Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2008

If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number: **CSE112**

Project Title: Monitoring for resistance to transgenic cotton

Project Commencement Date: 01/07/05 **Project Completion Date:** 30/06/08

CRDC Program: 3 Crop Protection

Part 2 – Contact Details

Administrator: Lori Cook
Organisation: CSIRO Plant Industry
Postal Address: Underwood Avenue, Floreat Park, WA 6014
Ph: 08-9387 8991 **Fax:** 08-9333 6605 **E-mail:** Lori.Cook@csiro.au

Principal Researcher: Dr Sharon Downes, Research Scientist
Organisation: CSIRO Entomology
Postal Address: Locked Bag 59, Narrabri 2390
Ph: 02-6799 1576 **Fax:** 02-6793 1186 **E-mail:** Sharon.Downes@csiro.au

Supervisor: Dr Rod Mahon, Principal Research Scientist
Organisation: CSIRO Entomology
Postal Address: GPO Box 1700, Canberra, ACT
Ph: 02-6246 4082 **Fax:** 02-6246 4082 **E-mail:** Rod.Mahon@csiro.au

Signature of Research Provider Representative: _____

Background

In the 1996/97 season the Australian industry adopted an insect-resistant variety of cotton (Ingard®) that is specific to the group of insects including the target *Helicoverpa* spp. but excluding natural predators and parasitoids. To prolong the efficacy of transgenic cotton against *H. armigera,* a resistance management plan (RMP) was implemented. This strategy was largely based on information from studies of the ecology and population genetics of *H. armigera*, and the outputs of computer simulation models that used biological information to predict the likelihood of resistance under different scenarios.

The industry adopted a necessarily conservative RMP for Ingard® due to the critical importance of preserving the Cry1Ac gene present in this variety until more robust two-gene transgenic cotton was available. A key component was to limit planting Ingard® to a maximum of 30% of the total area, thereby restricting the opportunity for moths to adapt to the toxin. Growers were also required to plant conventional crops alongside transgenic fields—these "refuges" harbor susceptible moths that should mate with potentially resistant individuals from the Ingard®, thereby diluting resistance in the population.

In the 2004/05 season Bollgard II® replaced Ingard® as the transgenic variety of cotton available to Australian growers. It improves on Ingard® by incorporating an additional insecticide protein (Cry2Ab) to combat *H. armigera.* Sequence information indicates that these genes are distantly related and the toxins they encode do not share a common binding site. Consequently it is thought unlikely that a single mechanism could confer resistance to both toxins. Due to the perceived difficulty for *H. armigera* to evolve resistance to both proteins within Bollgard II®, the RMP for transgenic cotton was relaxed to allow growers to plant up to around 95% of the total area to this product. Bollgard II® was well adopted, with an average of 70% planted area throughout the industry. Given the increased opportunity for moths to adapt to transgenic cotton, the enforced RMP will be rigorously tested immediately for the first time.

The cotton industry has sought to acquire early warnings of changes in sensitivity of insect populations to toxins that may signal the presence of resistance to transgenic varieties of cotton. The sensitivity of field-collected populations of *H. armigera* and *H. punctigera* to Bt products was assayed before and subsequent to the widespread deployment of Ingard® cotton expressing Cry1Ac in the mid-1990's. During CSE102C, baseline levels of susceptibility to Cry2Ab were established in preparation for replacement in the 2004/05 season of Ingard® with Bollgard II®. Preserving the efficacy of Cry1Ac and Cry2Ab is critical for the future of the industry, not only for the efficacy of the Bollgard II® varieties of cotton, but also for the long-term future of cotton varieties expressing Cry1Ac or Cry2Ab in combination with other effective toxins.

CSIRO Entomology has worked on resistance by *H. armigera* to transgenic cotton for many years, and presently maintains strains that are resistant to Cry1Ac or Cry2Ab. In both cases the forms of genes (alleles) that confer resistance have been isolated from field populations (see reports for CSE102C and CSE104C). Our work shows that resistant alleles in the field are rare for Cry1Ac but surprisingly common for Cry2Ab. *H. armigera* has an impressive track-record at evolving resistance to conventional insecticides. Therefore, while the combination of two genes in Bollgard II® should prevent the evolution of resistance by a target pest, it is not an impossible task for *H. armigera* to adapt to this technology.

Objectives

All of the objectives outlined in the original proposal were achieved. However, we note that no collections contained silverleaf whitefly and thus none were redirected to QDPI[1].

In response to concern from industry we included an additional objective[2] that involved assessing the resistance status of live larvae found in Bollgard II® crops that were collected to meet the objectives of CRC Project 1. 01.03.

In 2007/08 the Australian Cotton Growers Research Association granted permission to house Cry2Ab resistant Helicoverpa colonies at the Australian Cotton Research Institute. Subsequently F_1 tests were incorporated into the resistance monitoring program[3] (see "page 10" for a justification of this inclusion).

TABLE 1: The objectives, milestones, and performance indicators of CRDC Project CSE112. The text highlighted in bold font is additional to the original objectives.

No.	Objective	Milestone	Performance Indicator
1	Provide early warning of advent of Bt resistance	Screened eggs using the F_0 and F_2 methods **+ F_1 tests**[3]	Sensitivity of field populations to Bt rigorously assessed
2	Capture any genes conferring resistance to Bt	Reared subset from suspected resistant families	All suspected resistant strains reared to F_3 for further testing
3	Improve industry understanding of the program	Regular short articles in relevant publications	Improved interest from stakeholders in the program
4	Improve industry confidence in the program	Effectively and promptly communicated results	Excellent feedback from stakeholders
5	Streamline collection of material for several programs	Redirect leaves with silverleaf whitefly to QDPI[1]	Feedback from QDPI on receipt of material
6	**Test live larvae from Bollgard II plants for Bt resistance[2]**	**Screened collections of live larvae from Bollgard II using F_2 methods**	**Rigorous assessment of sensitivity of live larvae from Bollgard II plants to Bt**

Methods and Results

(i) Egg collections and allocation to screens

Our team at ACRI collected eggs in the Namoi and Gwydir Valleys, and we received collections from collaborators sampling Emerald, Darling Downs, Macquarie, Macintyre, Lachlan, and St George valleys. In 2005/06 we made one trip each to Bourke and Macquarie to sample eggs.

