Cotton

RESEARCH & DEVELOPMENT

Final Report

Project codes
Dan 118C and Dan 152C

Resistance Monitoring and Management of Bt Cotton

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Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

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

CRDC Project Number: **DAN 152C**

Annual Report: ☐ Due 30-Sep-03

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(or within 3 months of completion of project)

Project Title: Resistance monitoring and management of Bt cotton

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The points below are to be used as a guideline when completing your final report.

1. OUTLINE THE BACKGROUND TO THE PROJECT.

Key factors in managing transgenic Bt cottons for the future are to have an effective resistance management strategy. An essential component of any such strategy is to establish a resistance monitoring program. The core components of this project address resistance monitoring as well as examine the performance of transgenic cotton (two genes) in relation to resistance management.

NSW Agriculture initiated the industries Bt resistance monitoring program in 1993. Intensive monitoring of Australian field populations of Helicoverpa armigera and Helicoverpa punctigera began in 1997 following the commercial release of the Bt transgenic crop, Ingard®. This report summarises results of the Bt resistance monitoring program between 1999 and 2002 and discusses possible directions for future research. In conducting the resistance monitoring program eggs are collected from all cotton growing districts throughout the season.

It is known that expression of the Bt protein, that produces the toxin Cry1Ac, declines throughout the crop cycle. The highest levels of protein expression occur during the early squaring (pre-flowering) period of growth. It is expected that this period also corresponds to maximum selection pressure against the toxin Cry1Ac. Although the decline in the Bt expression during the last half of the crop cycle reduces field efficacy against *Helicoverpa spp* selection for resistance is still expected to occur, thus Cry1Ac is under constant selection pressure.

Developing future resistance management strategies

To develop an effective resistance management strategy it is important to understand the mechanisms of resistance as well as the possible behaviour changes that may occur within a resistant population as compared to a susceptible one. If the industry is to continue to use Dipel® and other foliar Bts, then the question of cross resistance between Bt proteins also needs to be evaluated.

To undertake this type of research it is important to develop colonies resistant to both Bt proteins used in transgenic cotton (i.e. Cry 1Ac and Cry 2Ab) and foliar Bts (Dipel®). To establish resistant colonies, surviving larvae have been reared in the laboratory. A colony with low to moderate resistance to MVP® (Cry 1 Ac), and lower order cross-resistance to fully expressing Bt transgenic cotton plants has been established. Further selection of the strain with MVP® and Ingard® plants should result in fully resistant colonies..

Two gene interaction

Two-gene (i.e. Cry 1Ac and Cry 2Ab) varieties are likely to be available commercially in the very near future . Two gene Bt cotton will still rely on the expression Cry 1Ac that forms the basis of Ingard cotton. In the last three seasons, it is clear that the expression of the Bt protein (Cry 1Ac), is not consistent throughout the season (Fit, 1998). In the development of a suitable resistance management

strategy it will be important to know the efficacy of different proteins at different crop growth stages and the impact of each or combined efficacy of both on *Helicoverpa spp.*

The commercially available Envirologix kit has been recognized as a tool to assess the level of expression of Cry 1Ac and Cry 2A (conclusions from project DAN 123C). Research conducted by the USDA proving that the Kit is user-friendly and providing acceptable results for field crop monitoring and management purpose. To assess the interaction between levels of Bt toxins and their efficacy, the level of expression of each toxin was monitored in the cotton plant at different periods of the season.

Knowledge of the interaction between Bt proteins will also assist in determining the potential risk of resistance the industry faces in the use of conventional foliar Bt's for "topping up" transgenic cotton plants.

2. LIST THE PROJECT OBJECTIVES AND THE EXTENT TO WHICH THESE HAVE BEEN ACHIEVED.

The project Dan 152C is a continuation of Dan 118C and as such for this report the aims have been combined into the following areas;

A. Bt resistance monitoring program

1 To continue the monitoring of resistance to conventional Bt (specifically Cry 1 Ac in MPV® and CryiAc and Cry2 genes in Dipel®) in field populations of both *Helicoverpa armigera* and *Helicoverpa punctigera* from across the Australian cotton belt .
2 Develop a number of Bt resistant *Helicoverpa armigera* colonies with specific resistance to MPV and Dipel.
3 Assess the field performance of different Bt cottons

These objectives have been achieved with regard to the continuing efforts in collecting field populations, screening for resistance, establishing resistant colonies and studies of resistant frequency in subsequent generations of resistant strains.

B. Developing future resistance management strategies

4 Study on resistance development and mechanisms of resistance
5 Support the development of future resistance management strategies for Bt cottons.

Cage experiments were conducted to study the feeding and foraging behaviour of *H.armigera* on conventional and Ingard® cotton. Extensive presentations and discussions occurred with the TIMS committee in the development of future resistance management strategies for Bt cotton.

3. HOW HAS YOUR RESEARCH ADDRESSED THE CORPORATIONS THREE OUTPUTS: SUSTAINABILITY, PROFITABILITY AND INTERNATIONAL COMPETITIVENESS, AND/OR PEOPLE AND COMMUNITY?

