gunning of NSW DPI, as part of the CRDC funded project DAN 172C (Gunning *et al.,* 2005) identified an esterase mediated cross resistance in *H. armigera* between pyrethroids and the Cry1Ac gene, one of the Bt genes expressed in Bollgard II. In addition, while accurate estimates of the frequency of resistance to Cry2Ab, the other Bt gene in Bollgard II, continue to be established, the CSIRO Bt resistance monitoring project has identified resistance associated genes in the field, with the data suggesting an increase in frequency in 2007/08 (Sharon Downes, pers comm.). Both these findings have serious implications for the control of *H. armigera* in the field using both conventional chemistry and transgenic cotton, and emphasise the need to continue insecticide resistance monitoring and associated resistance mechanism research, both An insecticide resistance management strategy (IRMS) is implemented in the Australian Cotton Industry to protect insecticides. This strategy relies on resistance monitoring data and mechanism research as part of assessing the success of the strategy as well as formulating changes to account for resistance development that may be detected, and for occurrence of cross resistance between different insecticides. This project aims to provide such data and any additional useful data from the monitoring program in the development of an effective strategy and guidelines for minimising the development of insecticide resistance.

In addition to resistance monitoring and mechanism research for chemicals currently registered for use on cotton, it is essential that new chemistries entering the industry have accurate dose-response data measured prior to their introduction. This accumulation of the baseline response allows for measurement of future changes and the detection of resistance development. Without this baseline data there is no means with which to detect resistance development until it is too late and field control problems or failures occur.

2. Objectives and extent to which they have been achieved

i) **Co-ordinate egg collection team based in Narrabri and provide information for egg collections in other cotton growing regions.** An egg collection team was successfully co-ordinated and deployed out of Narrabri in 2005/06, 2006/07 and 2007/08, regularly taking collections from the Upper and Lower Namoi and Gwydir Valley regions, with field trips to Bourke, Walgett, the Macquarie Valley, the Macintyre Valley and the Darling Downs also conducted. In 2005/06 CRDC funded collectors were co-ordinated to supply eggs from the Macintyre Valley and St George, with funding available from January 2007 for a collector in the Macintyre only in 2006/07. In 2007/08 CRDC supported collectors were co-ordinated together with Dr Sharon Downes, CSIRO, to provide collections to both the insecticide and Bt resistance monitoring projects from Emerald, the Darling Downs, St George, the Macintyre Valley and the Riverina/Murrumbidgee. Collections were also provided from the Macquarie, co-ordinated through the Macquarie RCEO, Sally Ceeney. Specific collecting protocols and guidelines were distributed to all collectors together with all equipment required for collecting and sending eggs to ensure collections were conducted to our requirements and arrived at ACRI in optimum condition.

ii) **Conduct insecticide resistance monitoring on insect samples collected across all cotton areas of Australia.** Insecticide resistance monitoring for *Helicoverpa armigera* and *H. punctigera* was conducted on field collected material from across most cotton growing regions during the 2005/06, 2006/07 and 2007/08 cotton seasons. An emphasis was placed on spinosad, indoxacarb and emamectin benzoate due to their importance within an IPM system as well as registration of the former two in a number of grains and pulse crops. Attempts were made to include all chemistries where practicable to ensure resistance information was as complete as possible.

iii) **Formulate and promote improved resistance management guidelines and strategies.** Resistance monitoring results were presented annually to the TIMS Committee as part of assessing the success of the IRMS. Dr Rossiter was involved in formulating improved resistance management guidelines incorporating secondary pests and crops other than cotton in her role as a member of the TIMS Insecticide Resistance Technical Panel and TIMS Bt Technical Panel. Resistance management strategies were promoted extensively to the cotton and associated broad acre industries through grower and consultant meetings, contributions to trial books and cotton tales, magazine publications, industry publications and the TIMS resistance roadshow in 2008 (see Section 8).

iv) **Establish insecticide resistant *Helicoverpa* spp. colonies using field survivors for use in further resistance research.** The few survivors to key insecticides resulting from the monitoring program were kept for rearing through and single pair mating to establish resistant colonies with varying success. Insecticide resistance frequencies to the insecticides of greatest interest about which little is known (ie spinosad, indoxacarb and emamectin benzoate) have been very low, making establishing colonies resistant to these difficult. Attempts were made to establish a chlorpyrifos resistant colony using a small number of field survivors, however despite outcrossing and several selection events, a stable resistant colony could not be established and after 20 generations the colony was weak and abandoned. Colonies resistant to other insecticides which have been more thoroughly studied in the past were established for use in investigating cross resistance. These have been utilised in dose response assays for a new insecticide recently registered in cotton as part of identifying potential cross resistance to insecticides currently in use.

v) **Investigate the inheritance of resistance and biochemical mechanisms of resistance to key IPM compatible chemistries by *Helicoverpa* spp.** This objective is dependent on establishment of resistant colonies from field survivors or lab selected colonies. See Objective 4. Preliminary investigations conducted on the mechanism of chlorpyrifos

resistance were inconclusive, with mechanisms unable to be confirmed before the colony was abandoned.

vi) **Investigate potential cross resistance between insecticides. Also investigate dose response of Bt resistant colonies to different insecticides.** Preliminary studies on the dose-response of Bt resistant colonies held by CSIRO in Canberra were conducted. This involved testing Cry 1Ac and Cry 2Ab resistant colonies with varying doses of most of the insecticides used in the resistance monitoring program. Discriminating doses were also used to test these colonies. These tests were used as a preliminary investigation of the range of dose concentrations required for accurate assessment of the possible presence of cross resistance between Cry 1Ac and Cry 2Ab toxins and specific insecticides.

   Cross resistance studies were conducted between two pyrethroids following findings of no survivors to the pyrethroid bifenthrin. Cross resistance was also investigated between colonies resistant to methomyl or fenvalerate and a new insecticide to be registered for use in cotton and other crops.

vii) **Identify the complete gene sequence of a possible resistance associated esterase.** Through collaboration with Dr Grant Herron and Martin McLoon at EMAI, Camden, a preliminary full esterase gene sequence has been determined. This sequence will be verified using proof reading polymerase to correct nucleotide-misincorporation errors and provide an accurate sequence for use in further investigations.

viii) **Using appropriate bioassay techniques, accumulate baseline dose-response data for new Helicoverpa insecticides.** Appropriate feeding and topical bioassay techniques were determined in preliminary assays in 2005/06 for a new insecticide from DuPont, Rynaxypyr. The first and/or second generation (+ some subsequent) of lab insect colonies established during the 2006/07 and 2007/08 cotton season were used to accumulate baseline dose-response data using these assays. This information shall provide the information necessary to determine a discriminating dose for use in resistance monitoring.

ix) **Support other Helicoverpa related research projects with field collected material and field collected laboratory reared insect colonies.** Field collected lab-reared insect colonies in each season have been maintained and made available for use by other Helicoverpa related projects.

3. Methodology and justification

3.1 Insect collection and laboratory rearing

The *Helicoverpa* spp. resistance monitoring program relies on the collection of insect eggs for lab rearing and resistance testing. Eggs are preferable as they can be collected and transported with relative ease. Unlike larvae, they are abundant in both sprayed conventional and transgenic cotton, have a lower risk of disease introduction to lab facilities and can be collected before exposure to insecticide. The disadvantage of egg collections are that they must be reared to 30-40 mg larvae for bioassay. In addition, *H. armigera* vs *H. punctigera* eggs cannot be distinguished, nor can Trichogramma parasitised white eggs, with both these factors potentially significantly affecting the number of *H. armigera* available for testing. *H. armigera* is the only species of the two to have developed field resistance to insecticides in Australian cotton, and monitoring focuses on this species, with *H. punctigera* included to a lesser extent. Despite these disadvantages, they remain the preferred collection material. Larvae and in some instances moths are also collected. These are not tested directly, but are laboratory reared and the next generation tested. The use of larvae or moths is rare however as in the case of larvae, the change in diet (from specific plant to artificial diet) as well as parasitism and disease results in high levels of mortality. Moths are rarely used as they are harder to collect and more prone to damage in transport, however are occasionally taken from light traps. These colonies may be used for other purposes also.

Helicoverpa spp. eggs were collected across all key cotton growing districts in Eastern Australia for the resistance monitoring program. Regular egg collections were taken from the Upper and Lower Namoi and Gwydir Valley regions, with field trips to Bourke, Walgett, the Macquarie Valley, the Macintyre Valley and the Darling Downs also conducted. In 2005/06 CRDC funded collectors were co-ordinated to supply eggs from the Macintyre Valley and St George, with funding available from January 2007 for a collector in the Macintyre only in 2006/07. In 2007/08 CRDC supported collectors were co-ordinated together with Dr Sharon Downes, CSIRO, to provide collections to both the insecticide and Bt resistance monitoring projects from Emerald, the Darling Downs, St George, the Macintyre Valley and the Riverina/Murrumbidgee. Collections were also provided from the Macquarie, co-ordinated through the Macquarie RCEO, Sally Ceeney. Collections were not structured in time and area sampled as egg pressure can be highly variable across both aspects. Collections were however co-ordinated as much as practicable to extend across the entire area of a region across the whole season, incorporating all cropping types including conventional and transgenic cotton, pigeon peas, maize, sorghum and other attractive crops and weed species. Areas without dedicated collectors in each season relied on contributions from growers, consultants, Industry Development Officers/Regional Cotton Extension Officers and District Agronomists and other industry representatives. These contributions were encouraged through direct verbal communication as well as written extension articles, however generally these collections were limited.

Egg collections were sent to ACRI and reared on an artificial soy flour based diet at 25 °C. Prior to testing larvae were speciated as *H. armigera* or *H. punctigera* using established visual identification techniques. Larvae that were not testing directly for resistance were reared through to pupae, involving a change to fresh diet in bigger containers for successful pupation of large healthy pupae. When pupation was complete, pupae were removed from diet, washed in a dilute bleach solution and transferred to lined rearing buckets. Insects from the same field collection were grouped as a colony. Moths were allowed to emerge in these buckets at 25 °C and 70 % relative humidity. Following initiation of moth emergence, a dilute honey solution was added to the buckets as a food source. The moth buckets were tended every two days, replacing liners and adding fresh honey solution. When all moths had emerged, egg covered liners were retained, placed in plastic bags and allowed to hatch at 25 °C. Larvae collected over two –three lays were used to establish the next generation.

3.2 Insecticide Resistance Monitoring

Resistance monitoring was conducted using established techniques, covering all chemistries used against *Helicoverpa* spp. but focussing on the softer chemistries that have a key fit in an IPM system in cotton as well as other crops. For *Helicoverpa armigera*, which has historically presented the greatest resistance threat, these include indoxacarb (Steward®), spinosad (Tracer®) and emamectin benzoate (Affirm®), with the former two registered for use in a number of grains and pulse crops also. Other insecticides tested included endosulfan, methomyl (carbamate), profenofos and chlorpyrifos (organophosphates), bifenthrin and fenvalerate (pyrethroids). Methoxyfenozide (Prodigy) was tested in 2005/06 for the second year since its registration in cotton in the 2003/04 season, however it was not tested in subsequent years due to its unavailability in Australia in 2006/07 and 2007/08. Chlorfenapyr (Intrepid) is also still registered for use in cotton however is likewise not commercially available, and therefore was also not included in the monitoring. Resistance monitoring for *H. punctigera* involved abamectin, endosulfan and pyrethroids (fenvalerate) which are the only chemistries with baseline data and discriminating doses established

Larvae were tested for resistance between 30 and 40 mg (3^{rd} – 4^{th} instar) using a discriminating dose bioassay. Discriminating doses ($LD_{99.99}$) for each insecticide, and the times to assessment were previously determined by Dr Robin Gunning, NSW DPI. A topical bioassay was employed, involving application of 1 µL of acetone dissolved technical grade insecticide to the dorsal surface of the insect. A feeding bioassay however was employed for methoxyfenozide which was found to have no topical contact toxicity (Dr Robin Gunning, pers comm.). This feeding bioassay involved adding the insects to diet previously overlayed with formulated insecticide and air dried. Insects were left for 2-4 days, with the time to mortality assessment dependent on the individual insecticide. Dead insects incorporated morbid insects; that is those insects that showed some signs of life (response when prodded), however very obviously were not going to survive. Insects that did survive a dose of insecticide were reared through to moth and used to establish a colony to confirm resistance status and for use in mechanism studies.

3.3 Resistance Management Strategy Formulation and Promotion

IRMS formulation and promotion involved the Transgenic and Insect Management Strategy (TIMS) committee. The formulation of resistance guidelines incorporated the resistance monitoring data and additional knowledge on resistance mechanisms and accounted for other pests of cotton and associated resistance issues. Reporting of resistance results and promotion of strategies was achieved through various written publications and oral presentations.

3.4 Establishment of resistant *H. armigera* colonies

Insects found to be resistant to key chemistries through the monitoring program were reared through to adults and either mated with each other where multiple resistant insects were found (of both sexes), or single pair mated with lab reared susceptible insects, with subsequent selection and outcrossing events to establish a resistant colony. Through this process a chlorpyrifos resistant colony was established. A number of other colonies resistant to various other older chemistries were also established and used for a number of purposes.

3.5 Resistance Mechanism and Cross resistance Studies

Established biochemical assays investigating enzyme systems involved in insecticide resistance were used to conduct preliminary studies on the chlorpyrifos resistant insect colony. The two enzyme systems focussed on were esterases and glutathione S-transferases (GSTs).

Cross resistance studies were conducted on various insecticide resistant colonies and a new insecticide undergoing registration in Australia, Rynaxypyr. Dose response data for this new

insecticide was collected using insect colonies that were resistant to profenofos, methomyl or fenvalerate. This data was compared with dose response data collected from field colonies to identify potential cross resistance between this insecticide and other insecticides. See Baseline Dose Response Data Accumulation for detailed methodology

Cross resistance studies were conducted between Bt toxins and insecticides utilising Bt resistant colonies held by CSIRO. A Cry 1Ac resistant colony (BX) and a Cry 2Ab colony (SP15) were tested with the discriminating dose of 8 different insecticides to identify potential cross resistance. Dose response assays were also conducted for a number of insecticides utilising these colonies and a Bt susceptible colony (GR). Discriminating dose assays were conducted as per the protocol for insecticide resistance monitoring. Dose response assays involved exposing 30 - 48 insect samples to a range of insecticide concentrations, also as per the protocol for insecticide resistance monitoring. Dose response assays were analysed by Probit analysis.

3.6 Esterase gene sequence identification

Molecular investigations of a possible resistance associate esterase gene were initiated during travel undertaken to Rothamsted Research Institute, England in 2006 (DAN 187). This resulted in a partial sequence identification encompassing the 5' end of the gene.

Standard molecular techniques were applied to identify the full esterase gene sequence. Fresh RNA was prepared by homogenising single larvae that had been placed in liquid nitrogen in 1 mL Tri-reagent (Sigma) according to the protocol. All experimental procedures from 3' RACE to analysing transformants were according to the manufacturer's protocol as supplied with the GeneRacer Kit (Invitrogen). 3' RACE was conducted using a number of primers determined from the partial gene sequence already identified. RACE products were excised from an agarose gel, and the products gel extracted before ligation into TOPO cloning vector and transformation into one-shot TOP10 competent cells and plating on LB Agar plates with 50 μg/mL ampicillen. Positive colonies were grown overnight in LB medium with 50 μg/mL ampicillen and plasmid DNA extracted using Genelute Plasmid Miniprep Kit (Sigma) according to the supplied protocol. Sequencing of plasmids containing 3' RACE products was conducted by Newcastle DNA using gene specific primers. Sequences were analysed using BioEdit (Ibis Biosciences) and DNAStar (Lasergene) software.