Eggs were collected from cotton plus all other crops present in a region that are hosts to Helicoverpa. Most collections were located after alerts from growers or consultants of the presence of high egg pressure, rather than by random sampling throughout a region. Variation in abundances of *H. armigera* and *H. punctigera* dictated the relative proportions and numbers of each species sampled at any point in time throughout the season.

The number of eggs submitted to the Bt resistance monitoring program from each valley is summarised for each season in Table 2. Among-year variation in the total number of eggs allocated to the program largely reflects differences in the "pressure" of Helicoverpa and hence opportunities for collection. Among-year variation in the total numbers of eggs received by non-ACRI collectors largely reflects the availability of paid dedicated collectors.

TABLE 2: The number of eggs allocated to the Bt resistance monitoring program during each year of the project. ACRI = team at the Australian Cotton Research Institute, DC = paid dedicated collector, VC = unpaid collector(s) (mainly consultants), RCEO = Regional Cotton Extension Officer

Valley	2005/06		2006/07		2007/08	
	No. eggs	Source	No. eggs	Source	No. eggs	Source
Lower Namoi	12267	ACRI	16716	ACRI	6697	ACRI
Upper Namoi	8718	ACRI	8650	ACRI	11175	ACRI
Gwydir	8194	ACRI	4851	ACRI	5869	ACRI
Bourke	1020	ACRI	0	-	0	-
Macquarie	2012	ACRI	0	-	746	RCEO
Emerald	934	DC	0	-	1593	DC
Darling Downs	4666	DC	395	VC	2007	DC
Macintyre	13166	DC	3878	DC	810	DC
Lachlan	114	VC	0	-	637	DC
St George	3827	VC	361	VC	7036	DC
TOTAL	**54918**		**34851**		**36485**	

In 2005/06 and 2006/07 we conducted F_0 screens against Cry1Ac and Cry2Ab and F_2 screens against Cry1Ac and Cry2Ab. We allocated around half of the larvae sampled per collection from each valley to the F_2 screens. The upper limit to the number of F_2 screens performed was determined by the availability of technical support relative to the workload necessary to conduct these tests, as well as the space available in our rearing room. Approximately 50% of allocated eggs successfully completed the F_2 testing regime. Our aim was to sample around 150 single mated pairs of moths (600 alleles) of each species against both Cry1Ac and Cry2Ab. The eggs that were not allocated to the F_2 screening program were assigned to F_0 screens.

In 2007/08 we conducted F_0 screens against Cry1Ac and Cry2Ab, F_2 screens against Cry1Ac and Cry2Ab, and F_1 screens against Cry2Ab. Until January we allocated material between the F_0 and F_2 screens as for the previous years. Afterwards, when an extension to our moth room was completed, we allocated 60 larvae (30 pairs)/species/week to the F_1 test. The eggs that were not allocated to the F_2 or F_1 screening program were assigned to F_0 screens.

(ii) Toxins used in our screens

The Cry1Ac toxin used in this work was produced from *Bacillus thuringiensis* strain HD73, provided by D. Pinnock (University of Adelaide, South Australia). The HD73 strain was grown on agar for 3-4 d at 28ºC, by which time sporulation and crystal formation was completed. The spores and crystals were scraped from the surface of the agar plate and suspended in distilled water. The suspension was centrifuged at 4ºC at 8000 g for 10 min. The supernatant was decanted and the pellet resuspended in distilled water. The suspension was sonicated to completely disperse the crystals and the concentration of Cry1Ac in the spore/crystal mixture was estimated using a standard procedure. Aliquots of the spore/crystal suspension were stored at -20ºC. This pure toxin was designated HD73. With the greater demands associated with the monitoring program, toxin production was outsourced to a large fermentation facility that produced a culture designated GHD73. The product was similar to the parent culture in its toxicity to *H. armigera*.

In all assays against Cry2Ab, dried and ground corn leaf material was used as the source of toxin. This corn powder was provided by Monsanto Company (St Louis, MO, USA) as a lyophilized *Zea mays* powder containing transgenically expressed *Bacillus thuringiensis* crystal protein, Cry2Ab2 at a concentration of 6 mg/g of powder.

(iii) F_0 screens against Cry1Ac and Cry2Ab

F_0 screens are likely to pick up only individuals that are homozygous resistant (RR) to Bt.

Using early 3rd instars in the F_0 screening program is favoured because it is difficult to identify (non-destructively) larval Helicoverpa spp. before the late 2nd instar. However, it is not logistically possible to adopt this method when the toxin is difficult to produce and/or a relatively high dose of the toxin is required to kill 3rd instars. This was the case with the corn powder used for the Cry2Ab screens. Thus, we developed a bioassay for neonates, and estimated the relative proportions of each species used in these assays based on the relative proportions of each species reared for the F_2 screens in a sub-sample of the same collection.

The eggs on collected leaves were transferred to artificial diet if they were destined for F_0 screens against Cry1Ac which used 3rd instars, or to empty 45 well trays if they were destined for F_0 screens against Cry2Ab which used unfed neonates. The eggs were placed at standard larval rearing conditions until they reached the required stage for testing when they were transferred to 24 well trays that contained contaminated diet. To maximize the numbers of insects available for testing during normal working hours we checked material twice daily during the week and daily on weekends and cooled moulting 2nd instars and black eggs overnight to slow development.

For both types of screen, larvae at the appropriate stage of development were placed into 24-well trays containing Bt treated diet. These trays were kept under standard larval rearing conditions until assessment. For each new batch of treated toxin a susceptible laboratory strain (ANGR for *H. armigera* and LHP for *H. punctigera*) was screened as a control for the assays.

Discriminating doses

In CSE102C, calibration of the GHD73 culture against *Helicoverpa* spp. was conducted using diet incorporation to assess development of early 3rd to 4th instar larvae over seven days. By incorporating GHD73 in diet at various concentrations we determined that 80µg/ml of diet and 120µg/ml of diet was the dose at which 98% of larvae from field strains of *H. armigera* and *H. punctigera* respectively were unable to develop from the 3rd to 4th instar over a period of 7 days.

For *H. armigera* and *H. punctigera* the discriminating concentration for Cry2Ab was 1 µg/cm^2 delivered in a 50 µl/well solution. For our susceptible strain of *H. armigera*, after 7 days at this concentration 99.6 ± 0.4% of larvae tested either died or did not grow beyond 1st instar and mortality was 96 ± 1.1% (n = 286 larvae over 6 assays). For 11 tested *H. punctigera* field strains collected during 2003 and 2004, 1 µg/cm^2 killed at least 94% of larvae (n = 63-72 larvae per population), and all larvae tested either died or did not grow beyond 1st instar.