Developing an effective resistance management strategy for transgenic cotton is vital for the future use of this technology. The outcomes from this project will be able to contribute to Outputs 1, specifically *Development of sustainable Integrated Pest Management Systems.*

4. DETAIL THE METHODOLOGY AND JUSTIFY THE METHODOLOGY USED.

1. Monitoring of field resistance to Bt proteins:

Three Bt products are used for screening field populations of Helicoverpa spp, namely MVP®, Dipel® and Xentari®.

Throughout each cotton season eggs of *Helicoverpa spp* are collected from cotton and other host crops across all the major cotton growing regions. To conduct the mortality assays the eggs are first transferred onto artificial diet, hatched and reared to early third instar larvae when they are visually sorted according to species. Then larvae are transferred onto "testing diet" made up from the same artificial diet mixed with the discriminating dose of the respective Bt products. The discriminating doses applied for MVP®, Dipel® and Xentari® were 3 ul/ml, 2mg/ml and 2mg/ml of diet, respectively (Table 1). Larval mortality was assessed after seven days for Dipel® and Xentari® and ten days for MVP®.

The early third instar larvae were used for screening because of the following reasons:

(1) Only at this stage , the speciation between *H. armigera* and *H. punctigera* is possible with greater certainty as compared to second instar , it is impossible to differentiate the two species at first stage (neonate).

(2) Due to the larger size of the larvae, insect handling is easier and mortality due to mishandling is much reduced as compared to younger larval stages .

During the monitoring program an increased level a *H.armigera* survival to MVP® was detected. These resulted in some controversy regarding the use of third instar mortality bioassays for resistance monitoring. No such concerns were raised until increased survival was detected. In an attempt to address these concerns several studies were conducted using a range different assay techniques. Results from these studies are provided in a report written in February 2002 for the CRDC commissioned review on resistance monitoring and management.

Results from this assessment indicated that there was no improvement in consistency between monitoring techniques, with the exception of a technique that involved assessing development of larvae from eggs to 3rd instar larvae. Using this technique in a monitoring program was not seen as practical or cost effective.

2. Develop a number of Bt resistant colonies.

H. armigera survivors of discriminating dose of MVP® (3ul/ml diet) were retained and bred to form F1 and F2 resistant strain (called Silver F1 and Silver F2). These colonies where bred over 6 generations, and reselected against MVP® during the F1, F2 (1.5ul/ml MVP®), and F5 (1.0ul/ml MVP®) generations. After this selection process the colony remained heterogenous for resistance and further reselection needed to occur before it could be used as a fully resistant test colony.

Attempts where made to develop a resistant colony for Dipel however due to the lack of surviving individuals from the discrimating dose this was not possible.

3. Field performance / Plant bioassays and Helicoverpa survival:

Performance of Bt cottons

Six cotton varieties including four one–gene, one two–genes stacked and one conventional variety were planted in field replicated trial in Oakville, Narrabri in collaboration with Delta Pine International to be used for assessing the performance of different Bt cotton. Four replicates were arranged in a randomised complete block design.

Newly opened terminal leaves were collected weekly, ten leaves per replicate. From each leaf, a leaf sample was collected and weighed. The concentration of Bt's protein(s) was expressed in ppm based on fresh weight. Each sample was analysed twice and the mean was used for statistical analysis. (Analysis was carried out at Grain Technology Service, Narrabri).

Through collaboration with Dr Neil Forrester, a field strain of *H. armigera* was collected from Southern Queensland during the early part of the cotton season. The objective of using a field susceptible strain instead of laboratory susceptible strain can not be over emphasised, as the result of the bioassay will reflect the realistic situation of crop- pest interaction under field conditions.

A bioassay using the Falcon petridish with one small leaf and one neonate per dish was carried out. One day old neonates were used and placed onto the leaf using the wet hair- brush. The Falcon petridish were self- locked and air tight to prevent drying of the leaf. Ten petridishes were used for each variety and each replicate. Efficacy was recorded as mortality at five days after introduction of the neonate onto the leaf.

Whole Plant Bioassay:

To satisfy the query that resistant colonies developed from screening on Bt formulation (ie MVP®) might not survive on transgenic cotton . An experiment was conducted from August to October , 2001 on whole plants.

Larvae from resistant, Silver (F4) and KO strains were placed on caged plants under greenhouse condition at ACRI . Two neonates were introduced into each caged plant at 50 days after sowing (squaring stage). Larval and pupal survivorship was monitored.

4. Study on resistance development and mechanisms of resistance:

To study resistance to Cry 1Ac, the resistant strains Silver F1 and Silver F2, a Cry 1Ac susceptible strain (KO) obtained from CSIRO (Canberra) and a field strain from Emerald, Queensland were bioassayed with MVP® and full dose/ mortality curves were obtained. Data was analysed by probit analysis (Probit 5).

To further study the mechanisms for Bt resistance, preliminary Envirologix test analysis of survivors and dead larvae from MVP screening revealed that dead larval contained higher levels of Cry 1Ac toxin than surviving larvae. To investigate possible reasons for this, collaborative research was initiated with Dr Robin Gunning (NSW Agriculture, Tamworth). The objective of this was to investigate the effects of esterases on Cry 1Ac. Susceptible and Silver F2 *H. armigera* homogenates were incubated with concentrations of purified Cry 1Ac. Total esterase activity was detected using 1–naphthyl acetate as a substrate. Incubates were also run on polyacrylamide gels and stained for esterase activity. Further details are provided within reports for project Dan 161C.