3.7 Baseline Dose Response Data Accumulation

Baseline dose response data focussed on Rynaxypyr, a new compound from DuPont registered for use in 2008/09 in cotton against *Helicoverpa* spp. Samples of Rynaxypyr were supplied by DuPont. Bioassays were conducted on 3rd instar (30 - 40 mg) larvae, with mortality assessed at 96 hours. F1 and F2 generation larvae were preferred, with some later generations also tested where previous generations were not available or assays were problematic. Where possible, strains were tested using both the feeding and topical bioassay, and were repeated in a subsequent generation to examine assay repeatability. Mortality incorporated morbid insects; that is those insects that showed some signs of life (response when prodded), however very obviously were not going to survive. Insect colonies representing samples from most of the cotton growing regions of eastern Australia were used in accumulating dose response data.

Feeding bioassay: Rynaxypyr (as commercial formulation) was supplied as a 350g ai/kg liquid and used in feeding dose response assays incorporated into the insect rearing diet. A stock solution and serial dilutions were prepared in de-ionised water. The bioassay consisted of 9 treatments (pesticide concentrations), with 30-48 larvae assessed at each concentration. Rynaxypyr concentrations used for *H. armigera* were 0 (control), 0,002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 ppm final concentration in diet. Lower concentrations were used for *H. punctigera* which was observed to have greater susceptibility (0, 0.0001, 0.0002, 0.0005, 0.001, 0.002, 0.005, 0.01, and 0.02 ppm final concentration in diet).

Insect rearing diet was prepared according to the standard rearing recipe, with an appropriate amount of water omitted (corresponding to the total volume of insecticide solution to be added). Diet incorporating each concentration of Rynaxypyr was prepared by adding 10 mL insecticide solution to 190 mL diet, followed by vigorous shaking. Diet was dispensed into rearing trays and allowed to cool and solidify. Larvae were added to diet and lids placed on trays. Bioassay trays were kept in an incubator at 25 °C, 12:12 hr light:dark until assessment.

Topical bioassay: Technical grade Rynaxypyr was supplied as a 96.45 % pure powder. A stock solution and serial dilutions were prepared in acetone. The bioassay consisted of 9 pesticide concentrations, with 30-40 larvae assessed at each concentration. Rynaxypyr concentrations used for *H. armigera* were 0 (control), 0,001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2 μg/μL. Concentrations used for *H. punctigera* were 0, 0.0002, 0.0005, 0.001, 0.002, 0.005, 0.01, and 0.02 μg/μL. Insecticide, 1 μL was applied to the dorsal surface of larvae in their rearing trays. Lidded trays were transferred to the incubator until assessment at 4 days. LC50 and LC99 values, expressed as ppm (feeding) or μg/μL (topical), 95 % Fiducial Limits (95 % FLs), slope and χ^2 values were determined by probit analysis using the program PROBIT5 (NSW Agriculture).

4. Results and discussion

4.1 Insecticide Resistance monitoring

Insecticide resistance monitoring was successfully conducted for the 2005/06, 2006/07 and 2007/08 seasons. Total data collected from field samples for monitoring purposes includes information on overall species complex at the time of sampling (Tables 1a-c). This information is used in the monitoring program and also has implications for resistance management, with *H. punctigera* easily controlled due to its susceptible status. In 2005/06 Helicoverpa pressure was high, with good collections as a result able to be received from areas that didn't have dedicated collectors. Pressure was substantially lower in 2006/07, with the majority of the collections coming from the collection team based at Narrabri. These samples were lower than the previous season highlighting the light pressure in this season. In 2007/08, pressure was moderate, with dedicated collectors in several valleys supplying collections that otherwise would not have been obtained if reliance was on voluntary collections.

Through all three seasons population species have generally been mixed with dominance by *H. punctigera* early season and late season composed mostly of *H. armigera*. One exception to this was around Hilston in 2005/06, where despite generally high pressure in other cotton growing areas, pressure was low and appeared to be dominated by *H. punctigera*. The very limited collection from this area was all *H. punctigera*, with the limited collection a result of the low pressure. In addition where large areas of maize were sampled, no *H .armigera* activity was observed, compared with other areas where maize throughout the season yielded large numbers of eggs.

Analysis of the resistance status of *H. armigera* from these collections has focussed on detecting any resistant individuals in the testing and reporting results in terms of whole numbers tested, with trend observations and statistical analysis between areas, years and within the season almost impossible due to the low numbers collected in some areas and in different seasons. Overall resistance monitoring results for *H. armigera* for the three seasons are summarised in Appendix 1: Tables 6a-c.

Tables 1a-c: % H. armigera from resistance monitoring collections

Source: crops that attract both Helicoverpa species: cotton, pigeon pea, chickpeas, sunflowers and mung beans - excludes maize and sorghum. Total insect numbers speciated are given in brackets. Blanks indicate no collections were received or collections were from sorghum, maize or other non *H. punctigera* hosts.

Table 1a: 2005/06

Region	Sept - Nov	Dec	Jan	Feb	March
Emerald		6 (133)	24 (1011)	50 (32)	
Darling Downs	71 (73)	20 (352)	69 (869)	95 (348)	
St George			43 (303)	79 (121)	
Macintyre		10 (973)	84 (2355)	91 (706)	
Gwydir	20 (1512)	20 (825)	94 (533)	99 (897)	
Bourke		0 (81)	4 (188)		
Walgett			45 (260)		
Lower Namoi	7 (194)	12 (1270)	74 (2310)	77 (397)	98 (91)
Upper Namoi		44 (936)	46 (198)	15 (121)	
Macquarie		2 (156)		51 (640)	
Riverina		0 (14)	0 (158)		

Table 1b: 2006/07

Region	Dec	Jan	Feb	March - April
Darling Downs		44 (36)	58 (359)	
St George		37 (102)		
Macintyre		69 (138)	90 (217)	
Gwydir	23 (169)	64 (407)		
Lower Namoi	2 (702)	45 (620)	67 (536)	83 (218)
Upper Namoi	1 (236)	55 (501)	39 (31)	100 (159)

Table 1c: 2007/08

Region	Sept - Nov	Dec	Jan	Feb	March-April
Emerald		15 (304)	27 (63)		
Darling Downs		6 (32)	66 (137)	10 (10)	
St George	29 (260)	70 (67)	46 (144)	20 (15)	
Macintyre		78 (40)	70 (98)	43 (69)	
Gwydir		30 (157)	34 (441)	45 (232)	95 (100)
Lower Namoi	38 (894)	31 (108)	26 (1444)	69 (911)	97 (767)
Upper Namoi		78 (516)	51 (434)	81 (341)	97 (154)
Macquarie			59 (29)	2 (47)	
Riverina		0 (18)	14 (70)	2 (44)	

Individual insecticides and resistance trends/observations for the previous three cotton seasons are discussed below.

Helicoverpa armigera

Spinosad (Tracer®)

The last three seasons have seen very few survivors detected to spinosad following a trend of decreasing resistance frequency since a peak in 2001/02. (Figures 1a and 1b). This reduction in resistance can be attributed to a combination of management strategies implemented to curb an increasing resistance trend, and reduced usage due to an increase in alternative soft options, and the widespread uptake of Bollgard II®. No survivors were found in a number of valleys in each year, however generally these were not highly sampled, with the more highly sampled areas yielding the occasional survivor. The exception to this was the Upper Namoi that had reasonable collections in all three years however no spinosad survivors were detected. Only one spinosad survivor has been found in this valley in the last 6 years (2003/04).

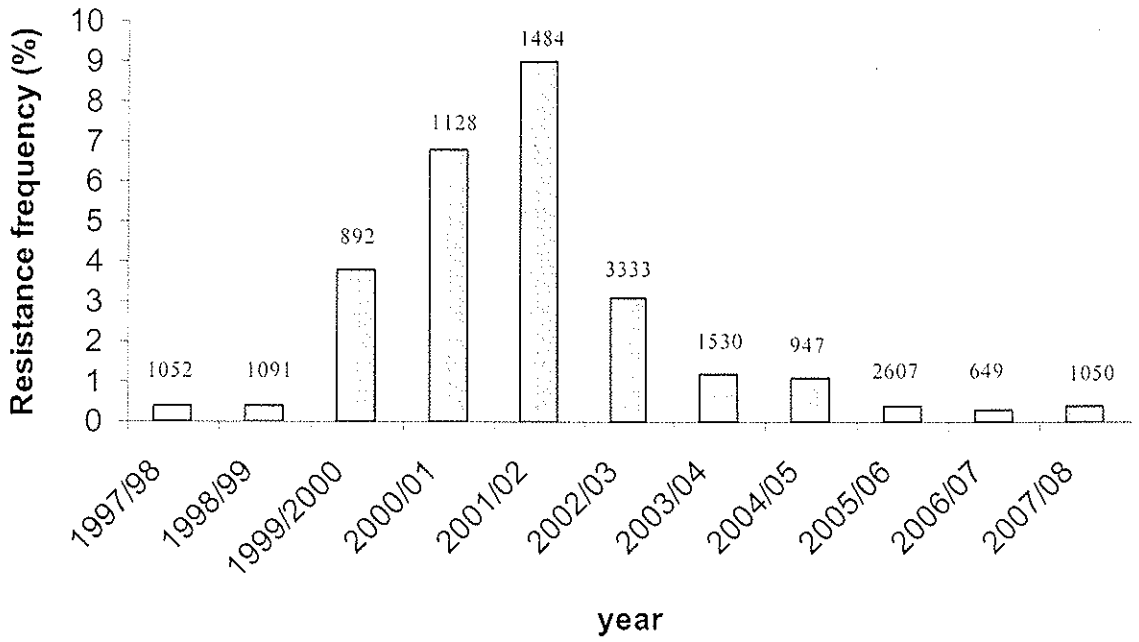

Figure 1a: Spinosad resistance frequencies, average of all valleys for each season (1997/98 – 2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

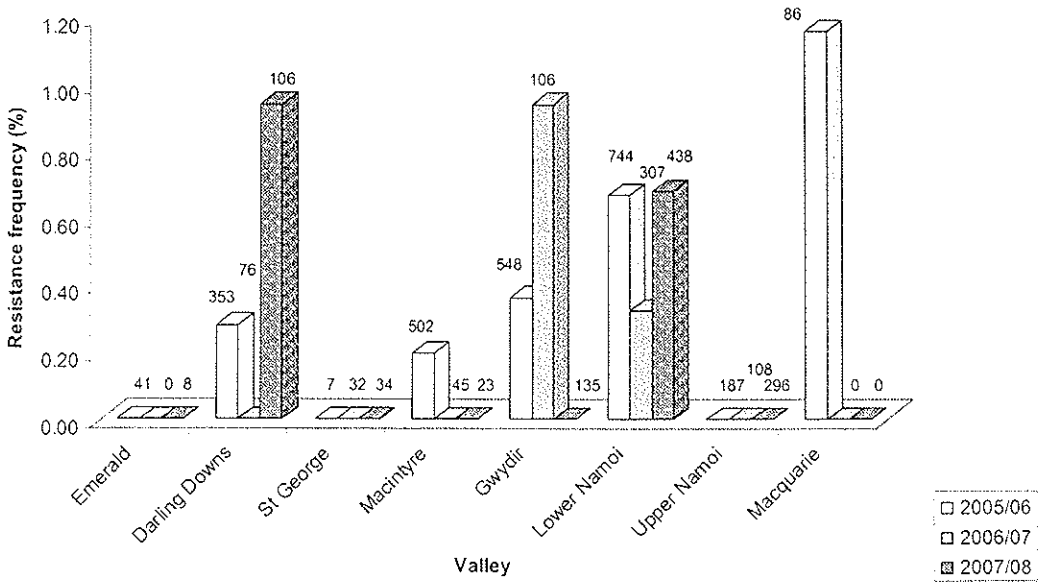

Figure 1b: Spinosad resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Indoxacarb (Steward®)

Indoxacarb resistant larvae were detected for the first time in 2002/03 in most valleys, and have continued to be detected, however at a decreasing frequency to the point where in 2007/08 only 1 survivor was detected in the Lower Namoi, the most highly sampled area. (Figures 2a & 2b). Like spinosad, while the results suggest that indoxacarb resistance is extremely low, the low level of sampling in the majority of areas and the detection of survivors in previous years means there is potential for selection (through a wide time period with the registration of both in a number of grains and pulse crops) and they remain an important component of the monitoring program to ensure the virtual absence of resistance remains.

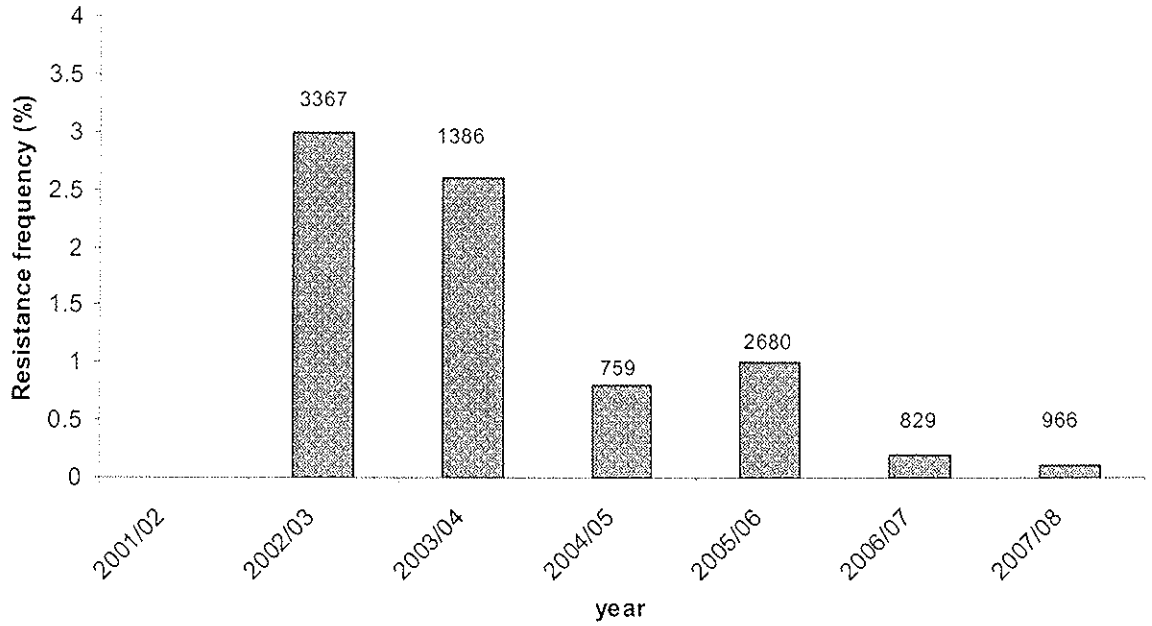

Figure 2a: Indoxacarb resistance frequencies, average of all valleys for each season. (2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