% F_0 individuals surviving discriminating dose

Tables 3 and 4 show the percentage of larvae that survived the F_0 screens for Bt resistance. The number of larvae tested is in the parentheses underneath the value for % survivorship. Data are provided separately for different regions, for Cry1Ac and Cry2Ab, and for *H. armigera* and *H. punctigera*. Around 2% survival is expected as a baseline for the doses of toxins used in the F_0 screens. It is critical to consider sample sizes when assessing the significance of survival estimates greater than 2%.

With one exception, in all sampled regions the season total survival of larvae tested in our program (i.e., the total number of survivors divided by the total number of individuals tested) is not substantially greater than 2%. The exception was for *H. armigera* from the Darling Downs in 2007/08; however, the 3.7% of survivors from this area is based on a small sample of 82 individuals. Thus, our data from the F_0 screens do not indicate any major deviations from the 2% survival expected from these screens.

*TABLE 3: The %***H. armigera** *larvae surviving the F_0 screens against Cry1Ac and Cry2Ab. T1=15 Nov-21 Dec, T2=22 Dec-19 Jan, T3=20 Jan-16 Feb, T4 =17 Feb-13 Apr.*

Toxin	Valley	*% F_0 individuals surviving discriminating dose (no. individuals tested)*														
		2005/2006					2006/2007					2007/2008				
		T1	T2	T3	T4	Total	T1	T2	T3	T4	Total	T1	T2	T3	T4	Total
Cry1Ac	Darling Downs	0.6 (318)	0.6 (167)	1.3 (234)	1.7 (119)	1.0 (838)	none tested	none tested	0.0 (8)	0.0 (6)	0.0 (14)	0.0 (8)	4.1 (49)	5.9 (17)	0.0 (8)	3.7 (82)
	Gwydir	0.0 (708)	1.0 (633)	0.7 (414)	none tested	0.5 (872)	0.0 (36)	0.6 (332)	1.3 (161)	4.6 (44)	0.9 (573)	0.0 (165)	0.9 (108)	0.0 (37)	0.9 (117)	0.5 (427)
	Lower Namoi	1.7 (1976)	0.4 (816)	0.7 (711)	0.7 (283)	0.6 (3786)	0.0 (10)	1.0 (824)	0.7 (1325)	1.0 (1464)	0.8 (3623)	1.4 (143)	1.6 (487)	0.0 (69)	2.5 (80)	1.5 (779)
	Macintyre	2.0 (49)	1.3 (1325)	1.1 (822)	0.6 (164)	1.2 (2360)	0.0 (41)	0.0 (82)	0.6 (182)	0.9 (249)	0.5 (554)	none tested	0.0 (42)	0.0 (38)	none tested	0.0 (80)
	Upper Namoi	0.9 (532)	2.0 (191)	1.0 (100)	2.0 (175)	1.7 (998)	0.0 (35)	0.5 (120)	0.5 (943)	1.2 (336)	0.7 (1434)	0.0 (68)	1.5 (546)	0.4 (242)	0.5 (224)	0.9 (1080)
	Macquarie	0.0 (2)	none tested	0.0 (178)	none tested	0.0 (180)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	Emerald	0.0 (13)	0.0 (39)	0.0 (43)	none tested	0.0 (95)	none tested	none tested	none tested	none tested	none tested	0.0 (20)	0.0 (16)	none tested	none tested	0.0 (36)
	Bourke	none tested	none tested	0.8 (256)	none tested	0.8 (256)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	St George	none tested	0.0 (14)	2.0 (220)	0.0 (235)	1.0 (469)	none tested	none tested	0.0 (12)	0.0 (34)	0.0 (46)	0.0 (149)	1.7 (231)	0.0 (40)	0.0 (41)	0.9 (461)
	Lachlan	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	0.0 (1)	0.0 (7)	0.0 (7)	none tested	0.0 (15)
Cry2Ab	Darling Downs	0.0 (98)	0.0 (5)	0.0 (72)	0.0 (74)	0.0 (249)	none tested	none tested	0.0 (18)	none tested	0.0 (18)	none tested	0.0 (16)	none tested	0.0 (2)	0.0 (18)
	Gwydir	0.0 (210)	0.0 (464)	0.0 (301)	none tested	0.1 (975)	0.0 (230)	0.0 (65)	0.0 (33)	none tested	0.0 (328)	0.0 (66)	0.0 (31)	0.0 (30)	1.4 (73)	0.5 (200)
	Lower Namoi	0.0 (187)	0.3 (363)	0.0 (417)	none tested	0.1 (967)	0.0 (24)	0.0 (301)	0.0 (430)	0.0 (429)	0.0 (1184)	0.0 (8)	0.6 (162)	0.0 (22)	0.0 (9)	0.5 (201)
	Macintyre	0.0 (1)	0.0 (717)	0.0 (350)	0.0 (24)	0.0 (1092)	0.0 (23)	none tested	0.0 (87)	0.0 (94)	0.0 (204)	none tested	none tested	none tested	none tested	none tested
	Upper Namoi	0.0 (350)	0.0 (102)	0.0 (8)	none tested	0.0 (460)	0.0 (11)	0.0 (120)	0.0 (255)	0.0 (182)	0.0 (568)	0.0 (13)	0.0 (184)	0.9 (117)	2.6 (77)	0.8 (391)
	Macquarie	none tested	none tested	0.0 (184)	none tested	0.0 (184)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	Emerald	0.0 (33)	0.0 (9)	0.0 (10)	none tested	0.0 (52)	none tested	none tested	none tested	none tested	none tested	0.0 (5)	0.0 (3)	none tested	none tested	0.0 (8)
	Bourke	none tested	none tested	0.0 (19)	none tested	0.0 (19)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	St George	none tested	none tested	0.0 (84)	none tested	0.0 (84)	none tested	none tested	none tested	none tested	none tested	0.0 (29)	0.0 (42)	0.0 (5)	none tested	0.0 (76)

*TABLE 4: The %***H. punctigera** *larvae surviving the F_0 screens against Cry1Ac and Cry2Ab. T1=15 Nov-21 Dec, T2=22 Dec-19 Jan, T3=20 Jan-16 Feb, T4 =17 Feb-13 Apr.*