Study into genetics of resistance:

To assess the genetic diversity of the resistant colony, Seventy pairs from the Silver F1 strain were bred and their offspring were used for testing on transgenic cotton leaves in Falcon® petridish. Forty neonates of each Silver F2 isoline were used for the test. One day old-fed neonate was placed in air–tight Falcon® petridish with newly opened transgenic cotton leaf. Insect mortality was assessed at 5 days after .

5. Development of resistant management strategies

All resistant monitoring and other research results where presented on regular intervals to the TIMS committee and technical sub committee for Ingard®. Upon detecting an increase in survival of *H. armigera* to MVP® a technical discussion groups was established to review the findings.

5. DETAIL RESULTS INCLUDING THE STATISTICAL ANALYSIS OF RESULTS.

1. Monitoring of field resistance to Bt proteins:

The percentage survival of *Helicoverpa armigera* and *H. punctigera* against the discriminating dose for each product from 1996/97 to 2001/02 seasons is presented in Table 2.

Monitoring for Dipel® resistance has been conducted since 1996 and there has been no change in susceptibility of field populations to this product. A similar result was obtained for Xentari®. Therefore there is no indication of the development of resistance to these Bt products. It should be noted that Dipel® and Xentari® contain a number of Bt toxins (Table 1) and resistance to these products would only occur if a mechanism developed that conferred cross resistance between Bt toxins.

As it was vital that field collected samples be prioritised for testing against the toxin Cry 1Ac, the TIMS sub-committee recommended that the level of testing of *H. armigera* against Dipel and Xentari be significantly reduced.

The level of survival of both species to MVP®, which contains the single Bt toxin (Cry 1Ac), increased significantly over the three years from 1999/2000. Average survival of *H. armigera* against MVP® for the crop season 1999/2000, 2000/01 and 2001/02 was 2.6%, 7.1% and 10.9% respectively. These results indicate that there has been a change in the susceptibility *H. armigera* to MVP (Cry 1Ac).

A closer examination of the increase in survival of *H. armigera* during 2000/01 indicates a significant increase in *H. armigera* survival in February and March. Eggs collected in February and March were generated from larvae surviving in crops during December and early January. This poses the question about the ability of refuge crops to dilute potential resistant moths emerging from transgenic crops during the first half of the crop cycle when expression of Cry 1Ac is at its highest level.

The level of survival of *H. punctigera* for the three year period was 2.3%, 3.7% and 11.8%. The small increases in survival during 1999/2000 and 2000/01 may have been expected, however the large increase in 2001/02 was not expected. If resistance in *H. punctigera* to MVP® followed a similar pattern in it's resistance to conventional chemistry it would be expected to remain low compared to *H. armigera.* One possible explanation for the increase in resistance to *H. punctigera* in 2000/01 is the lack of large infestations of the species on natural refuge, thereby allowing a local population to be exposed. Future testing in seasons where such infestations occur from western districts will determine if resistance in *H. punctigera* remains low as it has in conventional chemistry.

Appendices 1 to 4 show survival of larvae from different Australian cotton growing regions in the 2000/01 and 2001/02 cotton seasons against Dipel®, Xentari® and MVP®.

2. Develop a number of Bt resistant colonies.

In 2001, *H. armigera* survivors of the discriminating dose of MVP® (3ul/ml diet) were retained and bred to form Silver F1 and Silver F2. These Silver strains and a Cry 1Ac susceptible strain (KO) plus a field strain from Emerald, Queensland were bioassayed against MVP® and full dose/ mortality curves were obtained and analysed by probit analysis (Probit 5).

The results of the bioassays and probit analysis of these strains are shown in Table 3. The resistance factor (RF) based on LC99, of Silver F1 and Silver F2 was 118 and 187 fold respectively as compared to Emerald F4 strain, which was not selected for resistance having a RF of 77 fold. The RF based on LC50 was 37,48 and 15 for the Silver F1 and F2 and Emerald strains. There is not significant difference between the Silver F1 and F2 generations.

Bulks mating of the Silver F2 strain were also bioassayed with MVP® and full dose/mortality curves obtained. The results of the F2 isolines were compared with bulk–mating strains.(Table 4). One isoline, number 264, recorded a resistance factor (RF), 5836 fold. The resistant (Silver F4) and susceptible (KO) strains were crossed in September 2001. The probit analysis on the hybrid (Silko F1) developed from this cross had a resistance factor of 43, compared to 503 for Silver F4 and 415 for Silver F5 (Table 5).

Based on these results the inheritance of the resistant trait may be semi-dominant. However, it is obvious that the Silver strain is not homozygous for resistance. A homozygous strain needs to be developed before any firm conclusions are drawn.