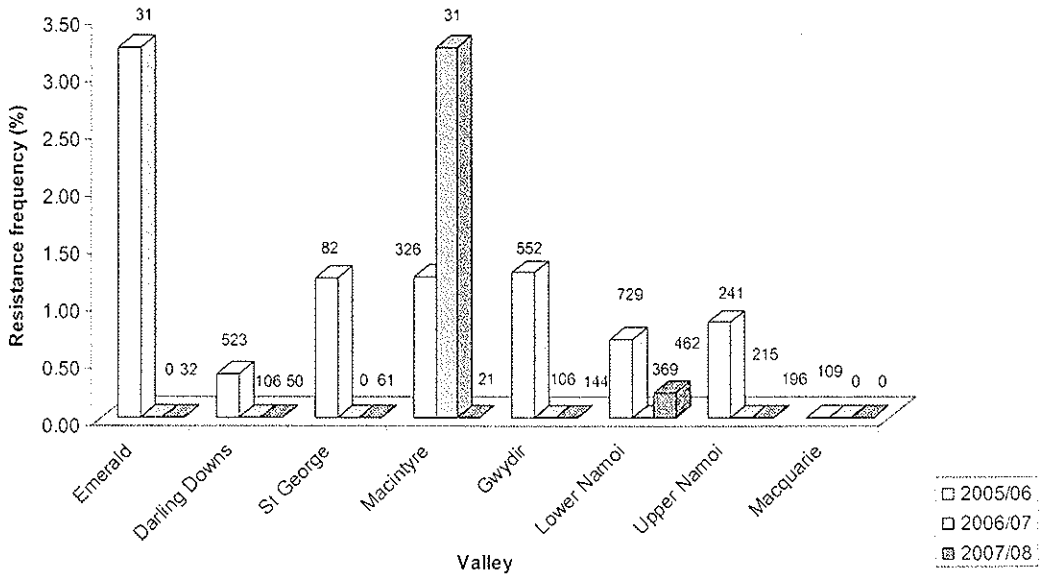

Figure 2b: Indoxacarb resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Emamectin Benzoate (Affirm®)

The 2002/03 season was also the first season that emamectin benzoate resistance was detected throughout the Australian cotton growing regions. Resistance frequencies have remained low and in some areas resistant larvae have not been detected at all (eg St George) (Figures 3a and 3b). Unlike spinosad and indoxacarb, emamectin benzoate is not registered for use against Helicoverpa in any other broad acre crops which minimises use and selection to cotton only, which has significantly decreased with the introduction of Bollgard II. It is however an important component of insect control within an IPM system and will continue to be monitored to ensure its efficacy continues.

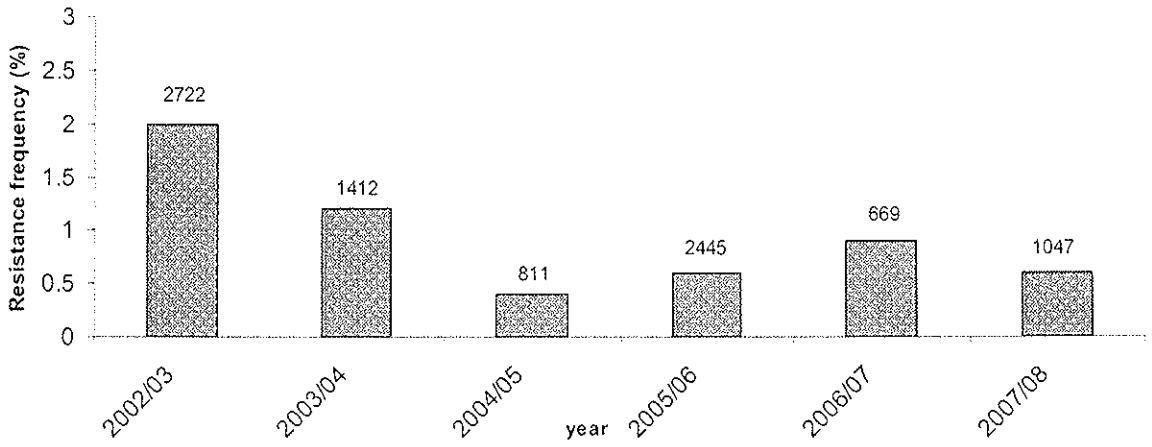

Figure 3a: Emamectin benzoate resistance frequencies, average of all valleys for each season. (2002/03-2004/05 data from previous monitoring project, L. Rossiter). Numbers on the graph indicate total larvae tested.

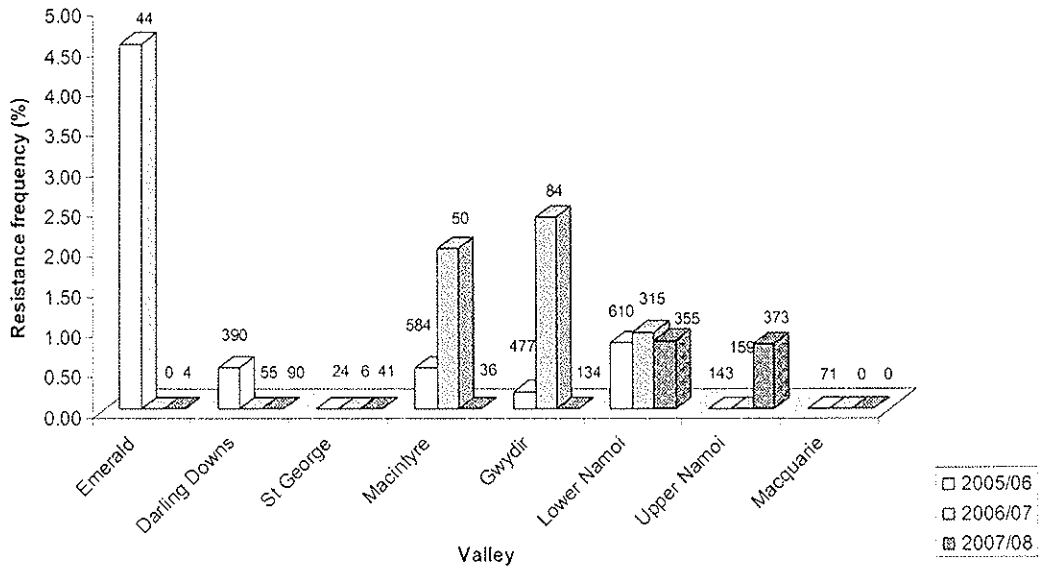

Figure 3b: Emamectin benzoate resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Chlorfenapyr (Intrepid®)

Chlorfenapyr resistance was last monitored in 2005/06. This was the first year that it was not commercially available in Australia and while is has retained registration it continues to be unavailable. Since its peak in 2001/02 (Figure 4), chlorfenapyr resistance declined significantly as a result of decreased use due to both restriction within the IRMS as well as reduced confidence in the product. There was minor use of the chemical in 2004/05, prior to discontinuation in 2005/06. While it is not currently part of the monitoring program, with larvae dedicated to insecticides actually in use in cotton, the ability to monitor resistance remains if it were to be reintroduced.

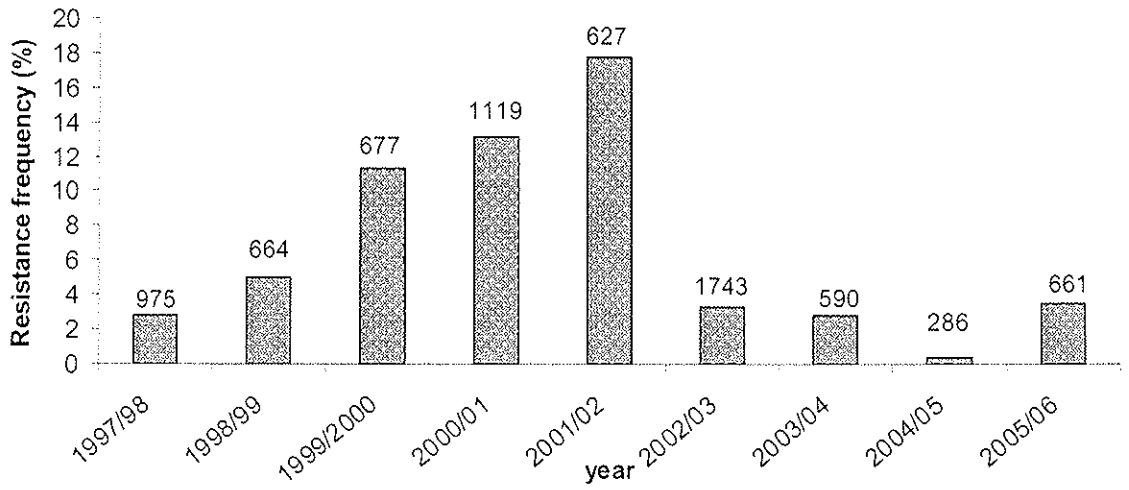

Figure 4: Chlorfenapyr resistance frequencies, average of all valleys for each season. (1997/98-2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

Methoxyfenozide (Prodigy®)

Methoxyfenozide was registered for use in cotton in 2003/04 however was only commercially available for two seasons (still registered, however no product has been available for purchase in Australia since 2005/06). Resistance monitoring was conducted in 2005/06 but has not continued since then. Since its introduction there has been no resistance detected to this insecticide.

Organophosphates (Chlorpyrifos and Profenofos)

Resistance to chlorpyrifos was detected again in 2002/03 following initial detection in 2001/02 (R. Gunning, pers comm.) and has continued to be found at very low frequencies with the odd survivor found in most valleys over the last three years (Figures 5a and 5b).

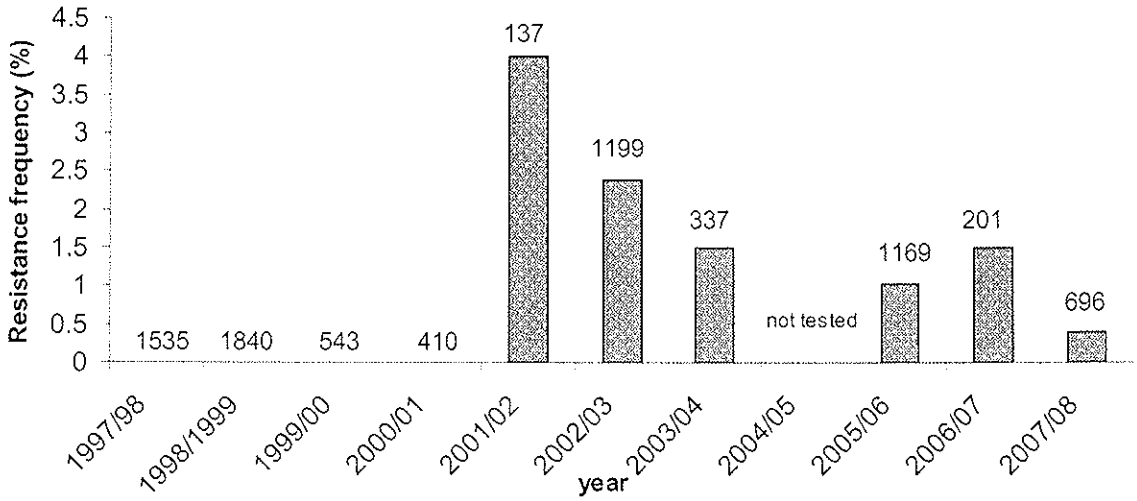

Figure 5a: Chlorpyrifos resistance frequencies, average of all valleys for each season (1997/98-2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

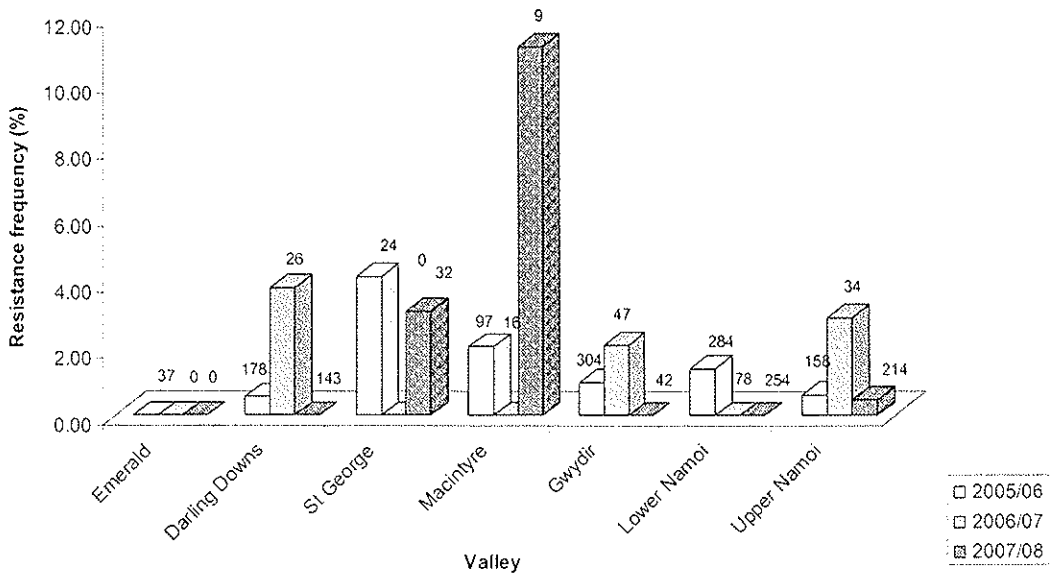

Figure 5b: Chlorpyrifos resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Unlike chlorpyrifos, profenofos resistance has been present in *H. armigera* populations in cotton growing areas since 1985. Although both insecticides are organophosphates, there is no cross resistance between the two chemicals. Resistance frequencies to profenofos have historically been moderate, however have declined in the last three years to the point where in 2007/08 only 1 survivor was detected. This was in the Gwydir, which is likely to have experienced the greatest pressure from insecticide use in cotton due to this area having the greatest area of conventionally sprayed cotton over the last three years. While monitoring was not particularly high in most areas in any year, this result does suggest a major turnaround in the frequency of profenofos resistance.