Toxin	Valley	*% F_0 individuals surviving discriminating dose (no. individuals tested)*														
		2005/2006					**2006/2007**					**2007/2008**				
		T1	T2	T3	T4	Total	T1	T2	T3	T4	Total	T1	T2	T3	T4	Total
Cry1Ac	Darling Downs	0.0 (156)	0.8 (123)	0.0 (103)	0.0 (14)	0.3 (396)	none tested	none tested	0.0 (6)	none tested	0.0 (6)	0.0 (5)	0.0 (39)	0.0 (13)	0.0 (95)	0.0 (152)
	Gwydir	0.1 (812)	0.0 (42)	0.0 (18)	none tested	0.5 (1755)	0.0 (200)	0.2 (460)	0.0 (55)	none tested	0.1 (715)	0.5 (218)	2.1 (239)	2.6 (38)	0.3 (307)	1.0 (802)
	Lower Namoi	1.4 (1165)	1.5 (194)	0.5 (416)	0.0 (63)	0.4 (1838)	0.4 (842)	0.0 (241)	0.1 (936)	0.0 (8)	0.2 (2027)	1.7 (60)	0.0 (128)	0.4 (261)	0.0 (60)	0.4 (509)
	Macintyre	0.1 (785)	1.4 (280)	0.0 (119)	0.0 (24)	0.4 (1208)	0.9 (227)	0.0 (10)	0.0 (33)	0.0 (42)	0.6 (312)	none tested	0.0 (23)	0.0 (14)	none tested	0.0 (37)
	Upper Namoi	1.1 (803)	1.0 (124)	0.0 (83)	none tested	0.0 (1010)	1.1 (521)	0.0 (202)	0.3 (402)	0.0 (34)	0.6 (1159)	none tested	1.2 (163)	2.2 (325)	2.7 (74)	2.0 (562)
	Macquarie	0.0 (115)	none tested	0.0 (232)	none tested	0.0 (347)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	Emerald	0.7 (150)	0.0 (107)	0.0 (20)	none tested	0.4 (277)	none tested	none tested	none tested	none tested	none tested	1.2 (242)	1.7 (118)	none tested	none tested	1.4 (360)
	Bourke	none tested	none tested	0.0 (182)	none tested	0.0 (182)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	St George	none tested	0.0 (14)	0.0 (220)	0.0 (1)	0.0 (235)	1.5 (68)	none tested	0.0 (96)	0.0 (3)	0.6 (167)	0.0 (93)	1.5 (205)	1.4 (278)	0.0 (14)	1.2 (590)
	Lachlan	none tested	0.0 (61)	none tested	none tested	0.0 (61)	none tested	none tested	none tested	none tested	none tested	0.0 (4)	2.7 (37)	1.1 (89)	none tested	1.5 (130)
Cry2Ab	Darling Downs	0.0 (47)	0.0 (2)	0.0 (26)	0.0 (17)	0.0 (92)	none tested	none tested	0.0 (6)	none tested	0.0 (6)	none tested	0.0 (5)	none tested	0.0 (18)	0.0 (23)
	Gwydir	0.0 (30)	0.0 (71)	0.0 (4)	none tested	0.0 (105)	0.0 (130)	0.0 (43)	0.0 (6)	none tested	0.0 (179)	none tested	0.0 (61)	0.0 (8)	0.0 (58)	0.0 (127)
	Lower Namoi	0.0 (30)	0.0 (119)	0.0 (135)	none tested	0.0 (284)	0.0 (222)	0.0 (300)	0.0 (55)	0.0 (26)	0.0 (603)	0.0 (17)	0.0 (33)	0.00 (38)	0.0 (1)	0.0 (89)
	Macintyre	0.0 (2)	0.0 (177)	0.0 (42)	0.0 (3)	0.0 (224)	0.0 (111)	none tested	0.0 (71)	0.0 (6)	0.0 (188)	none tested	none tested	none tested	none tested	none tested
	Upper Namoi	0.0 (248)	0.0 (81)	none tested	none tested	0.0 (329)	0.0 (131)	0.0 (105)	0.0 (169)	0.0 (16)	0.0 (421)	0.0 (6)	0.0 (24)	0.0 (107)	none tested	0.0 (137)
	Macquarie	none tested	none tested	0.0 (174)	none tested	0.0 (174)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	Emerald	0.0 (30)	0.0 (34)	0.0 (5)	none tested	0.0 (69)	none tested	none tested	none tested	none tested	none tested	0.0 (33)	0.0 (51)	none tested	none tested	0.0 (84)
	Bourke	none tested	none tested	0.0 (30)	none tested	0.0 (30)	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested	none tested
	St George	none tested	none tested	0.0 (57)	none tested	0.0 (57)	none tested	none tested	none tested	none tested	none tested	0.0 (26)	0.0 (26)	0.0 (76)	none tested	0.0 (128)

(iv) F_2 screens against Cry1Ac and Cry2Ab

F_2 tests generate isofemale lines that produce a proportion (1/16) of individuals which are homozygous for haplotypes present in their field-derived parents (see Figure 1).

Field-collected eggs were reared individually to adults in the laboratory. On maturation, pupae were collected, washed, sexed and set up in cages that contained pupae of the same sex from the same valley. Emerged adult moths (one male and one female) from the same valley were then placed into containers (750 ml) as single pairs. The use of single pairs (hereafter 'P_1 lines') ensured that four haplotypes were tested for the presence of resistance alleles. This technique was preferred over testing field-mated females because multiple mating occurs in Helicoverpa species which complicates the interpretation of data.

Containers housing the single pairs were checked every 2 days, and liners containing fertile eggs were collected and stored at 10ºC until the female had ceased ovipositing or at least 150 eggs were collected. At this time the liners were incubated at 25ºC which promoted hatching in approximately 3 days. We aimed to rear 135 neonates from each pair to establish individual isofemale lines. On maturation, pupae were collected, washed, sexed and equivalent numbers of males and females were placed in a 5 litre container and allowed to sib-mate in bulk.

Eggs were collected daily and stored at 10ºC. When at least 300 eggs had accumulated over an interval of at least 4 days, they were removed from the liners or cloths by washing in a 0.005 % solution of household bleach, filtered onto a paper disc with a suction funnel, and placed at 25 ºC to hatch. All F_2 lines were produced from at least 15 F_1 males and 15 F_1 female moths.