In collaboration with CSIRO's Dr Ray Akhurst and Dr Rod Mahon the Silver F5 was crossed with a susceptible (GR) strain. These results indicated no significant difference between the Silver cross and the susceptible strain. This was repeated with a non-selected Silver F8 strain with similar conclusions in 2002. The difference between the results at ACRI and Canberra may be due to the loss to resistance within the colony due to non selection against MVP® in its development to F8.

3. Field performance / Plant bioassays and Helicoverpa survival:

Expression of Bt proteins in Ingard® Crops:

Table 6 shows the contents of Bt toxins in one-gene Ingard® varieties and two-gene varieties during the cotton growing season. There was consistent difference between the concentrations of Cry1Ac from different varieties.

Expression of the second gene (Cry2A) was much higher than that of the first gene (Cry1Ac), the ratio of the Cry2A/Cry1Ac concentration ranges from 12.5 to 46 fold. The concentration of Cry2A did not decline with crop age to the same extent as that of Cry1Ac.

The results of bioassays are presented in table 7 and figure 1. The percentage of larval mortality on two-gene variety (DP50BX) was much higher than on the single-gene varieties. On single-gene varieties , the insect mortality significantly reduced as the crop ages .

Leaf bioassays of resistant strain:

The result of leaf bioassays on Silver F2 isolines is shown in Figure 3. Among the fourteen isolines (those that produced viable eggs among the seventy pairs at the

start), there was five isolines with showed 100% survival, seven isolines with survivorship ranging from 92.1 to 97.5%, one with 80% and one with 20% survival. The bulk-mating SF2 strain, field strain and susceptible strain showed 97.5%, 80% and 18.1% survival respectively. Thus, based on these results the inheritance of the resistant trait might appear to be dominant. However, due to high level of heterozygosity in the populations, these conclusion need to be taken with caution until a homogeneous strain is developed.

Whole plant Bioassays

Larvae from resistant Silver (F4) and KO strains were placed on caged plants under greenhouse condition at ACRI (August–October 2001). Two neonates were introduced into each caged plant at 50 days after seeding (squaring stage). Larval and pupal survival was monitored. The result is shown in table 8. Of the larvae exposed to transgenic cotton, 10.75% were able to survive through to pupation. Only 1.25% of the susceptible (KO) strain survived passed 7 days with non-surviving through to pupation. No significant differences were found in the pupal weights of larvae exposed to transgenic and conventional cotton. These results support that the Silver stain had developed resistance to Cry 1Ac that may allow some individuals to survive on plants. This work needs to continue.

4. Study on resistance development and mechanisms of resistant strain:

Susceptible and Silver F2 *H. armigera* homogenates were incubated with concentrations of purified Cry1Ac pro-toxin. Polyacrylamide gels (Figure 4), developed from this work showed that the resistant strain had greatly increased esterase activity and there were considerable differences in esterase banding patterns between the susceptible and resistant strain. Data in Figure 5 show that Cry1Ac inhibited esterase activity in the resistant strain, whereas there was no detectable binding of Cry1Ac to esterase in the susceptible strain.

Esterases are enzymes in *H. armigera* and other insect pests, which de-toxify many insecticides by hydrolysis and sequestration. Sequestration by esterases has been characterised as the primary cause of pyrethroid resistance in Australian *H. armigera.* While the mechanisms of Bt resistance in insects are not well understood, Bt toxin sequestration is recognised by research workers as a potential mechanism for Bt resistance.

Preliminary research by Dr Gunning, and Dr Graham Moores (IACR, Rothamsted, UK), has shown that activity of esterase in the gut of the Bt resistant *H.armigera* strain binds readily to the Cry1Ac pro-toxin. Given the greatly increased esterase activity in the resistant strain, it is likely that considerable amounts of Cry1Ac pro-toxin could be sequestered. The work has continued as part a the CRDC project DAN 161C.

Table 1: Products tested against *H.armigera* and *H.punctigera* in the Bt Resistance Monitoring Survey(DD=Discriminating Dose)

Dipel® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry 1Ac, Cry2A, Cry2B, spore

Xentari® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry1C, Cry1D, Cry 2B, spore

MVP/MVP2® (DD=3ul/ml)
Cry 1Ac encapsulated in dead *seudomonas flourescens* cells

Table 2: Summary of resistance monitoring survey 1996 - 2002

Bt Product	Year/1	*H. armigera*		*H. punctigera*	
		% Survival	Number Tested	% Survival	Number Tested
Dipel®	1996/97	0.3	6149	0.5	1788
	1997/98	0.7	7580	1.3	1699
	1998/99	0.6	9974	1.4	974
	1999/00	0.7	14295	0.2	1496
	2000/01	1.0	5143	0.5	1393
	2001/02	.2	2086	.4	1435
Xentari®	1996/97	0.4	4980	0.5	1155
	1997/98	0.2	3130	0.4	974
	2000/01	0.8	3698	0.6	1059
	2001/02	.5	847	.5	897
MVP®	1997/98*	0	2575	0.1	1217
	1999/00**	2.6	11275	2.3	1884
	2000/01**	7.1	11572	3.7	4385
	2001/02	10.9	2758	11.8	2250

* Data based on screening with neonates
** Data based on screening with 3rd instar larvae
1/Results of 96-99 survey from Dr N.W.Forester .