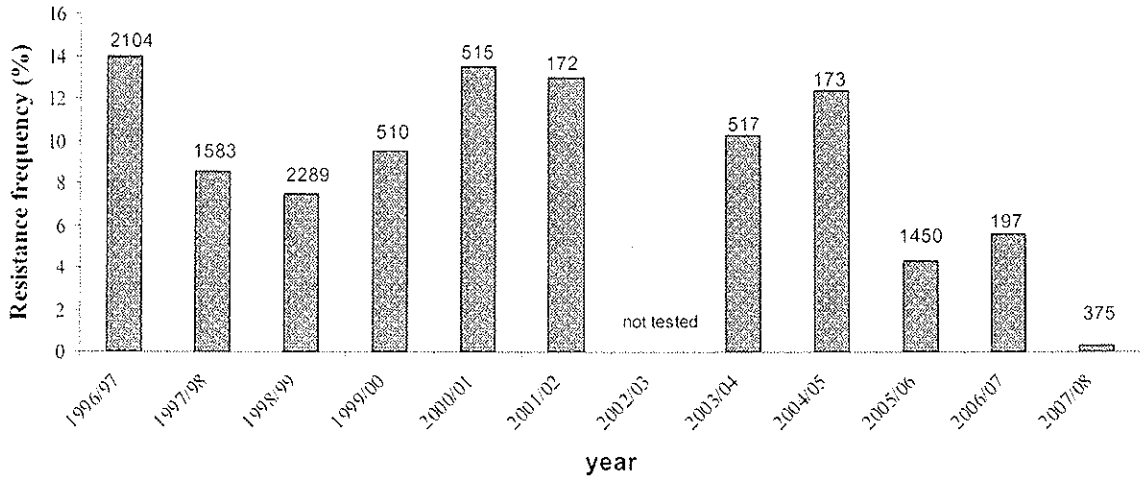

Figure 6a: Profenofos resistance frequencies, average of all valleys for each season (1996/97 – 2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

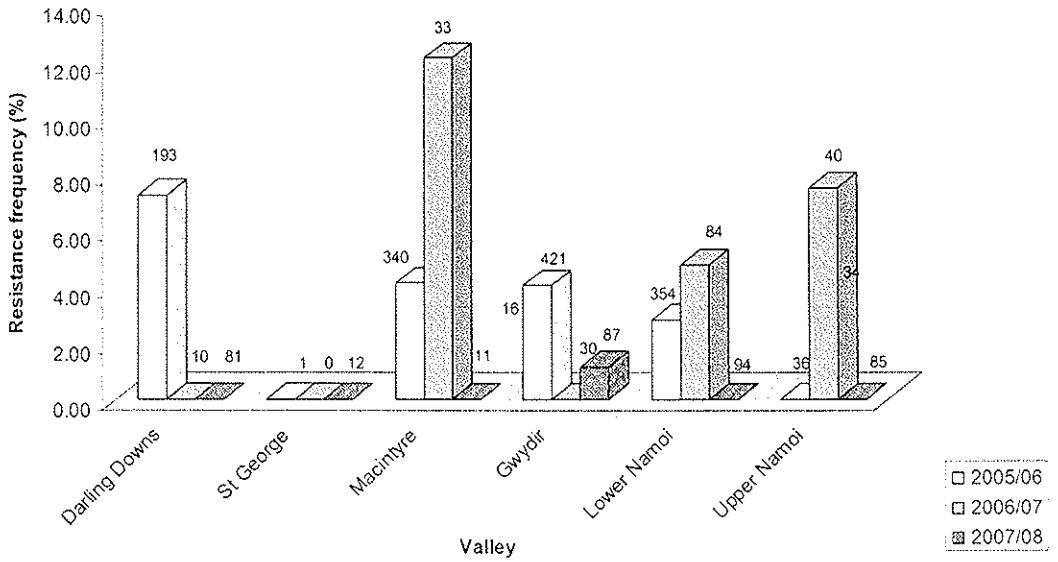

Figure 6b: Profenofos resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Endosulfan

Endosulfan resistance has been known to be present in *H. armigera* populations now for over thirty years (first reported in 1972/73), and variation in resistance frequency reflects overall use patterns in different areas. While resistance frequencies have been typically variable in different areas, they were generally moderate to high (Figure 7a). Monitoring in 2003/04 and 2004/05 was limited however followed a similar pattern of variation as previously observed, at slightly lower frequencies than in 2001/02 and 2002/03. Monitoring over the last three seasons, again involving low numbers tested, follows on from this, with the results suggesting further reductions in endosulfan resistance frequencies (Figure 7b). Given that endosulfan resistance has historically varied in response to use, the reduction in resistance over the last three seasons is likely to be a result of the reduction in use due to high uptake of Bollgard II.

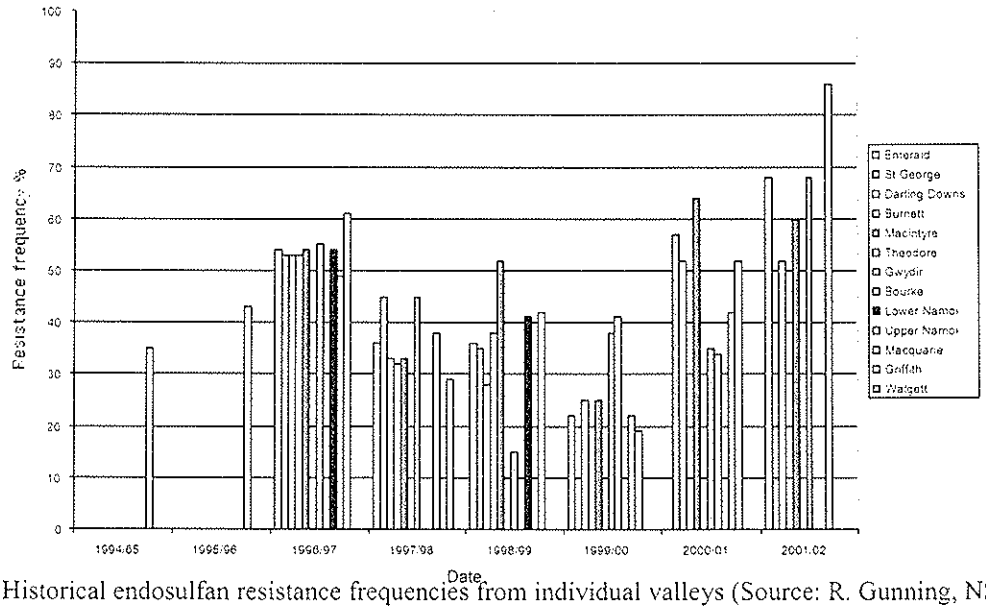

Figure 7a: Historical endosulfan resistance frequencies from individual valleys (Source: R. Gunning, NSW DPI, pers comm.)

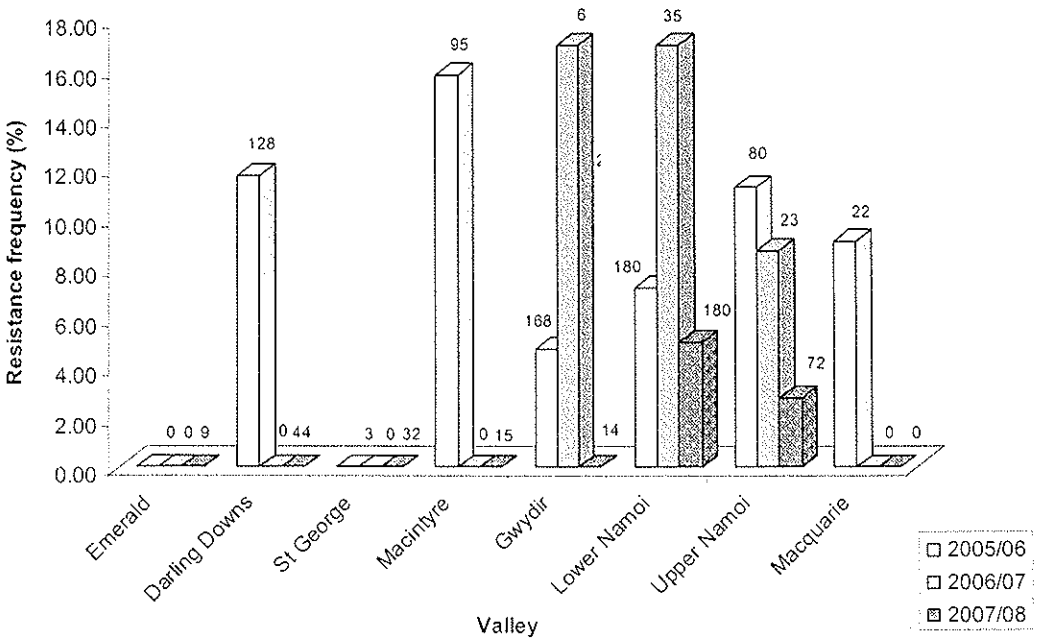

Figure 7b. Endosulfan resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Pyrethroids

Pyrethroid resistance is also established in *H. armigera* populations throughout the cotton growing regions of Australia, at variable but generally high frequencies. Monitoring has historically involved the pyrethroid fenvalerate, which while not registered for use in cotton, provided a good indicator of the level of general pyrethroid resistance. Monitoring over the last three years however has only involved the pyrethroid bifenthrin which is registered for use in cotton (Talstar®) only. Resistance to this pyrethroid has decreased significantly in the last three seasons, to the point where in 2007/08 no survivors were detected. To examine if this finding extended to other pyrethroids, fenvalerate was introduced back into the monitoring with an overall resistance frequency of 41 % (442 larvae tested). The survivors from this testing were kept to form a colony which was then tested with bifenthrin, with no survivors detected. These results confirm that the reduction in resistance to bifenthrin is specific to this insecticide and does not extend to other pyrethroids, with the fenvalerate results indicating that general pyrethroid resistance remains at a high frequency.

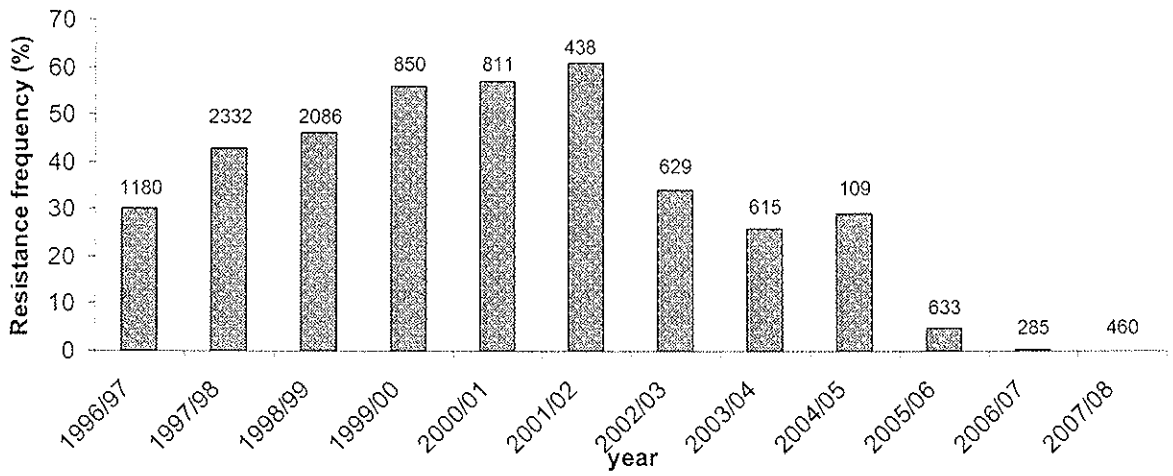

Figure 8: Bifenthrin resistance frequencies, average of all valleys for each season (1996/97-2001/02 data from previous monitoring project, R. Gunning, 2002/03-2004/05 L. Rossiter). Numbers on the graph indicate total larvae tested.

Methomyl (carbamate)

Methomyl (carbamate) resistance has been present at high frequencies throughout all cotton growing regions for over ten years, with typical frequencies of 60 % or more. The last year that methomyl was tested using reasonable numbers of larvae was 2004/05 with resistance frequencies across four valleys averaging between 40 and 60 %. Over the last three years, monitoring has been limited with insect material utilised to concentrate on the newer softer chemistries most used. The high pressure in 2005/06 allowed for reasonable numbers to be tested in this year, which suggested that resistance was still widespread and at a high frequency (Figure 9). If insect numbers allow, in 2008/09, a larger number of insects shall be tested with methomyl to determine if, like the other older insecticides, there has been a decrease in resistance frequencies.

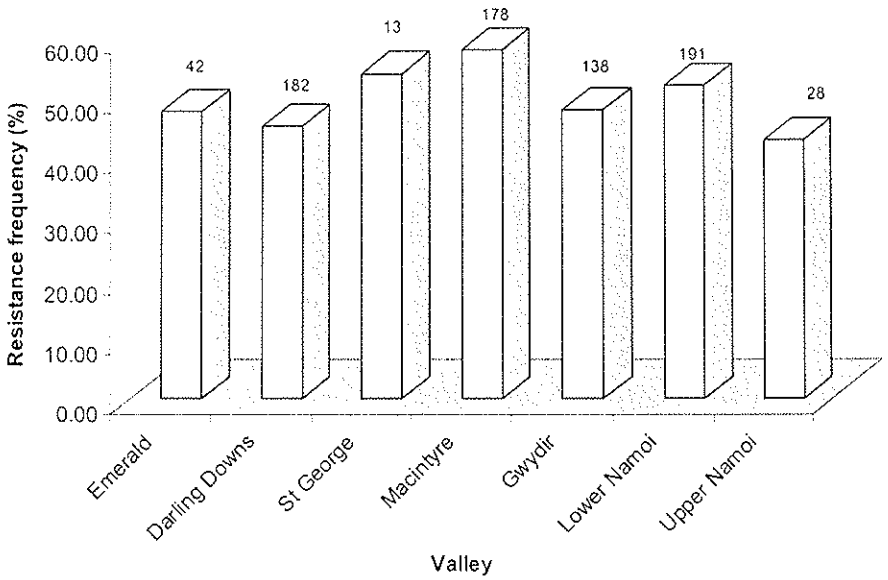

Figure 9: Methomyl resistance frequencies for several valleys in 2005/06. Numbers on the graph indicate total larvae tested.

Helicoverpa punctigera

Abundant and moderate-heavy *H. punctigera* pressure early and mid season in two of the three seasons (2006/07 was lower) ensured there was sufficient monitoring in most areas to assess if resistance is present or developing in these populations. There was no suggestion that resistance in *H. punctigera* was developing to any of the three insecticides.

Endosulfan

Endosulfan resistance has been detected in previous years at very low frequencies, with no indication that resistance was increasing. Monitoring in 2005/06-2007/08 confirmed that this is still the situation (Figure 10), with migration of very large numbers in 2005/06 resulting in detection of almost no resistance in these populations, with only a small number of survivors over the three years.

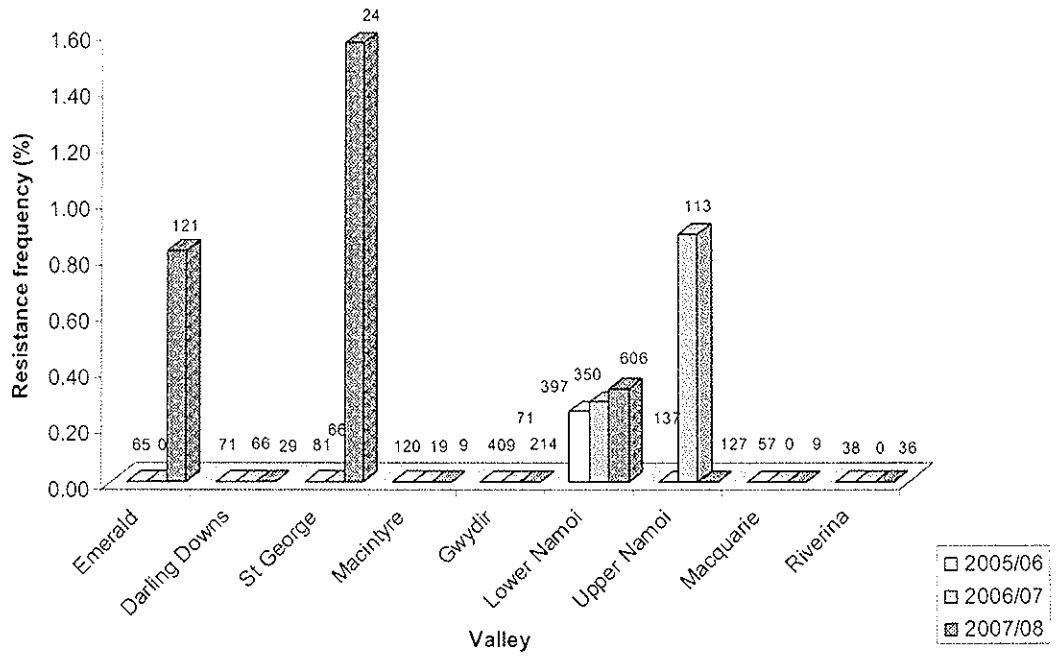

Figure 10: Endosulfan resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Pyrethroids (Fenvalerate)

Pyrethroid resistance has also been detected in previous years at low frequencies. Over 2005/06 to 2007/07 a small number of survivors were detected in those valleys most highly sampled (Gwydir and Upper and Lower Namoi), with no survivors detected in the other areas (Figure 11).