Assays were conducted in 45 well (2.7 cm^2) trays which contained approximately 2 ml of rearing diet that was overlaid with an aqueous solution of toxin at the selected concentration, and then allowed to air dry. Concentrations were calculated as µg of toxin per cm^2 of diet surface. After the addition of one neonate larvae per well, trays were heat sealed and maintained at 25 °C and 45-55 % RH. We aimed to expose 90 neonate larvae (two 45 well trays) to each toxin for each isofemale line (mean = 86.1, range = 42-90). After 7 days the larvae were scored as being alive (exhibiting normal movement) or dead (dead, moribund, uncoordinated movement), and the growth stage (instar) of all survivors was recorded.

The discriminating concentration for Cry1Ac was 0.5 µg/cm^2 delivered in a 50 µl/well solution. This concentration was confirmed as appropriate after examining the concentration-mortality responses of neonates from 12 *H. punctigera* field strains collected between 2003 and 2004 as reported in the final report for CRDC Project CSE102C. In all populations, a concentration of 0.5 µg/cm^2 of Cry1Ac killed at least 96 % of larvae (n = 69-72 larvae per population).

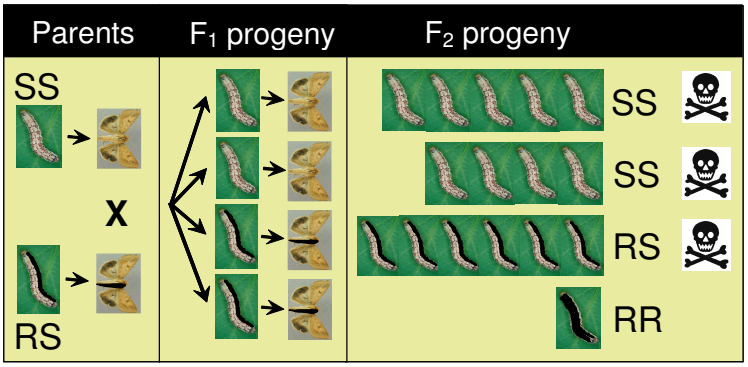


Figure 1: F_2 screen for rare resistance alleles. Parents are collected in the field as eggs, and here one of them is indicated with one copy of the resistance allele. Their F_1 progeny are sib-mated to produce the F_2 generation. If resistance is completely recessive, in the F_2 generation only 1/16 of the larvae will be homozygous for the resistance allele (RR), and the remaining homozygous susceptible (SS) and heterozygote progeny(RS) will be killed by the dose of toxin.

Discriminating doses

For *H. armigera* and *H. punctigera* the discriminating concentration for Cry2Ab was 1 µg/cm^2 delivered in a 50 µl/well solution. For our susceptible strain of *H. armigera*, after 7 days at this concentration 99.6 ± 0.4% of larvae tested either died or did not grow beyond 1st instar and mortality was 96 ± 1.1% (n = 286 larvae over 6 assays). For 11 tested *H. punctigera* field strains collected during 2003 and 2004, 1 µg/cm^2 killed at least 94% of larvae (*n* = 63-72 larvae per population), and all larvae tested either died or did not grow beyond 1st instar.

For *H. armigera* the discriminating concentration for Cry1Ac was 0.25 µg/cm^2 of GHD73 delivered in a 50 µl/well solution. After 7 days this concentration killed 95.7 ± 1.8 % of our susceptible laboratory strain (*n* = 628 larvae in 10 assays) and no surviving larvae grew beyond 2nd instar. For *H. punctigera* the discriminating concentration for Cry1Ac was 0.5 µg/cm^2 of HD73 delivered in a 50 µl/well solution. For 12 tested *H. punctigera* field strains collected between 2003 and 2004, this concentration killed at least 96 % of larvae (*n* = 69-72 larvae per population) and no surviving larvae grew beyond 2nd instar (see final report for CRDC Project CSE102C.

Alleles conferring resistance to Cry1Ac

We screened for Cry1Ac resistance, 900 and 468 alleles in 2005/06, 522 and 712 alleles in 2006/07, and 772 and 1,142 alleles in 2007/08 for *H. armigera* and *H. punctigera* respectively. We isolated no cases in either *H. armigera* or *H. punctigera* of alleles conferring resistance to Cry1Ac (see Table 5). For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry1Ac since the program began in 2002/03 is 0/2,974. For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry1Ac since the program began in 2002/03 is 0/3,402.

These data suggest that in both species, the frequency of alleles conferring resistance to Cry1Ac remains low after 12 years exposure to cotton containing this toxin (initially Ingard® and now Bollgard II®). Perhaps the conservative RMP imposed for Ingard® was effective in minimising any increase in frequency of genes conferring resistance to Cry1Ac during the period that it was grown, i.e., 1996 - 2004. Alternatively, characteristics of Cry1Ac resistance present in the population may not promote its evolution.

It is also possible that our methods did not detect genes that were present in sampled populations but this situation is improbable. The F_2 screens are likely to detect non-trivial forms of resistance that are determined by a single locus. It is not infallible, and some forms of resistance may not be detected, particularly if they are due to more than one locus. However such forms of resistance develop infrequently in the field, but remain possible outcomes of selection. The probability of obtaining false negatives using the employed testing regime is very low and therefore we consider that our estimates of the frequency of resistance to both toxins are robust.

Alleles conferring resistance to Cry2Ab

We screened against Cry2Ab 900 and 468 alleles in 2005/06, 522 and 712 alleles in 2006/07, and 772 and 1142 alleles in 2008/09 for *H. armigera* and *H. punctigera* respectively.

In *H. armigera* during 2005/06, 2006/07 and 2007/08 we isolated 4, 5 and 4 cases respectively of alleles conferring high level resistance to Cry2Ab (see Table 5). Of these 13 cases, none exhibited cross-resistance to Cry1Ac. For *H. armigera,* the cumulative frequency of alleles conferring resistance to Cry2Ab since the F_2 screen program began in 2002/03 is 16/2,974 (0.0054). The F_2 data for *H. armigera* show no significant relationship between frequency of Cry2Ab resistance alleles and time, and no significant differences among years in frequencies (in all cases $P > 0.05$; but see below for F_1 screens).

In *H. punctigera* during 2005/06, 2006/07 and 2007/08 we isolated 0, 2 and 5 cases respectively of alleles conferring high level resistance to Cry2Ab (see Table 5). Of these 7 cases, none exhibited cross-resistance to Cry1Ac. For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the F_2 screen program began in 2002/03 is 8/3414 (0.0023). When we account for the differences in sampling effort between seasons, the F_2 data show a statistically significant increase in frequency of Cry2Ab resistance alleles in *H. punctigera* over time (GLIM, $F_{1,4} = 14.5$, $P = 0.019$).