Table 3: LC99, LC50 and Resistant factor (RF) of various *H.armigera* strains tested against MVP.

Strain[1]	*Selection*	LC99(ul/ml)	LC50(ul/ml)	RF[3]	*Slope*
Silver F1	+[1]	201	3,099	118 fold	1.06
Silver F2	+[1]	321	4.033	187	1.02
Silver F3	–	324	1.399	189	.81
Silver F4	-	860	1.674	503	.71
Silko F1(SF4 X KO)[3]	-	73	0.421	43	0.86
Silver F5	+[2]	711	2.122	415	0.76
Emerald F4 (Field strain)	–	132	1.303	77	.96
KO	–	1.71	.084	1	1.48

1/ Selected at 1.5 ul/ml diet.
2/ Selected at 1.0 ul/ml diet.
3/ Resistance Factor based on LC99.

Table 4: Content of *B. thuringiensis* proteins (ppm) in first opened leaves during the 00-01 crop season.[1]

Variety	6/12	13/12	22/12	28/12	8/1	15/1	18/1	24/1	2/2	07/2	12/2	19/2	26/2	21/03
289I (CryIA)	2.22c	2.19c	2.82c	2.51c	3.25c	2.43d	2.45cd	2.62b	2.96c	3.25c	2.23c	2.10c	1.99b	1.63b
50B (CryIA)	1.21a	0.90a	2.24a	1.71a	2.75a	1.26a	1.87a	1.54a	2.50a	2.68a	1.91a	1.29a	1.60a	1.23a
50BX(CryIA)	1.06a	0.91a	2.15a	2.41c	2.78a	1.71b	2.05b	1.52a	2.53a	2.63a	1.81a	1.29a	1.55a	1.31a
NuCot37(CryIA)	3.42d	2.97d	3.28d	3.41d	4.07d	2.41d	3.56d	2.86c	2.94c	3.64d	2.98d	2.23d	2.49c	2.06c
V16I(CryIA)	1.99b	1.41b	2.31b	2.16b	3.09b	2.06c	2.33c	1.62a	2.76b	2.78b	2.11b	1.63b	1.60a	1.24a
Mean(CryIA)	**1.98**	**1.676**	**2.56**	**2.44**	**2.988**	**1.974**	**2.452**	**2.032**	**2.738**	**2.996**	**2.208**	**1.708**	**1.846**	**1.494**
50BX(CryIIA)	**17.33**	**19.4**	**50.78**	**30.02**	**50.26**	**59.24**	**69.28**	**47.42**	**50.9**	**68.27**	**63.77**	**51.25**	**47.35**	**59.93**
DAS[2]	32	39	48	54	60	67	75	81	90	95	100	107	114	137

1. Means in a column followed by common letter are not significant difference at 5% level (Genstat 4.2)
2. DAS = Day after seeding.

Figure 1 : Mortality of *H. armigera* larvae tested on transgenic cotton leaves at 5 day after introduction .

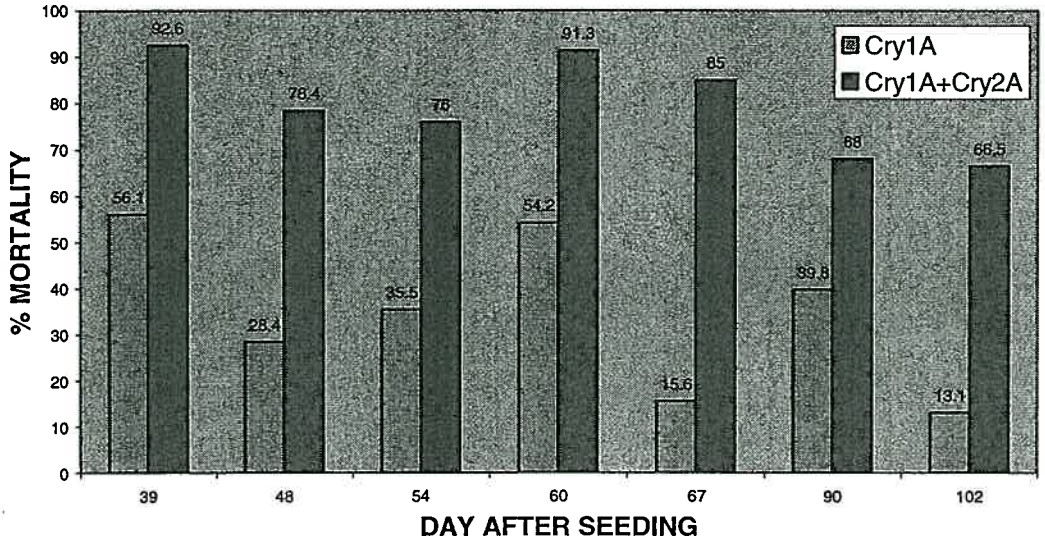

Table 5: Percent mortality of first instar larvae of *H. armigera* at 5 days after feeding on leaves of transgenic and conventional cotton varieties during the 00-01 crop season.[1]