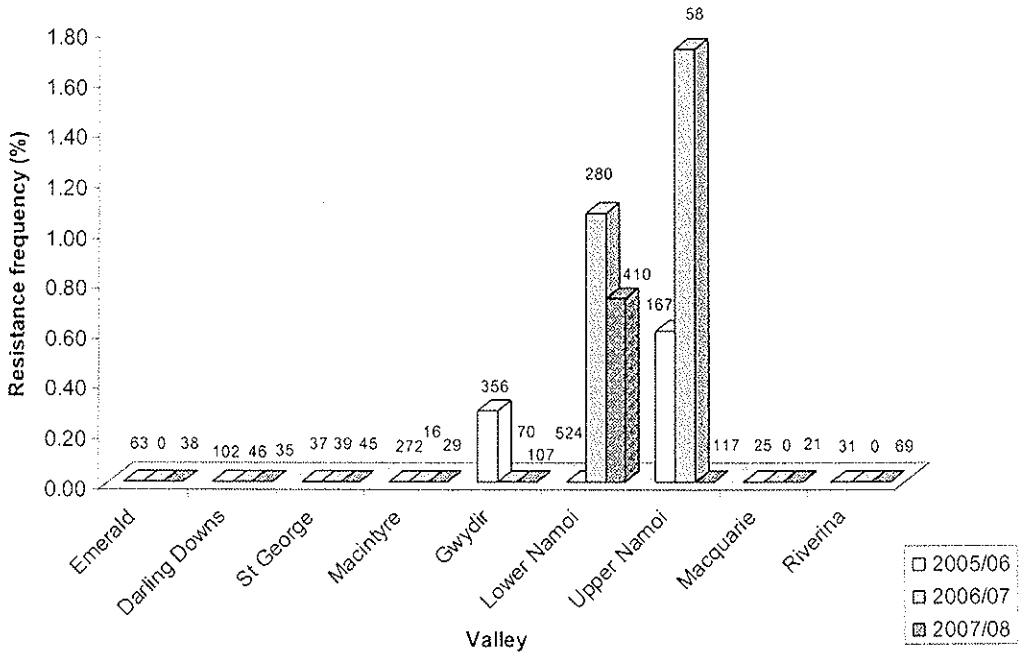

Figure 11: Pyrethroid (fenvalerate) resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

Abamectin

Disregarding small sample size, abamectin resistance was observed at low frequencies in all valleys over the last three years (Figure 12). With the exception of Emerald, at least one survivor was detected in every valley in at least one of the years (except Macquarie where only 16 insects were tested in the three years). Despite the findings of occasional survivors over all areas, there is no indication that resistance is developing beyond this very low frequency.

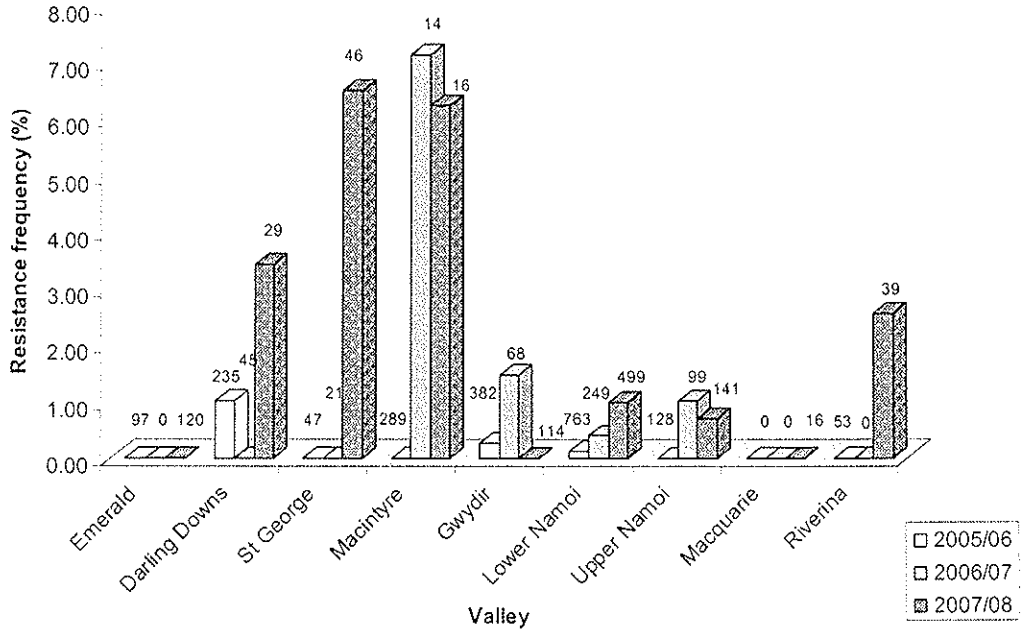

Figure 12: Abamectin resistance frequencies for each valley in 2005/06, 2006/07 and 2007/08. Numbers on the graph indicate total larvae tested.

4.2 Establishment of resistant *H. armigera* colonies and resistance mechanism and cross resistance studies

Over the three years, a number of colonies were established with using survivors of the resistance monitoring program. The only colonies to successfully establish were resistant to profenofos, methomyl and fenvalerate, established using multiple survivors rather than single pair matings of occasional survivors. These colonies were established and maintained so as to be available if required for other research, such as cross resistance studies in the event that a colony resistant to one of the key IPM compatible insecticides was formed. These colonies were used in dose response assays involving a new insecticide compound, Rynaxypyr (for details see section 4.4). A fenvalerate resistant colony was also used in 2007/08 to investigate cross resistance to bifenthrin (as detailed in section 4.1).

No colonies were successfully established through single pair matings involving indoxacarb, spinosad or emamectin benzoate survivors. Attempts were made to establish a chlorpyrifos resistant colony using a small number of field survivors, however despite outcrossing and several selection events, a stable resistant colony could not be established and after 20 generations the colony was weak and abandoned. Prior to abandoning this colony, esterase and glutathione S-transferase assays were conducted on insects from this colony. The level of activity of each of these enzymes was measured and compared with activity of insects from a susceptible colony (KO). No difference in activity was observed for either enzyme. These results suggest another mechanism may have been responsible for chlorpyrifos resistance, either cytochrome P-450 monooxygenase activity or an insensitive target site mechanism. Neither of these mechanisms were examined before the colony was closed. Due to the instability of the colony, in that individual selection events using the discriminating dose only managed to select for a small number of survivors, it is also possible that the insects tested in the enzyme assays were susceptible (colony a mix of susceptible and resistant insects). In the event that a chlorpyrifos resistant colony is established in the future, these enzyme systems would be re-investigated.

Preliminary studies on the dose-response of Bt resistant colonies held by CSIRO in Canberra were conducted. The initial response of the Bt resistant strains, BX (Cry1Ac) and SP15 (Cry 2Ab) to discriminating doses of insecticide did not indicate that either of the strains were resistant to any of the insecticides tested (Table 2) with 100 % mortality to all insecticides except profenofos. The finding of one SP15 survivor to profenofos was attributed to likely be a problem with the assay, with a higher frequency of resistance expected if there was cross resistance between profenofos and Cry 2Ab. These assays were followed up with dose response assays involving some of the insecticides against these two strains and a Bt susceptible strain (GR), with insect numbers limiting testing all insecticides against all strains. The results of these (Table 3) were inconclusive due to both an incomplete data set (assays limited by insect availability) as well as some assays requiring repeating due to the inappropriate range of insecticide concentrations used. This latter problem was expected as for some insecticides it was unknown the range of concentrations that would give a response from 0 to 100 % mortality, with the discriminating dose providing the only guide. Of the strains that provided good data for analysis, there was no difference in the response of SP15 to the susceptible GR, for emamectin benzoate or bifenthrin, based on overlapping 95 % Fiducial Limits of LD_{50} values.

It was anticipated that this work would be repeated and a full data set analysed, however a number of factors resulted in this not occurring (including the need to travel to Canberra to do the assays as well as the principal researcher going on maternity leave for over 4 months). This work is to be repeated however in the current project. The availability of SP15 (with CSIRO) at ACRI, Narrabri shall facilitate this.

Table 2: Mortality of Bt resistant (SP15 and BX) strains and susceptible (GR) strains to discriminating doses of 8 different insecticides (numbers in brackets are number of larvae tested)

Insecticide	D. Dose (µg/µL)	Susceptible GR	SP15 (Cry 2Ab resistant)	BX (Cry 1Ac resistant)
Emamectin benzoate	1	100 % (65)	100 % (72)	100 % (66/66)
Spinosad	10	100 % (68)	100 % (72)	100 % (66/66)
Indoxacarb	6	100 % (72)	100 % (72)	100 % (65/65)
Bifenthrin	0.1	100 % (72)	100 % (71)	100 % (64/64)
Methomyl	1	100 % (48)	100 % (69)	100 % (65/65)
Profenofos	1	100 % (47)	98.5 % (69)	100 % (60/60)
Chlorpyrifos	20	100 % (60)	100 % (70/70)	100 % (61/61)
Endosulfan	12.5	100 % (39)	100 % (69/69)	100 % (60/60)

Table 3. LD_{50} (µg/µL) values of Bt resistant (SP15 and BX) and Bt susceptible (GR) strains (95 % Fiducial Limits are given in brackets).

Insecticide	GR (susceptible)	SP15 (Cry 2Ab)	BX (Cry 1Ac)
Spinosad	-	-	-
Indoxacarb	0.0089 (0.0064-0.0124)	0.0090 (0.0069-0.0118)*	0.0069 (0.0041-0.0116)*
Emamectin benz	0.0010 (0.0006-0.0018)	0.0023 (0.0014-0.0035)	-
Chlorpyrifos	0.8193 (0.5972-1.1239)*	0.3351 (0.0966-1.1634)*	0.4380 (0.3088-0.6211)
Bifenthrin	0.0009 (0.0006-0.0012)	0.0009 (0.0006-0.0013)	-
Fenvalerate	0.0099 (0.0071-0.0138)	0.0138 (0.0108-0.0175)*	0.0154 (0.01100-0.0215)*
Endosulfan	-	0.1877 (0.1359-0.2592)	-
Profenofos	0.1166 (0.0275-0.4942)*		-
Methomyl	-	0.1161 (0.07128-0.1890)*	-

* data unacceptable and needs repeating, (χ^2 value too high, or too few data points to accurately measure response)
- assay not conducted.

4.3 Esterase gene sequence identification

A full length preliminary esterase gene sequence was obtained using RACE techniques on a partial gene sequence that had been identified during travel undertaken in DAN 187. The 5' end of the gene had already been determined, so 3' RACE was conducted to obtain the full sequence. 3' RACE products were obtained using 4 different primer combinations, with several plasmids containing each product sequenced. Sequence data was analysed using BioEdit and DNASTAR programs, with all sequences aligned to detect possible errors in sequencing and to determine a putative esterase gene based on consensus of alignment. The full length sequence was determined to be 1662 bp long (554 amino acids), which fits within the range of other insect esterase sequences.

The final gene and protein sequence entered into the Web based BLAST Program (http://www.ncbi.nlm.nih.gov/BLAST/) that finds regions of local similarity between sequences using sequence databases of genes identified worldwide. This search indicated a high homology to other insect esterase sequences as well as insect acetylcholinesterase sequences, which share many similar motifs with general esterases. Despite the similarities in sequence, the mechanisms of resistance conferred by the two enzymes are different and it was imperative that the sequence was esterase derived and not an acetylcholinesterase. The sequence was confirmed as being part of an esterase gene through the absence of Trp 84, an amino acid with a Torpedo number of 84, that is conserved in all acetylcholinesterases but not present in esterase sequences.

The full length protein sequence is given in Figure 13, showing an alignment with a number of resistance associated esterase sequences from insects (sequences obtained from Genbank). This alignment shows a reasonable degree of conservation of amino acids within insect esterases, particularly towards the 5' end of the sequence.

This putative esterase gene sequence shall be confirmed using PCR involving a proof reading polymerase to minimise error and obtain an accurate gene sequence for use in further research.

Figure 13. Alignment of *H .armigera* esterase protein sequence (labelled possible full sequence June 08.pro) with other resistance associated esterase sequences from insects. Amino acids matching the consensus are shaded.

```
   LEVAXKIKKFYFGDKTVSKENLDXFXSLXSDXSFGYGTQRTLQ-R--ANKGXPVYLYRFGYDSE---NSY Majority
            430       440       450       460       470       480       490
   LQVANAVKNFYFGDNTVSTDNLDLFVSYSSNASIGYHAQRFANKW--ANIGKKTYFFKENFFTE------ possible full sequence June 08.pro
   RVVNDLVKASF---KQVKKIGMDVFFSGPIDL------TQRFLAR---HNKGHPVYYYKLSYQS------- apis carboxylesterase 2 protein.pro
   KEVGKQMKKFYYGETEPSFENREGYLTLMTDKLFLHGLHRTILSRLNSKKPSKTFLYRFSVDSDTY-NHY C. quinquefasciatus esta2 protein.pro
   LEMGAKIKKAHVTGETPTADN---EMDLCSHIYEWFPMHRLLQLRFNHTSGTPVYLYRFDFDSEDLINPY lucilia cuprina aest3 protein.pro
   ---AEKIKTEYFGNSTISNETLPQLTKLYSDTYFLNGIKSTL-----SRHEGEKYVYKFGYEGS---YSI nilaparvata est1 protein.pro
   FKTAQDIKEFYFGDKPISKETKSNLSKMISDRSFGYGTSKAAQ-HIAAKNTAPVYFYEFGYSGN---YSY myzus persicae FE4 protein.pro

   RIVFSGXGVKYGGSHADH-DLTFY-FFKNXFN------KXSXDYKXIKKMVGLIATFA-KNGNPNSEEIX Majority
            500       510       520       530       540       550       560
   WNVFGQQGVKYGLQEASHFDMPFYVFYPNDQN--WTVDTSSQQYALVQKITTAIANFA-KNSDPSTDTI- possible full sequence June 08.pro
   ---------KYAMHK-----------FKGN----------------------PLNDYQSDDMSAISPEIT apis carboxylesterase 2 protein.pro
   RIVFCDKNVR-GTAHAD--DLSY--IFKNVFN--DPPAKDTFEHRAMMNMVGLFSTFASNNGNPNGEQIN C. quinquefasciatus esta2 protein.pro
   RIMRSGRGVK-GVSHAD--ELTY--FFWNQLA--KRMPKESREYKTIERMTGIWIQFATT-GNPYSNEIE lucilia cuprina aest3 protein.pro
   SQLLSGDPTYRNGVC--HADDLFYLFPMKPFLGLRVGSETEKDKEISAKFVDLITNFV-IEGNPNSKSEP nilaparvata est1 protein.pro
   VAFF-DPKSYSPGSSPTHGDETNYVLKVDGFTVY----DNEEDPKMIKTMVNIWATFI-KSGVPDTEN-S myzus persicae FE4 protein.pro

   E-----W-PVIKXSAXXFKCLNISNDGLFEIXXPE---IKNWXSAYEKXN--NLY----            Majority
            570       580       590       600       610
   -----TW-PAYTSSEKAY--VTFENDDVTVGYGPDDQDYIFWKDTYEKAGV-NF.                possible full sequence June 08.pro
   ----VRR-PVYQQDELNHLC-----------------------KYAKFNE-ALYC                apis carboxylesterase 2 protein.pro
   E-----W-ESIATPAGPFKCLNINNDGLQFIEYPEQERMKFWDSLYSKDK---LY                 C. quinquefasciatus esta2 protein.pro
   GMENVSW-DPIKKSDEVYKCLNISDE-LKMIDVPEMDKIKQWESMFEKHR--DLF                 lucilia cuprina aest3 protein.pro
   SI-----WTPSSKDVD----FLSISTEGNFEMKK-------NFPGA                          nilaparvata est1 protein.pro
   EI-----WLPVSKNPADLFRFTKITQQQTEEAREQSTMAIMNFGVAYHYQNILNIMCQMT            myzus persicae FE4 protein.pro
```

4.4 Baseline dose response data accumulation

2005/06

The feeding and topical bioassays were developed and refined using lab colonies in 2005/06. This included where possible replicating assays using the same colony (same generation) as part of identifying if the assays were robust and the methods could produce repeatable results. The results for the two assay methods are presented in Tables 4a and 4b. Both methods had reasonable slope values and produced data of good fit as indicated by the with χ^2 values. Converting the topical bioassay LC_{50} values from µg/µL to ppm, suggested that Rynaxypyr was more toxic when administered in the feeding assay (eg 1860/61N Ha F1, topical = 0.012 µg/µL = 12ppm, feeding = 0.022/0.020 ppm). The difference in toxicity could not be accurately determined however as the topical assay administered an exact dose of insecticide (in 1 µL), while the amount eaten in the feeding bioassay would have varied between individuals and was not quantified to determined the exact amount of toxin ingested.