In both species, the first isolates of Cry2Ab resistance were detected prior to significant opportunities for selection by Bollgard II®, so we believe that the resistance alleles were present at a detectable frequency prior to the introduction of Bt-cotton that expresses this protein.

TABLE 5: The number of lines in the F_2 screen that scored positive for carrying a resistance allele to Cry1Ac or Cry2Ab. Data are presented separately for each species and year but have been combined for all crops (i.e., conventional cotton, Bollgard II® cotton, mungbean, sunflowers, chick pea, maize, and pigeon pea).

Species	Year	Cry1Ac F_2 screen		Cry2Ab F_2 screen	
		alleles tested	scored positive	alleles tested	scored positive
Helicoverpa punctigera	2002/03	8	0	8	0
	2003/04	60	0	60	0
	2004/05	1012	0	1024	1
	2005/06	**468**	**0**	**468**	**0**
	2006/07	**712**	**0**	**712**	**2**
	2007/08	**1142**	**0**	**1142**	**5**
	Total	3402	0	3414	8
Helicoverpa armigera	2002/03	136	0	132	1
	2003/04	280	0	284	2
	2004/05	364	0	368	0
	2005/06	**900**	**0**	**900**	**4**
	2006/07	**522**	**0**	**522**	**5**
	2007/08	**772**	**0**	**772**	**4**
	Total	2974	0	2978	16

(v) F_1 screens for SP15-like Cry2Ab resistance in H. armigera

In 2004 we developed protocols for testing the frequency of resistance using a shorter version of the F_2 method called an F_1 test. F_1 screens can detect heterozygote individuals (RS). They involve testing the offspring of single-pair matings between moths from a Cry2Ab resistant strain maintained in the laboratory (SP15) and moths raised from eggs collected from field populations (Figure 2). They take around 5 weeks to complete (c.f. 10 weeks for F_2 screens), and therefore a major benefit of employing these tests is the ability to increase throughput.

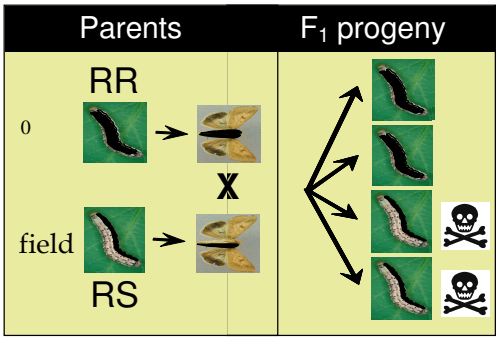


Figure 2: F_1 screen for rare resistance alleles. One parent is collected in the field as an egg, and here it is indicated with one copy of the resistance allele. It is mated to a moth from a Cry2Ab resistance laboratory strain that is known to carry two copies of the allele. If the wild moth carries the same Cry2Ab resistance allele as the SP15-like resistant colony, in the F_1 generation 50% of the larvae will be homozygous for the resistance allele (RR), and the remaining heterozygote progeny (RS) will be killed by the dose of toxin.

The F_1 method assumes that the various isolates of Cry2Ab resistance alleles detected thus far are of the same kind. These protocols were immediately adopted by Monsanto for *H. armigera*. During the following two years we determined that the same mechanism appeared to confer resistance in all of the isolates of Cry2Ab detected in *H. armigera* to date (see final report for CRDC Project CSE109); this has now been verified for 9 out of 9 tested isolates. In 2007/08 we began F_1 tests to determine the frequency of this SP15-type of Cry2Ab resistance for *H. armigera*. This work was also performed at Black Mountain on a small scale in 2006/07 and more extensively in 2007/08 as part of CRDC Project CSE005.

The methods that we adopted are identical to those described above for the F_2 screens except that we tested the F_1 generation produced from a cross between a Cry2Ab resistant moth and a field collected moth (see Figure 2), and we aimed to expose 65 neonate larvae (one and a half 45 well trays) to each toxin for each isofemale line.

In both laboratories, the results from 2007/08 confirmed previous findings from Monsanto that the frequency of Cry2Ab resistance alleles in *H. armigera* using F_1 screens is up to 6 times higher than that determined with the F_2 tests: 2007/08 CSIRO data F_1 screen = 22/686 alleles (0.03), F_2 screen = 4/772 alleles (0.005). Currently, we believe that the frequencies obtained from the F_1 screens are likely to most accurately reflect the situation in the field. Both CSIRO and Monsanto are working to better understand the differences between the F_1 and F_2 screens.

Since 2004/05, Monsanto have used the F_1 protocol developed by CSIRO to screen *H. armigera* for SP15-like Cry2Ab resistance alleles. In the three years from 2004/05 to 2006/07, the frequency of resistance detected in the field remained fairly consistent, with no significant differences between years (Fisher's Exact Test, P = 0.64: Table 6). However, the combined data from CSIRO and Monsanto for 2007/08 indicates a deviation from this trend with alleles conferring resistance to Cry2Ab in *H. armigera* being significantly higher than previous years (P = 0.003: Table 6).

TABLE 6: The number of lines in the F_1 screen that scored positive for carrying an SP15-like Cry2Ab resistance allele. Data have been combined for all crops (i.e., conventional cotton, Bollgard II® cotton, mungbean, sunflowers, chick pea, maize, and pigeon pea). BM= Black Mountain, NBR = Narrabri.

Year	Source	Cry2Ab F_2 screen	
		alleles tested	scored positive
2004/05	Monsanto	294	4
2005/06	Monsanto	878	13
2006/07	Monsanto, CSIRO BM	372	3
2007/08	Monsanto, CSIRO BM, CSIRO NBR	874	26
	Total	**2418**	**46**

(vi) 'Survivors from Bollgard II® plants'

During 2005/06, 2006/07 and 2007/08 there were reports from early-January until late-February of surviving larvae at threshold levels in Bollgard II® fields on some properties in Emerald, Lower Namoi, Upper Namoi, Darling Downs, Gwydir, Macquarie, Macintyre and St George. All affected fields were at mid-flowering to late-flowering.