Variety	6/12	13/12	22/12	28/12	8/01	15/01	2/02	12/02/	19/02	26/02	6/03	21/03
289i	25.0c	48.8a	21.4a	44.0b	64.9b	10a	51.0c	21.4b	9.5a	23.4b	2.4a	12.2a
50B	20ab	43.9a	30.6b	24.0a	62.4b	7.5a	34.0a	9.5a	11.9a	27.6b	33.2c	14a
50BX	67.5d	92.6c	78.4d	76.0c	97.3c	85.0c	68.0d	78.4d	66.5c	68.0d	49.9d	85.3
Nu37	25c	70.7c	38.0c	46.0b	62.4b	10.0a	34.0a	9.5a	11.9a	36.1c	4.8ab	21.9b
V16i	15a	60.9b	23.8a	28.0a	27.5a	35.0b	40.4b	28.5c	19.0b	14.9a	9.5b	17.1ab
Mean	30.0	63.4	38.4	43.6	61.7	29.5	45.5	29.5	23.8	19.5	19.9	30.2
DAS[2]	32	39	48	54	60	67	90	100	107	114	122	137

1. Means in a column followed by common letter are not significant difference at 5% level (Genstat 4.2)
2. DAS = Day after seeding

Figure 2: Polyacrylamide gels showing effects of Cry1Ac on esterase activity in Cry1Ac -susceptible and Cry1Ac- resistant Australian *H. armigera*

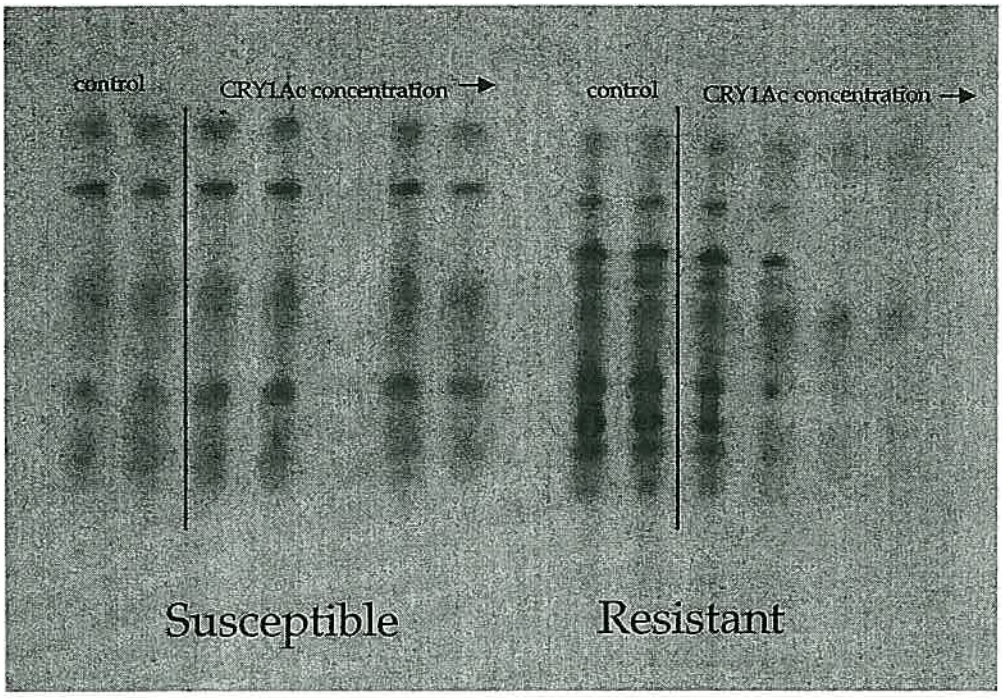

Figure 3: Results of leaf bioassay of the F2 single pair mating isolines and bulk-mating strains of *H. armigera*