Data for two replicates of three colonies was obtained for the topical assay, with two of these having statistically similar LC_{50} values (as determined by overlapping 95 % Fiducial Limits (FLs). The third colony, 1782M F2 had an approximate two-fold difference in LC_{50} values, with 95 % FLs almost overlapping. This topical bioassay is a standard method that has been used successfully in the resistance monitoring program for *Helicoverpa* spp. for over 20 years due to its repeatability as well as ease of use when screening large number of insects. These factors, combined with the results which were considered acceptable in regard to replication, supported the continued accumulation of dose response data using this method.

Four insect colonies were replicated for the feeding assay. There was no significant difference in the LC_{50} values obtained for three of these colonies, as determined by their overlapping 95 % FLs. The fourth colony, Emerald c'pea F1 was replicated three times, with a maximum two fold difference in LC_{50} values. While the 95 % FLs of the highest and lowest LC_{50} values did not overlap for this one colony, the data was considered acceptable to continue use of this assay method for accumulating baseline dose response data for Rynaxypyr.

The 2005/06 results were not incorporated with the other two years of data as the methods underwent refinement before a final protocol was employed in 2006/07 and 2007/08.

2006/07 and 2007/08

Dose response data for both feeding and topical assays are presented in Tables 5a and 5b for 2006/07 and 2007/08. A small number of *H. punctigera* colonies were included in the colonies, with this species proving to be more susceptible to Rynaxypyr than *H. armigera*. There was a range of LC_{50} values for both the topical and feeding bioassays, however for all strains, the highest concentration tested resulted in 100 % mortality. Inclusion of a number of insecticide resistant strains (field selected and then lab reared under selection) indicated that there was no cross resistance between methomyl or fenvalerate and Rynaxypyr. Bellata/BJ methomyl rep 1 F3 did have the highest LC_{50} value for the feeding bioassay, however this was not significantly different to a number of other susceptible colony values as determined by overlapping 95 % Fiducial Limits.

Various methods can be used to assign a discriminating dose, using LC_{95} – $LC_{99.9}$ values, such as choosing the highest $LC_{99.9}$ value or highest 95 % FL value, doubling the $LC_{99.9}$ (Sabatini et al., 2001), or combining all data and taking the LC_{99} value (Nauen & Konanz, 2005). Regardless of the method, the discriminating dose must be so that it is not too low resulting in a large number of false positives and not too high that low level resistance is not detected. Based on the data presented in Tables 5a and 5b, and the observation of 100 % mortality at the highest doses tested, for the purposes of monitoring resistance to Rynaxypyr, a discriminating dose of 0.5 µg/µL and 1 ppm is proposed for topical and feeding bioassays

respectively. This, and the method employed for resistance monitoring shall be further discussed with colleagues before implementation in the 2008/09 resistance monitoring program.

Table 4a Baseline susceptibility to Rynaxypyr applied topically, 2005/06

Year	Population	n	Probit Analysis Parameters			
			Slope ± SE	LC_{50} ($FL_{95\%}$) µg/µL	$LC_{99.9}$ ($FL_{95\%}$) µg/µL	χ^2 (df)
2005/06	1392D Ha F4	310	2.16 ± 0.31	0.0062 (0.0043-0.0088)	0.176 (0.0753-0.409)	7.81 (5)
	1860/61N Ha F1	252	1.98 ± 0.12	0.012 (0.0083-0.017)	0.458 (0.141-1.486)	0.89 (4)
	1782M Ha F2 rep 1	324	1.63 ± 0.10	0.0087 (0.0061-0.012)	0.720 (0.250-2.071)	2.17 (6)
	1782M Ha F2 rep 2	288	1.87 ± 0.18	0.019 (0.013-0.026)	0.876 (0.320-2.394)	3.98 (5)
	1667G Ha F4 rep 1	324	1.80 ± 0.26	0.0050 (0.0034-0.0074)	0.280 (0.110-0.712)	12.2 (6)
	1667G Ha F4 rep 2	324	1.43 ± 0.18	0.0025 (0.0014-0.0043)	0.391 (0.117-1.303)	7.34 (6)
	1671N Ha F3	252	1.71 ± 0.15	0.0026 (0.0016-0.0044)	0.181 (0.0470-0.698)	1.71 (4)
	Emerald c'pea Ha F1 rep 1	432	2.22 ± 0.10	0.0062 (0.0048-0.0079)	0.160 (0.0765-0.334)	1.47 (5)
	Emerald c'pea Ha F1 rep 2	344	1.59 ± 0.23	0.0080 (0.0057-0.011)	0.763 (0.210-2.761)	9.38 (5)
	1610Gu Ha F2	240	1.91 ± 0.27	0.0085 (0.0058-0.012)	0.374 (0.118-1.186)	5.87 (5)

Table 4b Baseline susceptibility to Rynaxypyr using diet incorporation feeding assay, 2005/06

Year	Population	n	Probit Analysis Parameters			
			Slope ± SE	LC_{50} ($FL_{95\%}$) ppm	$LC_{99.9}$ ($FL_{95\%}$) ppm	χ^2 (df)
2005/06	1860/61N Ha F1 rep 1	280	1.60 ± 0.21	0.022 (0.015-0.033)	2.029 (0.573-7.176)	8.05 (5)
	1860/61N Ha F1 rep 2	240	1.94 ± 0.30	0.020 (0.014-0.029)	0.824 (0.270-2.517)	9.66 (4)
	1860/61N Ha F2	200	2.51 ± 0.34	0.028 (0.019-0.043)	0.506 (0.171-1.497)	4.92 (3)
	1782M Ha F2	280	2.01 ± 0.23	0.018 (0.012-0.026)	0.654 (0.260-1.647)	6.67 (5)
	1667G Ha F4	336	2.21 ± 0.22	0.021 (0.015-0.028)	0.544 (0.245-1.205)	6.88 (5)
	1667G Ha F7	200	2.01 ± 0.25	0.019 (0.012-0.029)	0.700 (0.205-2.387)	3.92 (3)
	Namoi U+L Ha F3	240	2.32 ± 0.25	0.022 (0.015-0.031)	0.488 (0.214-1.114)	5.15 (4)
	Lower Namoi Ha F4	288	2.19 ± 0.25	0.022 (0.016-0.031)	0.598 (0.254-1.409)	6.09 (4)
	1522M Ha F6 rep 1	336	2.49 ± 0.33	0.018 (0.014-0.022)	0.320 (0.150-0.681)	9.02 (5)
	1522M Ha F6 rep 2	210	2.39 ± 0.26	0.016 (0.012-0.022)	0.337 (0.125-0.907)	4.25 (5)
	Emerald c'pea Ha F1 rep 1	336	1.79 ± 0.19	0.019 (0.014-0.026)	1.091 (0.387-3.072)	5.50 (5)
	Emerald c'pea Ha F1 rep 2	280	2.01 ± 0.20	0.031 (0.023-0.042)	1.141 (0.471-2.767)	5.12 (5)
	Emerald c'pea Ha F1 rep 3	336	2.29 ± 0.16	0.039 (0.031-0.051)	0.930 (0.456-1.894)	3.53 (5)
	Emerald c'pea Ha F2	320	2.17 ± 0.24	0.031 (0.024-0.040)	0.857 (0.387-1.896)	8.57 (6)

Table 5a Baseline susceptibility to Rynaxypyr applied topically, 2006/07 and 2007/08

Year	Population	N	Probit Analysis Parameters			
			Slope ± SE	LC_{50} ($FL_{95\%}$) µg/µL	$LC_{99.9}$ ($FL_{95\%}$) µg/µL	χ^2 (df)
2006/07	Bellata c'pea/faba Hp F5	210	2.41 ± 0.36	0.00056 (0.00041-0.00078)	0.0114 (0.00408-0.0319)	6.02 (4)
	2001N Ha F2	134	2.68 ± 0.30	0.0022 (0.0012-0.0041)	0.0329 (0.00994-0.109)	1.27 (3)
	Gatton Chickpea Ha F3	240	1.27 ± 0.17	0.0026 (0.0015-0.0045)	0.787 (0.169-3.664)	6.62 (6)
	2079N Ha F1	252	1.70 ± 0.17	0.0027 (0.00178-0.00395)	0.187 (0.0491-0.712)	2.73 (4)
	2132Gu Ha F2	320	1.89 ± 0.18	0.00325 (0.00236-0.00448)	0.149 (0.0647-0.344)	5.89 (6)
	Bellata chickpea Ha F3	132	1.68 ± 0.35	0.0042 (0.0024-0.0072)	0.306 (0.0550-1.707)	8.09 (6)
	2145N Ha F1	320	1.76 ± 0.13	0.0043 (0.0031-0.0058)	0.257 (0.103-0.643)	3.70 (6)
	2056N Ha F2	320	1.72 ± 0.15	0.0044 (0.0032-0.0060)	0.297 (0.108-0.818)	4.54 (6)
	Goondi Corn Ha F2	288	1.42 ± 0.19	0.0052 (0.0036-0.0077)	0.843 (0.226-3.148)	9.05 (6)
	2043N Ha F2	320	1.58 ± 0.08	0.0052 (0.0038-0.0073)	0.504 (0.180-1.414)	1.68 (6)
	2059Gu Ha F2	320	1.62 ± 0.14	0.0054 (0.0039-0.0074)	0.465 (0.174-1.241)	5.46 (6)
	Bellata chickpea Ha F5	319	2.05 ± 0.17	0.0061 (0.0045-0.0081)	0.205 (0.0911-0.461)	3.76 (5)
	Goondi Corn Ha F1	365	1.50 ± 0.16	0.0082 (0.0061-0.0112)	1.030 (0.349-3.041)	8.20 (6)
	2132Gu Ha F1	324	1.48 ± 0.21	0.00978 (0.00697-0.0137)	1.282 (0.388-4.236)	11.4 (6)
2007/08	2302N Hp F2	205	1.65 ± 0.30	0.00013 (0.000046-0.00034)	0.010 (0.0017-0.060)	3.20 (4)
	CS SG Hp F4	380	1.60 ± 0.29	0.00016 (0.000089-0.00029)	0.015 (0.0047-0.046)	1.60 (5)
	2007 SG chickpea Hp F2	202	2.72 ± 0.35	0.00021 (0.00013-0.00036)	0.0030 (0.0011-0.0084)	2.26 (3)
	Darling Downs Hp F2	222	2.58 ± 0.51	0.00022 (0.00014-0.00037)	0.0037 (0.0013-0.010)	5.68 (3)
	2483M Ha F1	228	1.52 ± 0.34	0.00054 (0.000185-0.00156)	0.062 (0.010-0.374)	5.13 (4)
	2474Gu Ha F2	276	1.28 ± 0.15	0.00062 (0.000244-0.00157)	0.174 (0.028-1.070)	2.19 (5)
	CS St George Ha F2	262	1.47 ± 0.22	0.00090 (0.000410-0.00196)	0.125 (0.017-0.891)	2.60 (4)
	2474Gu Ha F1	276	1.75 ± 0.31	0.00096 (0.000520-0.00176)	0.060 (0.015-0.238)	5.33 (4)
	2414Gu Ha F2	276	1.74 ± 0.24	0.00123 (0.000723-0.00209)	0.079 (0.026-0.239)	5.64 (5)
	2331TW Ha F3	320	1.41 ± 0.15	0.00150 (0.0009-0.00251)	0.253 (0.070-0.913)	4.28 (6)
	CS UN Ha F3	360	1.60 ± 0.24	0.00168 (0.00109-0.00260)	0.153 (0.049-0.480)	9.70 (6)
	CS SG Harl Ha F3	320	1.97 ± 0.20	0.00188 (0.00130-0.00272)	0.074 (0.028-0.195)	4.28 (4)
	2007 Emerald chickpea Ha F2	320	1.70 ± 0.26	0.00218 (0.00148-0.00321)	0.155 (0.047-0.507)	8.08 (5)
	CS Gwydir Ha F3	320	1.45 ± 0.15	0.00231 (0.00149-0.00359)	0.335 (0.075-1.494)	3.32 (5)
	CS Macintyre Ha F3	320	1.89 ± 0.20	0.00235 (0.00166-0.00332)	0.108 (0.037-0.318)	4.22 (5)