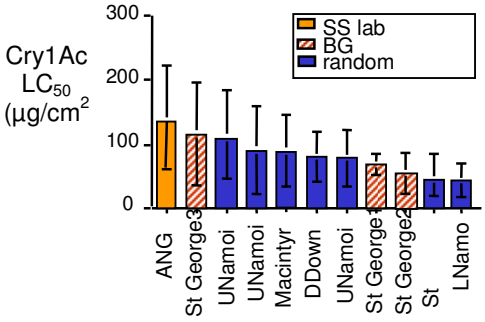
We received for testing 320 (50% *H. armigera*), 231 (30% *H. armigera*), and 763 (75% *H. armigera*) larvae that were collected on Bollgard II®. The majority of these larvae were medium and large in size. The larvae were collected from a number of properties within each valley. We have not presented this breakdown because it probably does not relate to the incidence of living larvae on Bollgard II® among properties and valleys but instead reflects differences in the readiness of consultants or growers to submit larvae to the program.

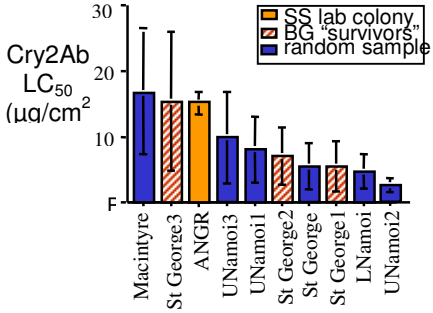
Mr Gavin Whitburn is currently working with CSIRO, CCA members and Monsanto to survey the proportion of Bollgard II in each valley that reached threshold levels of Helicoverpa, and the proportion of threshold levels that were sprayed to control Helicoverpa. These data will be forwarded to the CRDC at the completion of the project.

Responses of survivors vs. non-survivors to Cry1Ac and Cry2Ab

In 2007/08 we allocated a majority of larvae collected from three sites at St George to create three *H. armigera* colonies that were comprised entirely of survivors on Bollgard II from three separate properties. We scored the responses of these colonies to Cry1Ac and Cry2Ab across a range of toxin concentrations and compared this to our Bt-susceptible laboratory strain as well as several other strains that we created from egg collections from cotton in a number of valleys, including St George. The highest concentration tested was similar to the discriminating dose used in our screens, and the subsequent 6 concentrations were each half the strength of the previous one. Our assay therefore covered a large range of concentrations that should detect survival of the toxin ranging from a high level resistance to some tolerance.

The vertical axis on the graphs below shows, for Cry1Ac and Cry2Ab respectively, the concentration that killed 50% of insects from each tested strain (known as the LC_{50}). The LC_{50} values have been ordered from highest to lowest. Against both toxins the responses of the survivor colonies are not significantly different from the response of our Bt susceptible laboratory strain or strains made up of randomly collected eggs from cotton in a number of different valleys.





F_2 screens with survivors

All larvae that were successfully reared on artificial diet in the laboratory to healthy moths were assigned to the F_2 screening component of the Bt monitoring program. Of these "survivors" we screened against Cry1Ac and Cry2Ab 124 and 108 alleles in 2005/06, 24 and 168 alleles in 2006/07, and 132 and 84 alleles in 2008/09 for *H. armigera* and *H. punctigera* respectively.

In the sample of *H. punctigera* we did not detect alleles conferring resistance to Cry1Ac or Cry2Ab. Since the program began (2005/06) the cumulative frequency of alleles in *H. punctigera* survivors that confer resistance to Cry1Ac and Cry2Ab is 0/360 for both toxins.

In the sample of *H. armigera* we did not detect alleles conferring resistance to Cry1Ac. Since the program began the cumulative frequency of alleles in *H. armigera* survivors that confer resistance to Cry1Ac is 0/280. During 2005/06, 2006/07 and 2007/08 in *H. armigera* we isolated 2, 0 and 1 case(s) respectively of alleles conferring high level resistance to Cry2Ab. Since the program began the cumulative frequency of alleles in *H. armigera* survivors that confer resistance to Cry2Ab is 3/280. This frequency is not significantly different from the cumulative frequency of alleles conferring Cry2Ab resistance in the random sample (Fisher's Exact test, $P > 0.05$). Thus survivors do not carry alleles that confer resistance to Cry1Ac and to Cry2Ab any more often than general Helicoverpa spp. populations.

Several other factors support the notion that larvae were not able to survive on Bollgard II due to a physiological resistance to Bt. First, two copies of the Cry2Ab allele are required to resist

the toxin but we can conclude from our bioassays that the positive survivors are heterozygotes (RS). Second, all of the positive lines are susceptible to Cry1Ac. Third, more than 99.9% of the sample was susceptible to Cry1Ac and Cry2Ab.

Qualitative ELISA tests of host plants

For a subset of larvae that were collected as survivors on Bollgard II® we have leaf samples from the host and surrounding plants. As part of CRC Project 1.01.03, we used qualitative ELISA tests to analyze this material for the presence or absence of Cry1Ac and Cry2Ab. For completeness, and because this component will be incorporated into the Bt resistance monitoring program from 2008, we have included the ELISA data here (as well as in the final report for CRC Project 1.01.03). Note that our analyses were performed on a biased sample because only plants supporting larvae were tested.

In 2006/07 a total of 7 samples of the 168 tested scored negative for either Cry1Ac or Cry2Ab. In one sample from the Lachlan valley the same leaf tested negative for both Cry1Ac and Cry2Ab. In one sample from the Lower Namoi the leaf tested negative for Cry1Ac and positive for Cry2Ab. In 5 samples from St George the leaf tested positive for Cry1Ac and negative for Cry2Ab.

Our data suggest that of the samples taken from Bollgard II® plants that were hosts, or nearby hosts, of the collected surviving Helicoverpa larvae, at least one of the two Bt proteins was present in 99.4% of cases, Cry1Ac was absent and Cry2Ab was present in < 1% of cases, and Cry1Ac was present and Cry2Ab was absent in 2.9% of cases.

In 2007/08 a total of 4 leaf samples of the 295 tested scored negative for Cry1Ac or Cry2Ab. In the samples from the Macintyre valley the same leaf tested negative for both Cry1Ac and Cry2Ab. In two samples from the Lower Namoi and one sample from St George the plants tested positive for Cry1Ac and negative for Cry2Ab.

Our data suggest that 98.6% of the Bollgard II® plants that were hosts, or nearby hosts, of the sampled surviving Helicoverpa larvae, contained at least one of the two Bt proteins. Only one plant did not contain both proteins. No plants contained Cry2Ab only.