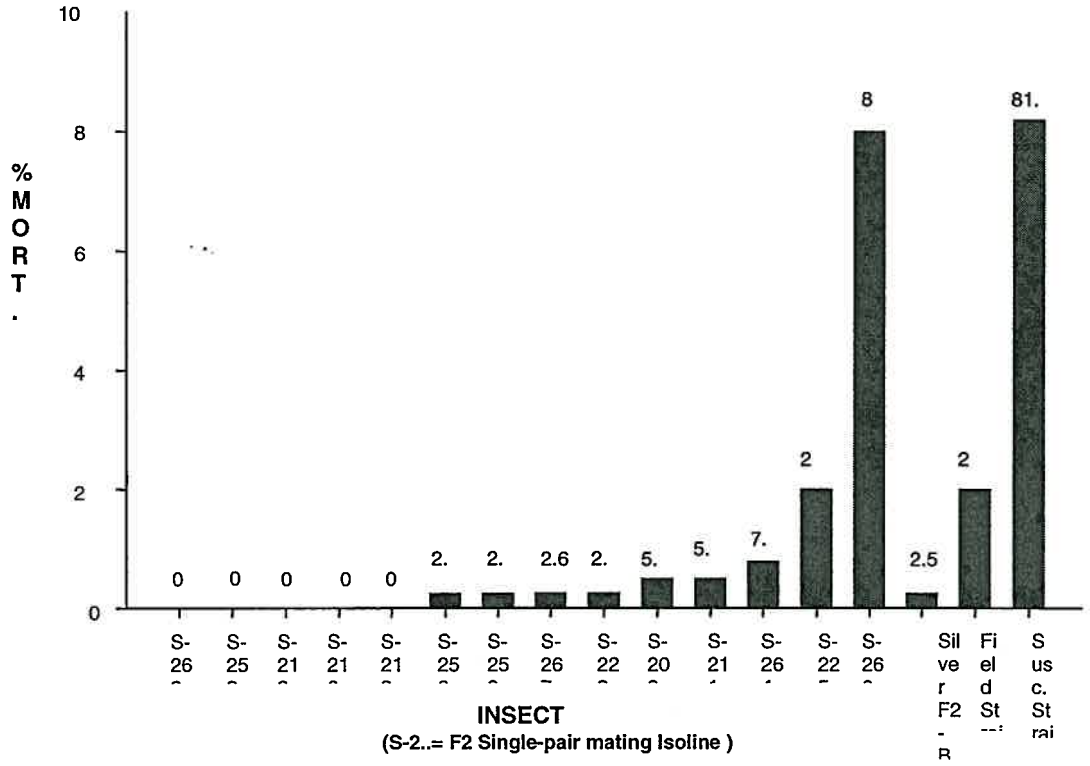

Table 6: Percentage survivorship of *H. armigera* on whole plants in greenhouse conditions[1]

Insect	Plant Type[2]	No. of Plants	Larval Survival		Pupal Survival	Pupal Weight (mg)
			7 DAI[3]	14 DAI		
Silver F4	Ingard	200	25.50	13.25	10.75	325.1
Silver F4	Convention	40	92.0	80.0	55.0	327.1
KO	Ingard	40	1.25	0	0	-
KO	Convention	40	82.0	78.0	57.0	304.5

1. Two first instar larvae were introduced to each caged plant at 50 days old.
2. Ingard: Sioct 289i, Convention: Sicot 189
3.DAI=Day after introduction .

6. Discuss the results, and include an analysis of research outcomes compared with objectives. What are the "take home messages"?

Ingard® cotton provides a valuable tool for the management of *Helicoverpa spp* in cotton. This technology has become the backbone for IPM strategies being adopted by the industry. Maintaining susceptibility of insect populations to Bt toxins as well as to new chemistries is essential for the industry's sustainability.

Over the past two cotton seasons, the NSW Agriculture Bt resistance monitoring program has shown that field *H. armigera* have become less susceptible to the Bt endo-toxin Cry1Ac and this indicates resistance to this toxin may have developed. Further selection for a more virulent strain of *H. armigera* is required to validate the significance of these findings.

The variation in expression of Bt toxin in crops during the crop cycle may have contributed significantly to the change in *H. armigera* susceptibility to Cry1Ac. The use of refuges for production of susceptible field population may have had a significant effect on dilution of the resistance frequency during the last half of the growing season. However peaks of *H. armigera* survival in February tend to indicate that the current refuge strategy has little effect on resistant populations that may develop early in the season.

The actual "erosion" of efficacy of transgenic crops due to development of resistance is difficult to measure. The current change in *H. armigera* susceptibility and subsequent survival on transgenic crops would not be detected by commercial field checks for helicoverpa infestations. Also, any loss of efficacy in transgenic crops would be difficult to establish as the majority of transgenic cotton crops are regularly sprayed with larvicides after flowering. The use of non–Bt conventional insecticides in transgenic crops would mask the reduced efficacy.

While the mechanisms of Bt resistance in insects are not well understood, enzymatic sequestration of toxin is recognised by Bt research workers overseas as a potential resistance mechanism. In the case of Bt resistant *H. armigera*, it is likely that considerable amounts of Cry1Ac pro-toxin are being sequestered. Esterase sequestration however might be only one of a number of mechanisms involved in Bt resistance. Improved information on mechanisms and the mode of inheritance is required urgently.

A number of the assumptions that were made in the development of the resistance management strategy for Bt cotton have proved to be incorrect (Daly and Olsen, 2000; Tabashnik et al. 2000). Improving our understanding of the mechanisms and genetics of resistance to Cry1Ac and new Bt toxins is seen as a priority to in the development of future resistance management strategies. The impact of resistance to Cry1Ac on the future use of two gene cotton containing both Cry1Ac and Cry2Ab, also needs to be determined.

After the introduction of Bollgard II, monitoring for resistance to Cry1Ac needs to continue as well as that for Cry2Ab.

Caution is therefore required in *Helocoverpa spps* management to avoid further loss of efficacy of Bt protein Cry1Ac which can cause significant disadvantage when the two-gene transgenic varieties are deployed in the future.

7. **Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry and future research needs.**

A number of the assumptions that were made in the development of the resistance management strategy for Bt cotton have proved to be incorrect (Daly and Olsen, 2000; Tabashnik et al. 2000). With the detection of increased survival of *H. armigera* to Cry 1Ac it is vital to improve our understanding of the mechanisms and genetics of resistance to Cry1Ac and new Bt toxins. Without this work the development of effective future resistance management strategies for the future will be jeopardised. The impact of resistance to Cry1Ac on the future use of two gene cotton containing both Cry1Ac and Cry2Ab, also needs to be determined. After the introduction of Bollgard II, monitoring for resistance to Cry1Ac needs to continue as well as that for Cry2Ab.