	2326N Ha F1	240	3.06 ± 0.32	0.00274 (0.00204-0.00369)	0.029 (0.012-0.071)	2.52 (3)
	2646N Ha F2	359	1.83 ± 0.19	0.00305 (0.00216-0.00429)	0.158 (0.064-0.391)	6.45 (6)
	2483M Ha F3	308	1.97 ± 0.17	0.00314 (0.00234-0.00421)	0.124 (0.050-0.307)	4.04 (5)
	CS Darling Downs Ha F4	360	2.08 ± 0.21	0.00324 (0.00237-0.00444)	0.104 (0.050-0.216)	7.12 (6)
	2331TW Ha F1	280	1.97 ± 0.25	0.00326 (0.00218-0.00489)	0.126 (0.047-0.336)	6.03 (4)
	2007 Emerald chickpea Ha F1	432	1.68 ± 0.13	0.00327 (0.00239-0.00449)	0.242 (0.110-0.533)	6.06 (6)
	CS SG Harl Ha F4	384	1.39 ± 0.094	0.00348 (0.00238-0.00507)	0.632 (0.176-2.273)	2.21 (5)
	2008 Fenval surviv Ha F3	320	1.84 ± 0.22	0.00352 (0.00250-0.00497)	0.179 (0.0712-0.451	6.82 (5)
	CS SG Ha F3	360	1.91 ± 0.11	0.00378 (0.00280-0.00510)	0.167 (0.076-0.366)	2.63 (6)
	2648N Ha F2	320	1.63 ± 0.20	0.00408 (0.00286-0.00582)	0.344 (0.090-1.310)	5.36 (5)
	CS Gwydir Ha F2	360	2.07 ± 0.23	0.00443 (0.00333-0.00589)	0.147 (0.068-0.317)	8.24 (6)
	CS St George Ha F3	274	1.61± 0.18	0.00478 (0.00327-0.00700)	0.425 (0.121-1.499)	5.12 (6)

Table 5b Baseline susceptibility to Rynaxypyr using diet incorporation feeding assay, 2006/07 and 2007/08

Year	Population	N	Probit Analysis Parameters			
			Slope ± SE	LC_{50} ($FL_{95\%}$) ppm	$LC_{99.9}$ ($FL_{95\%}$) ppm	χ^2 (df)
2006/07	Bellata c'pea/faba Hp F5	240	3.70 ± 0.48	0.00244 (0.00192-0.00311)	0.0173 (0.00937-0.0319)	6.93 (4)
	Gatton C'pea Ha F2	200	4.69 ± 0.76	0.0073 (0.0057-0.0093)	0.0339 (0.0200-0.0577)	6.68 (3)
	Bellata Chickpea Ha F5	200	3.19 ± 0.43	0.0112 (0.0078-0.0162)	0.108 (0.0369-0.319)	3.47 (3)
	2145N Ha F1	200	2.34 ± 0.28	0.0123 (0.0083-0.0182)	0.269 (0.0804-0.900)	3.40 (3)
	2043N Ha F2	240	3.34 ± 0.38	0.0135 (0.0104-0.0174)	0.117 (0.0623-0.221)	4.42 (4)
	2079N Ha F2	200	2.88 ± 0.37	0.0145 (0.01074-0.0195)	0.179 (0.0709-0.451)	3.90 (3)
	Bellata Chickpea Ha F3	280	2.72 ± 0.24	0.0156 (0.0122-0.0201)	0.224 (0.120-0.417)	4.52 (5)
	Goondi Corn Ha F1	280	2.76 ± 0.37	0.0158 (0.0124-0.0202)	0.217 (0.112-0.420)	10.2 (5)
	2059Gu Ha F2	240	2.67 ± 0.28	0.0171 (0.0130-0.0224)	0.255 (0.119-0.548)	4.30 (4)
	2003D Ha F2	320	1.51 ± 0.12	0.0211 (0.0152-0.0295)	2.563 (0.807-8.138)	3.88 (6)
	2056N Ha F2	240	2.79 ± 0.11	0.0212 (0.0164-0.0274)	0.283 (0.133-0.604)	0.59 (4)
	2132Gu Ha F1	320	2.81 ± 0.38	0.0221 (0.0171-0.0286)	0.289 (0.161-0.517)	12.2 (6)
	2001N Ha F3	320	1.96 ± 0.22	0.0240 (0.0181-0.0318)	0.957 (0.369-2.482)	7.78 (6)
	2132Gu Ha F2	240	2.48 ± 0.41	0.0279 (0.0209-0.0373)	0.513 (0.211-1.246)	8.92 (4)
	2002D Ha F3	240	3.01 ± 0.23	0.0304 (0.0236-0.0390)	0.335 (0.166-0.676)	2.23 (4)
	Goondi Corn Ha F2	280	2.62 ± 0.28	0.0335 (0.0259-0.0434)	0.532 (0.252-1.126)	5.37 (5)
	2056N Ha F1	384	1.72 ± 0.19	0.0374 (0.0281-0.0497)	2.522 (0.975-6.526)	10.98 (6)
	Bellata/BJ methomyl rep1 F3	240	1.93 ± 0.22	0.0430 (0.031-0.060)	1.808 (0.520-6.281)	3.78 (4)
2007/08	CS SG Hp F4	360	2.39 ± 0.19	0.00137 (0.00107-0.00177)	0.0282 (0.0134-0.0592)	4.57 (6)
	CS UN Ha F3	264	2.49 ± 0.30	0.0058 (0.0043-0.0078)	0.105 (0.042-0.262)	4.28 (4)
	2317SG Ha F2	240	4.12 ± 0.38	0.0064 (0.0052-0.0085)	0.038 (0.021-0.070)	2.00 (3)
	2008 fenval surviv Ha F1	280	2.06 ± 0.39	0.0065 (0.0046-0.0091)	0.2166(0.0618-0.759)	8.20 (4)
	2007 Emerald Chickpea Ha F2	240	4.28 ± 0.32	0.0074 (0.0058-0.0093)	0.040 (0.022-0.071)	3.78 (4)
	2498N Ha F1	304	1.56 ± 0.17	0.00741 (0.00460-0.0119)	0.756 (0.204-2.799)	3.41 (5)
	CS SG Harl Fenval surv Ha F4	320	2.61 ± 0.28	0.0084 (0.0064-0.0110)	0.1339 (0.0674-0.266)	5.75 (5)
	CS Macintyre Ha F3	240	4.34 ± 0.19	0.0098 (0.0078-0.012)	0.052 (0.028-0.095)	0.469 (3)
	2317SG Ha F3	228	2.29 ± 0.22	0.011 (0.0078-0.016)	0.259 (0.082-0.825)	2.00 (4)
	2483M Ha F1	296	1.94 ± 0.13	0.011 (0.0079-0.015)	0.442 (0.164-1.189)	2.14 (5)
	CS SG Harl Ha F2	280	2.25 ± 0.24	0.012 (0.0088-0.017)	0.298 (0.104-0.850)	3.16 (4)

	CS ST George Ha F2	316	2.33 ± 0.10	0.012 (0.0093-0.016)	0.269 (0.126-0.576)	1.00 (5)
	CS St George Ha F3	344	2.15 ± 0.30	0.012 (0.0093-0.016)	0.353 (0.145-0.859)	10.98 (6)
	2474Gu Ha F2	264	2.57 ± 0.28	0.012 (0.0094-0.017)	0.206 (0.094-0.451)	4.53 (4)
	2326N Ha F2	280	3.30 ± 0.29	0.014 (0.011-0.018)	0.128 (0.064-0.255)	2.95 (4)
	2483M Ha F2	360	1.97 ± 0.15	0.015 (0.011-0.019)	0.568 (0.238-1.356)	3.95 (6)
	2414Gu Ha F2	320	2.37 ± 0.24	0.016 (0.012-0.020)	0.333 (0.140-0.792)	4.48 (5)
	2007 Emerald Chickpea Ha F1	360	2.03 ± 0.25	0.016 (0.012-0.021)	0.560 (0.246-1.273)	9.31 (6)
	CS Gwydir Ha F3	320	2.75 ± 0.15	0.016 (0.013-0.021)	0.227 (0.113-0.457)	1.57 (5)
	2474Gu Ha F1	280	2.59 ± 0.27	0.017 (0.013-0.022)	0.268 (0.127-0.566)	4.62 (4)
	CS SG Ha F3	304	2.08 ± 0.25	0.017 (0.013-0.023)	0.565 (0.204-1.570)	5.81 (5)
	CS SG Harl Ha F4	280	2.56 ± 0.16	0.018 (0.014-0.024)	0.307 (0.138-0.685)	1.54 (4)
	2008 fenval surviv Ha F3	268	2.74 ± 0.32	0.0199 (0.0152-0.0260)	0.2790 (0.1179-0.6604	4.68 (4)
	2646N Ha F2	320	1.95 ± 0.18	0.020 (0.014-0.026)	0.791 (0.289-2.160)	3.76 (5)
	CS Gwydir Ha F2	320	2.24 ± 0.20	0.0226 (0.0172-0.0298)	0.569 (0.263-1.228)	4.65 (5)
	CS Macintyre Ha F2	344	1.80 ± 0.17	0.024 (0.018-0.032)	1.361 (0.539-3.436)	6.42 (6)

4.5 Resistance management strategy formulation and promotion

Resistance monitoring data were presented to the TIMS committee in 2006, 2007 and 2008 as part of assessing the IRMS, used primarily by the cotton industry. The 2006/06 IRMS had minor amendments, including a shortening of the endosulfan window (label restriction), and an earlier window for Tracer, in response to industry request. The 2006/07 IRMS remained unchanged, as it did for the 2007/08 season in regard to insecticide use, however the resistance monitoring results were used to support an amendment to the guidelines regarding pupae busting, which utilised the Helicoverpa Diapause Induction and Emergence Tool developed by QDPI&F to assess the risk to resistance carryover by pupae at different times. Importantly this amendment better aligned pupae busting recommendations in cotton with other crops such as autumn finishing pulses. Resistance monitoring data was used in assessing the risk of the amendment in relation to resistance management. This amendment will be annually reviewed by TIMS, relying on the resistance monitoring data to determine if changes such as this impact on resistance management. In 2008, the resistance monitoring data was again used by TIMS to support an extension to the use period for abamectin in cotton which will apply to the 2008/09 season. The IRMS was disseminated through the DPI publication Cotton Pest Management Guide, and is also available on the Cotton CRC website.

Promotion of improved insecticide resistance management strategies is vital in ensuring appropriate resistance measures are applicable in an ever evolving industry. This objective was completed through involvement with the TIMS committee in formulating the annual IRMS, with further extension to the industry via the Resistance Roadshow (May 2008), grower and consultant meetings, researcher meetings and written extension articles (see Section 8). A highlight of these publications has been the promotion of resistance management specifically at grain and pulse growers, as part of bringing together cotton with these industries. While the IRMS is focussed on the cotton industry which reference to pulses, good resistance management within cotton obviously has benefits for other crops. Likewise however it is important that insecticide use within grains and pulses supports measures taken in cotton.

4.6 Support other Helicoverpa related research projects with insect material

This project maintains an insecticide susceptible and field derived *H. armigera* and *H. punctigera* colonies for use within the project, which are available to support other related projects. Regular support has been provided to a DPI projects investigating Helicoverpa semiochemicals (CRC 1.05.04). The colonies have also been used casually by various other NSW DPI and CSIRO researchers at ACRI.

The project has also supported a PhD student at the University of Queensland, Corinna Lange. This project involves looking at *H. punctigera* populations, and H. *punctigera* larvae have been collected and sent to her after testing for insecticide resistance.

H. armigera have also been supplied to UNE for teaching purposes on a number of occasions.

5. Outcomes

Output contribution to planned outcomes:

Outcome 1: Measure of the success of insecticide resistance management strategies promoted to the Australian Cotton Industry. Resistance monitoring data used by TIMS committee in assessing and modifying IRMS.

The insecticide resistance monitoring data was used directly by the TIMS committee to assess the success of management strategies and to modify the IRMS. The data was also used to support amendments to the IRMS. Without this information the committee could not confidently endorse any amendment to the IRMS, nor could it measure if the strategy was having any benefit. Without this information, promotion of the strategy would be difficult, with little incentive for cotton growers to apply insecticides according to the IRMS.

Outcome 2: Increase knowledge of effective insecticide resistance management in cotton production. Formulation of improved resistance management strategies encompassing all aspects of cotton production, including all pest insects and associated resistance issues, IPM principles and the practicalities of production.

This outcome is also reliant on the insecticide resistance monitoring data and its use by TIMS and dissemination to the industry along with resistance management guidelines. The data provides a measure of field resistance frequencies which can be compared over time and between areas to determine if the strategies in place are appropriate and managing resistance. Resistance monitoring over the last three years suggests that strategies in place (supported by other factors) have contributed to the observation of declining resistance frequencies.

Formulating improved resistance management strategies does not necessarily involve modification to insecticide use within the IRMS. The resistance monitoring data in conjunction with the Helicoverpa Diapause Induction and Emergence Tool developed by QDPI&F was used in 2007 to modify the IRMS in regard to pupae busting guidelines. In 2008 an amendment was made to the abamectin window, which while involving an insecticide use period change, was in response to a request for use on mites, which the monitoring data for *H. punctigera* supported.

Outcome 3: Dose-response data accumulated to increase scientific knowledge of the response of *Helicoverpa* spp. to different insecticides. Appropriate bioassay technique and discriminating dose used for accurate resistance detection before field problems develop.

The development of appropriate topical and feeding bioassays and employment of these assays over two seasons for Rynaxypyr directly contributes to this outcome. The dose response data for over 25 field derived lab colonies representing most cotton growing regions shall be used to assign a discriminating dose to Rynaxypyr for use in resistance monitoring from its initial registration in the 2008/09 cotton season.

Outcome 4: Increased knowledge of how insects overcome different insecticides with different modes of action. Increased effectiveness of IRMS achieved with information on resistance mechanisms. Possible development of rapid bioassays for detecting resistant individuals in the laboratory / field.

This outcome was dependent on the establishment of resistant insect colonies, which was inhibited by the low frequency of resistance to the key IPM insecticides of greatest interest. Preliminary work was done using a chlorpyrifos resistant colony, but due to its instability (in terms of ability to select and maintain resistance within the colony) the results were inconclusive.

Outcome 5: Increased knowledge on how different resistance mechanisms overcome the toxic effects of different insecticides. Increased effectiveness of management strategies for insecticide resistance by accounting for cross resistance between chemistries.

This outcome was also largely dependent on colonies resistant to key IPM compatible chemistries. Some cross resistance studies were done as part of the accumulation of baseline dose response data for Rynaxypyr which suggested that insects resistant to pyrethroids or methomyl shall be effectively controlled by this insecticide. The inclusion of new insecticides within the IRMS depends on this information, as if cross resistance is present between other insecticides, efficacy of a new insecticide can be compromised and in such circumstances its placement within the IRMS may require greater strategy.

Fenvalerate resistant *H. armigera* colonies were also used to determine that the absence of resistance to bifenthrin (also a pyrethroid) was specific to this insecticide, and that general pyrethroid resistance remains at a high frequency.

Outcome 6: Increased knowledge on both resistance to insecticides and Bt toxins for use in development of alternative control measures (both insecticidal and transgenic) in the future. Increase effectiveness of both insecticide and Bt resistance management strategies.

Discriminating dose assays and dose response assays on Bt resistant and susceptible strains using different insecticides initiated investigation to meet this outcome. The results of this were not finalised and shall be completed to provide the information outlined in the outcome.

Outcome 7: Investigation of the role of this esterase in resistance, with possible future identification of other esterases and their properties and role in resistance. More precise mechanism of resistance determined and properties identified with implications for possible resistance diagnostic development, and future control methods.

Identification of a preliminary esterase gene sequence is the first step in realising this outcome. Knowledge of the sequence shall allow for extensive further investigation of the genetic and biochemical properties of this gene and its protein product in relation to insecticide resistance for incorporation into the IRMS.