8. **Detail how your research has addressed the Corporations three Outputs: Sustainability, and/or Profitability & International Competitiveness, and/or People & Communities?**

Developing an effective resistance management strategy for transgenic cotton is vital for the future use of this technology. The outcomes from this project will be able to contribute to Outputs 1, specifically *Development of sustainable Integrated Pest Management Systems.*

9. **Describe the project technology (eg. commercially significant developments, patents applied for or granted licenses etc).**

NA

10. **Provide a technical summary of any other information developed as part of the research project. Include discoveries in methodology, equipment design, etc.**

NA

11. **Detail a plan for the activities or other steps that may be taken;**

The CRDC commissioned review of resistance research and management recommended a number of changes to established in the future. It is vital that collaborative programs be maintained in addressing resistance management to Bt cottons.

12. List the publications arising from the research project.

-Ho T. Dang ; JW Holloway and N. Forrester.2000. Monitoring Susceptibility to Bt Toxins in Australian Helicoverpa species .Proceeding of the annual meeting of the Australian Cotton Consultant Association . May 16 &17, 2000. Narrabri .

-Ho T. Dang ; J.W. Holloway, A. Shlack and L. Wilson. 2000. Comparison of Insecticides efficacy against Helicoverpaspecies in conventional and Ingard Cotton .Proceeding of the annual meeting of the Australian Cotton Consultant Association . May 16 &17, 2000. Narrabri ..

-Ho T. Dang and R. Gunning . 2001. Resistance to *Bacillus thuringiensis* (Bt) delta-endo toxin Cry1Ac in Australian *Helicoverpa armigera*(Lepidoptera:Noctuidae) .In:Proceeding Resistance 2001: The challenge. September 21-24 ,2001,IACR,Rodhemsted,UK.

-Ho T. Dang and R. Gunning .2001. Evidences of Resistance *Bacillus thuringiensis* (Bt) toxin Cry1Ac in Australian *Helicoverpa armigera* (Lepidoptera:Noctuidae) .In:Proceeding of the 4th Pacific Rim conference on the biotechnology *Bacillus thuringiensis* (Bt) and environmental impact .Australian National University , November 11-16 ,2001, Canberra .

-Ho T. Dang , R. Gunning and I. Christian.2001. Resistance monitoring , doing our part. Australian Cotton Grower.Novemver-December ,2001 . PP 18-20 .

13. Are changes to the Intellectual Property register required?

NA

Over the past two cotton seasons, the NSW Agriculture Bt resistance monitoring program has shown that field *H. armigera* have become less susceptible to the Bt endo-toxin Cry1Ac and this indicates resistance to this toxin.

Average survival of *H. armigera* against MVP® for the crop season 1999/2000, 2000/01 and 2001/02 was 2.6%, 7.1% and 10.9% respectively. The level of survival of *H. punctigera* for the three year period was 2.3%, 3.7% and 11.8%. The variation in expression of Bt toxin in crops throughout the crop cycle may have contributed significantly to the change in *H. armigera* susceptibility to Cry1Ac.

Preliminary research by Dr Gunning, and Dr Graham Moores (IACR, Rothamsted, UK), into the mechanisms of resistance in surviving insects has shown that activity of esterase in the gut of the Bt resistant *H.armigera* strain binds readily to the Cry1Ac pro-toxin. Given the greatly increased esterase activity in the resistant strain, it is likely that considerable amounts of Cry1Ac pro-toxin could be sequestered and this may be the primary cause of resistance.

The actual impact on efficacy of transgenic crops due to development of resistance is difficult to measure. The current change in *H. armigera* susceptibility and subsequent survival on transgenic crops would not be detected by commercial field checks for *Helicoverpa* infestations. Also any reduction of efficacy in transgenic crops would be difficult to establish due to the fact that the majority of transgenic cotton crops are regularly sprayed with larvicides after flowering. The use of non–Bt conventional insecticides in transgenic crop would mask the reduced efficacy

One gene, Ingard® cotton has been a valuable part of the cotton IPM program for several years and a two gene (Bollgard II), cotton will shortly be registered. Maintaining susceptibility of insect populations to Bt proteins as well as to new chemistries is essential for the industry's sustainability. Transgenic cotton has become the backbone for IPM strategies being adopted by the industry.

Improving our understanding of the mechanisms and genetics of resistance to Cry1Ac and new Bt toxins is seen as a priority for future research. The impact of resistance to Cry1Ac on the future use of two gene cotton (with Cry1Ac and Cry2Ab) needs to be determined.

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Bruce E. Tabashnik, Amanda L.Patin,Timothy J.Dennehy ,Yong-Biao Liu,Maria A. Sims,and Larry Antilla.2000. Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. Proceedings of the National Academy of Sciences of the US of America . PP 12980-12984.

Daly Joanne and Karen Olsen .2000. Genetics of bt Resistance . In: proceedings of the Australian Cotton Conference ,Brisbane ,August,2000,PP 4.