6. Technical Advances, methodology discoveries and changes to IP

Technical Advances: This project was a research project and not of a technical or commercial nature

Other research information and IP: The data from the dose response assays conducted for Rynaxypyr shall be used to determine a discriminating dose for use in future resistance monitoring. This shall be initially covered by NSW DPI IP, however shall be published and in the public domain in the near future. The esterase gene sequence shall also be publicly available on the internet accessible databank Genbank once the accurate sequence is confirmed.

7. Conclusions

Insecticide resistance monitoring was successfully conducted in 2005/06, 2006/07 and 2007/08. The objective of this monitoring was to detect resistance to chemistries used against *Helicoverpa* spp. and monitor trends and changes in resistance frequency. These results were utilised by TIMS as part of assessing the success of the IRMS and formulating changes.

Conclusions from this monitoring were:

- Very low resistance frequencies were detected to the IPM compatible chemistries of indoxacarb, spinosad and emamectin benzoate.
- Resistance is still present in field populations to those chemistries that *H. armigera* is known to have developed resistance to, including endosulfan, methomyl and organophosphates (profenofos), however frequencies appear to have declined over the last three seasons, quite significantly for some insecticides.
- While resistance to the pyrethroid bifenthrin is very low, this is specific to this insecticide and does not extend to other pyrethroids, with widespread general pyrethroid resistance still present.
- Very low frequency detection of resistance to endosulfan, pyrethroids and abamectin by *H. punctigera.*
- Information recorded on species complex across time and space within cotton growing regions which has implications for the monitoring project and also for resistance management.

These results have direct implications for insecticide use within both the cotton industry as well as grains/pulses. The IPM compatible insecticides can continue to be used with confidence of good efficacy. For a number of the other older insecticides, these may also provide reasonable control. It is important to note however that the reduction in resistance frequency can probably be largely attributed to reduced insecticide use since the introduction of Bollgard II. It is important that insecticides continue to be used within the IRMS to ensure that their effectiveness remains, particularly if usage increases. With the reduction in resistance frequency to a number of the older insecticides, the industry is in a well placed position to retain those insecticides if required within an IPM system.

Attempts to investigate resistance development within a *H. armigera* population with a focus on the key IPM compatible insecticides met limited success. Difficulties with establishing resistant colonies for investigation hindered this investigation and resulted in no definite outcomes. Colonies resistance to older insecticides however were utilised in various ways, including investigating bifenthrin cross resistance to other insecticides. The finding that the reduction in bifenthrin resistance is specific to this pyrethroid only was an important distinction from other pyrethroids as basing pyrethroid efficacy recommendations on this one pyrethroid would have been an inaccurate message to extend to the cotton industry.

The preliminary sequence of a possible resistance associated esterase gene was successfully identified. This is an important first step in further investigations of the gene and its protein properties that may lead to increased information on the ways insects confer resistance.

Baseline dose response data was accumulated over two seasons for the new Helicoverpa insecticide Rynaxypyr. This data was used to assign a discriminating dose for this insecticide to allow for its incorporation in the resistance monitoring program. Resistance monitoring for this insecticide shall begin in 2008/09 following its registration for use in cotton.

8. Extension

The results of this project have been disseminated using various methods as outlined below. In addition the TIMS committee has been annually updated on the status of insecticide resistance by *H. armigera* as part of assessing and formulating management strategies. The methods of extension outlined shall continue to be utilised. Aspects of the project shall be further disseminated to the scientific community through the publication of peer reviewed scientific articles (in preparation).

8.1 Publications

Book Chapter

Andow, D.A., Fitt, G.P., Grafius, E.J., Jackson, R.E., Radcliffe, E.B., Ragsdale, D.W. & Rossiter, L. (2008). Pesticide and Transgenic Plant Resistance Management in the Field. In M.E. Whalon, D. Mota-Sanchez and R.M. Hollingworth (Eds) Global Pesticide Resistance in Arthropods'. CAB International.

Conference and meeting abstracts and oral presentations

Rossiter, L., Gunning, R. & McKenzie, F. (2008). Silver Anniversary of Resistance Management in the Australian Cotton Industry: An overview and the current situation for *Helicoverpa armigera*. In Proceedings, 14th Australian Cotton Conference, Gold Coast, QLD, 12-14 August 2008.

Rossiter L. (2008). Silver Anniversary of Resistance Management: An overview and the current situation for *H. armigera*. Northern Farming Systems IPM Researchers Forum, 25-26th June 2008, Toowoomba, abstract and oral presentation.

Rossiter, L. (2007). Helicoverpa insecticide resistance monitoring 2006/07. Northern Farming Systems IPM Researchers Forum, 24-25th July 2007, Toowoomba, abstract and oral presentation.

Rossiter, L. (2007). Pupae busting in conventionally sprayed cotton: can it be removed in a cotton farming system dominated by Bollgard II? ACGRA Research and Extension in Bt resistance and management meeting, 20th June 2007, oral presentation.

Rossiter, L. & Kauter, G. (2006). *Helicoverpa armigera* insecticide resistance management - key considerations for 2006/07. In Proceedings, 13th Australian Cotton Conference, Gold Coast, QLD, 8-10 August 2006.

Extension articles

Rossiter L (2008). Helicoverpa insecticide resistance: Management for the grain industry. *Australian Grain* 18 (3): 44-46.

Rossiter, L. (2008). Survey reveals minimal change in frequency of pesticide resistance. In Leonard, E (Ed.) Groundcover Integrated Pest Management Supplement, May- June 2008 pg 10.

Rossiter, L., Murray, D., Miles, M., Downes, S., Wilson, L., and Kauter, G. (2007). Better pupae busting decisions in sprayed conventional cotton. *The Australian Cotton Grower* 28 (6): 21-22.

Downes, S., Rossiter, L., Parker, T., McKenzie, F. & Staines, T. (2007). How to collect Helicoverpa for resistance testing. *The Australian Cotton Grower* 28 (7): 48-49

Rossiter, L. (2007). Resistance on the slide. in Boehm R. (Ed.) Spotlight on Cotton R&D, Spring 2007 pg 17.

Kauter, G. & Rossiter, L. (2006). TIMS ratifies 2006-07 insecticide resistance management strategy. *The Australian Cotton Grower*, 27(4): 22-24.

Downes, S., Mahon, R., Rossiter, L., Farrell, T., Wilson, L. & Kauter, G. (2006). How will the drought affect Helicoverpa resistance management? *The Australian Cotton Grower*, 27(7): 60-62.

Downes, S., Rossiter, L., Farrell, T., Wilson, L. and Kauter, G. (2005). Managing resistance: your IRMS and RMP questions answered. *The Australian Cotton Grower*, 26(**6**): 10-14.

Rossiter, L., Downes, S. and Mahon, R. (2005). Helicoverpa: species mix, parasitism and resistance monitoring. . *The Australian Cotton Grower*, **26 (6):** 66-69

Contributions to extension publications and manuals

Rossiter, L., Farrell, T., Larsen, D., Kauter, G., Downes, S., Wilson, L. Murray, D. & Miles, M (2007). Insecticide Resistance Management Strategy (IRMS) for 2007/08. In Farrell T. (Ed) Cotton Pest Management Guide 2007/08, pages 37-41.

Rossiter, L. (2007). *Helicoverpa* spp. Insecticide resistance monitoring results 2006/07. In O'Halloran, J (Ed). Gwydir Valley Cotton Season & Trial Book 2006/07. NSW Dept. of Primary Industries and Cotton CRC.

Rossiter, L., Murray, D., Miles, M., Downes, S., Wilson, L. & Kauter, G (2007). IRMS 2007/08 Post harvest pupae destruction in sprayed conventional cotton. In Farrell T. (Ed) Cotton Pest Management Guide 2007/08 pg 42

Rossiter, L., Farrell, T., Larsen, D., Kauter, G., Downes, S., and Wilson, L. (2006). Insecticide resistance management strategy (IRMS) for 2006-2007. In Farrell, T. (Ed) *Cotton Pest Management Guide*, 2006-07. NSW Dept. of Primary Industries.

Rossiter, L., Farrell, T., Larsen, D., Pyke, B., Kauter, G., Downes, S. & Wilson, L. (2005). Insecticide resistance management strategy (IRMS) for 2005/06. In Farrell T. & Johnson A. (Eds) *Cotton Pest Management Guide 2005/06*, pages 32-36

Downes, S. and Rossiter L. (2005) Bt and Insecticide Resistance Monitoring. In Vaessen, S. (Ed). *Southern New South Wales Cotton Trial Book*. NSW Dept. of Primary Industries and Cotton CRC.

Contributions of insecticide resistance monitoring results to Cotton Tales newsletters compiled by Industry Development Officers and District Agronomists across all cotton growing valleys, 2005-2008.

Invited Oral presentations at field days and grower meetings

TIMS Resistance Roadshow 2008. Oral presentations to growers and consultants at five locations in NSW and QLD:

'Helicoverpa Insecticide Resistance Monitoring Results, 2007/08'.

Presentation of 2005/06 insecticide resistance monitoring results and draft IRMS to CCA AGM, 16th May 2006.

Presentation of 2005/06 insecticide resistance monitoring results and draft IRMS during 2 seminars at 2006 Moree Trade Show, May 24-25th 2006

Media presentations

Insecticide Resistance Management Strategy CSD Website: 14th June 2006

Lawson, A. (2006). Chemical Treatments survive heliothis test. The Land Newspaper, June 1, 2006.

On-Line Resources

EXECUTIVE SUMMARY

Resistance is one of the greatest threats to effective pest control in the Australian Cotton Industry, both against insecticides as well as transgenic cotton. This project has continued a long term insecticide resistance monitoring program for both *H. armigera* and *H. punctigera* over the last three seasons. Other aspects of the project include cross resistance and resistance mechanism research, accumulation of dose response data for new insecticides and resistance management formulation and promotion.

Insecticide resistance monitoring was successfully conducted in 2005/06, 2006/07 and 2007/08. The objective of this monitoring was to detect resistance to chemistries used against *Helicoverpa* spp. and monitor trends and changes in resistance frequency. The data is used in the assessment and formulation of the IRMS utilised by the cotton industry to delay and manage insecticide resistance. Conclusions from this monitoring were:

- Very low resistance frequencies were detected to the IPM compatible chemistries of indoxacarb, spinosad and emamectin benzoate.
- Resistance is still present in field populations to those chemistries that *H. armigera* is known to have developed resistance to, including endosulfan, methomyl and organophosphates (profenofos), however frequencies appear to have declined over the last three seasons.
- While resistance to the pyrethroid bifenthrin is very low, this is specific to this insecticide and does not extend to other pyrethroids, with widespread general pyrethroid resistance still present in the field.
- Very low frequency detection of resistance to endosulfan, pyrethroids and abamectin by *H. punctigera*.

These results have direct implications for insecticide use within both the cotton industry as well as grains/pulses which a number are registered for use in. The IPM compatible insecticides can continue to be used with confidence that the products will provide good control. While the resistance frequency to most of the older insecticides appears to have declined, it is important to note that the reduction in resistance frequency can probably be largely attributed to reduced insecticide use since the introduction of Bollgard II. Resistance is still present and detectable in the field allowing for selection when insecticides are used. For this reason it is important that insecticides continue to be used within the IRMS to ensure that their effectiveness remains, particularly if their overall use increases.

As part of continuing to investigate the features of resistance mechanisms, an esterase gene has been successfully sequenced. This information shall be used to characterise the properties of this gene and its protein product and its possible role in insecticide resistance. This shall enable investigation of resistance to move from the biochemical characterisation of resistance enzymes to investigating the features of the genes that drive these enzyme systems.

In addition to resistance monitoring and mechanism research for chemicals currently registered for use on cotton, it is essential that new chemistries entering the industry have accurate dose-response data measured prior to their introduction. For the last two seasons this data has been collected for a new insecticide registered for use in cotton in 2008/09, Rynaxypyr. This research has enabled an appropriate bioassay and discriminating dose to be determined for use in resistance monitoring which will begin in its first year of registration. This shall allow for measurement of future changes and the detection of resistance development before it is evident in the field.

The final aspect of this research is the formulation and promotion of resistance management strategies and principles. The assessment and formulation of the IRMS by TIMS has utilised resistance monitoring data which has enabled changes to be made to the IRMS at the request of the industry. The resistance monitoring data was also used to support an amendment to the

Table 6c: 2007/08 *Helicoverpa armigera* resistance frequencies during the season, with number of larvae tested in brackets

Valley			Endosulfan	Steward	Tracer	Affirm	Pyrethroid (Talstar)	Pyrethroid (fenvalerate)	Chlorpyrifos (OP)	Profenofos (OP)	Carbamate
	% *H. armigera*	% *H. punctigera*	% resistance	% resistance	% resistance	% resistance	% resistance	% resistance	% resistance	% resistance	% resistance
Emerald											
December	15	85		0 (22)	-	-	-	-	-	-	-
Darling Downs											
November	100*	0	-	0 (30)	1 (80)	0 (75)	0 (12)	-	0 (80)	0 (40)	-
December	6	94	-	-	0 (18)	0 (20)	-	-	0 (31)	0 (20)	-
January	66	34	0 (35)	-	-	-	-	11 (9)	0 (42)	0 (21)	-
St George											
November	29	71	-	0 (15)	-	-	-	-	-	-	-
December	70	30	0 (11)	-	-	-	-	-	0 (17)	-	-
January	46	54	0 (17)	-	-	0 (10)	-	-	0(12)	-	-
February	20	80	-	-	0 (14)	0 (11)	0 (12)	-	-	-	-
March	100*	0	-	0 (33)	0 (11)	0 (15)	0 (13)	32 (19)	-	-	-
Macintyre											
December	78	22	-	-	-	0 (12)	-	-	-	-	-
January	70	30	0 (12)	0 (16)	0(16)	0 (14)	-	-	-	-	-
February	43	57	-	-	-	0 (10)	-	-	-	-	-
Gwydir											
December	30	70	-	0 (53)	-	0 (41)	-	-	-	0 (41)	-
January	34	56	-	-	0 (34)	0 (13)	0 (57)	-	-	0 (10)	-
February	45	55	-	0 (28)	0 (68)	0 (62)	0 (14)	-	0 (28)	0 (22)	-
March	95	5	-	0 (50)	0 (24)	0 (18)	0 (23)	-	-	7 (14)	-
Lower Namoi											
November	38	62	5 (19)	-	0 (64)	0 (23)	-	-	-	-	-
December	31	69	8 (13)	0 (23)	-	0 (10)	-	-	-	-	14 (21)
January	26	74	0 (58)	0 (112)	0 (74)	1 (80)	-	-	0 (122)	0 (41)	-
February	69	31	7 (68)	0 (148)	2 (126)	0 (58)	0 (53)	31 (35)	0 (50)	0 (40)	20 (15)
March	97	3	17 (12)	1 (175)	0 (165)	1 (184)	0 (68)	49 (74)	0 (66)	-	-
Upper Namoi											
December	78	22	3 (33)	0 (80)	0 (99)	2 (162)	-	-	0 (146)	0 (39)	-
January	51	49	0 (11)	0 (43)	0 (140)	0 (32)	0 (30)	-	0 (63)	0 (30)	-
February	81	29	8 (13)	0 (36)	0 (42)	1 (104)	0 (145)	39 (62)	-	0 (16)	-
March	97	3	0 (15)	0 (36)	0 (15)	0 (75)	-	-	-	-	-

- * denotes where collections were only from maize or sorghum which only attracts *H. armigera* species
- - denotes where values are not included where the numbers of insects tested was less than 10 in that month.