TABLE 7: The number of Bollgard II® host plants containing surviving larvae that scored positive for the* cry1Ac *or* cry2Ab *gene using qualitative ELISA. Data for each season have been summarised according to valley and separately for the two toxins. The total leaf samples include those that were the host of the larvae at the time of collection and those that immediately surrounded the host plant. We scored a sample as negative only if duplicate samples from that leaf proved negative.

			No. positive samples	
Year	Valley	Total leaf samples	Cry1Ac	Cry2Ab
2006/07	Gwydir	7	7	7
	Macintyre	68	67	67
	Lower Namoi	32	32	30
	St George	188	188	187
	Total	**295**	**294**	**291**
2007/08	Lower Namoi	4	3	4
	St George	159	159	154
	Lachlan	5	4	4
	Total	**168**	**166**	**162**

Outcomes

Describe how the project's outputs will contribute to the initial planned outcomes.

During the project we used F_0 screens to challenge ~ 27,000 *H. armigera* and 1,800 *H. punctigera* to Cry1Ac and Cry2Ab, and F_2 screens to challenge 2,194 alleles of *H. armigera* and 2,322 alleles of *H. punctigera* to Cry1Ac and Cry2Ab. This data enabled us to achieve our main outcome of rigorously assessing the sensitivity of field populations of Helicoverpa to Cry1Ac and Cry2Ab toxins to detect early signs of the development of resistance to genetically modified cotton.

Please describe any:

a) Technical advances achieved:

This project is not of a technical nature thus there were no significant commercially developments, patents applied for or granted licenses arising from this work.

b) Other information developed from research (e.g., discoveries in methodology, equipment design, etc.):

This project follows from CRDC Project CSE102C in which we developed sensitive bioassay protocols for monitoring resistance in *Helicoverpa* spp. to Cry1Ac and Cry2Ab; these refined methods were utilised in this project. We did not develop any methodology or equipment specifically for the work performed as CRDC Project CSE112.

c) Required changes to the Intellectual Property register:

No changes to the IP register are required.

Conclusions

- For *H. armigera,* the assumed frequency of Cry2Ab resistance alleles in populations may be substantially (up to 6 times) higher than previously thought.
- In 2007/08 there was a significant increase in the frequency of Cry2Ab resistance alleles obtained using F_1 screens compared to previous seasons for *H. armigera.*
- Since the introduction of Bollgard II the frequency of Cry2Ab resistance alleles obtained using F_2 screens has also increased in *H. punctigera*.
- Despite these findings, Bollgard II should continue to provide excellent protection against Helicoverpa provided that the industry manages its stewardship responsibilities.
- There have been no reported field failures of Bollgard II and the occasional occurrence of threshold levels of Helicoverpa in some Bollgard II fields is not due to Bt resistance.
- Although survivors on Bollgard II are not currently resistant, it would be useful to control them so that they are not exposed to low doses of toxin which can select for resistance in the future.
- We need to verify the extent and distribution of fields with Helicoverpa survivors, and determine whether it's possible to predict if a particular field will have a problem. Gavin Whitburn is currently working with CCA members to collect this data.
- Despite a poor history in developing resistance to conventional insecticide, the industry needs to regard *H. punctigera* as a potential risk of developing resistance to Bt.

Extension Opportunities

Detail a plan for the activities or other steps that may be taken:

(a) To further develop or to exploit the project technology:

This project is not of a technical nature.

(b) For the future presentation and dissemination of the project outcomes:

The outcomes of this project have been disseminated in full to industry via written articles and oral presentations (see "Publications" below), and end of month and season reports (see "Online resources" below).

(c) For future research:

This project has been ongoing since the mid-1990's. It is critical that this research continues in order to determine future trends in the frequencies of Cry2Ab resistance alleles. These data will be pivotal for informing TIMS on the merit of recommending changes to the RMP for Bt cotton that would enhance the longevity of Bollgard II® and any other transgenic technology that makes use of Cry1Ac or Cry2Ab. If additional transgenic varieties become available that employ new toxins the resistance monitoring program should be extended to assess frequencies of resistance genes for such toxins. It would be preferable that pre-release resistance data are obtained for those toxins.

The longer term success of growing cotton in Northern Australia will depend on several issues including Bt resistance management. For the main pest, *Spodoptera litura*, it will be important to develop sensitive bioassays against Cry1Ac and Cry2Ab, and begin screening field populations to establish baseline frequencies of resistance alleles.

Publications arising from the research project

Refereed articles in popular science magazines and industry publications

Mahon R, Downes SJ, 2008, GM longevity under threat. CRDC Spotlight Magazine Winter:4-6

Mahon R, Downes SJ, Olsen K, Parker T, 2008, Stakeholders must remain vigilant to manage Bt resistance. Farming Ahead 193:1-3

Baker G, Tann C, Downes SJ, 2008, Research comments: Entomology, Cotton Seed Distributors Trial Book, pp. 49-52

Downes SJ, Wilson L, Kauter G, Farrell T, 2008, Preamble to the Resistance Management Plan (RMP) for Bollgard II® for 2008-2009, Cotton Pest Management Guide (Ed T Farrell), pp. 39-46

Downes SJ, Rossiter L, Parker T, McKenzie F, Staines T, 2007, How to collect *Helicoverpa* for resistance testing. The Australian Cottongrower Dec-Jan 28:48-49

Mahon R, Downes SJ, Olsen K, Parker T, 2007, An update on Bt resistance in *Helicoverpa armigera* in Australia. The Australian Cottongrower Dec-Jan 28:10-12

Rossiter L, Murray D, Miles M, Downes SJ, Wilson L, Kauter G, 2007, Better pupae busting decisions in sprayed conventional cotton. The Australian Cottongrower Oct-Nov 27:21-22

Baker G, Tann C, Downes SJ, 2007, Research comments: Entomology, Cotton Seed Distributors Trial Book, pp. 45-47

Downes SJ, Wilson L, Kauter G, Farrell T, 2007, Preamble to the Resistance Management Plan (RMP) for Bollgard II® for 2007-2008, Cotton Pest Management Guide (Ed T Farrell), pp. 36-44

Downes SJ, Mahon R, Parker T, Staines T, 2006, WANTED ALIVE: Large *Helicoverpa* larvae from Bollgard II® plants. The Australian Cottongrower Dec-Jan 27:8-10

Downes SJ, Mahon R, Rossiter L, Farrell T, Wilson L, Kauter G, 2006, How will the drought affect the way that *Helicoverpa* resistance is managed? The Australian Cottongrower Dec-Jan 27:60-62

Baker G, Tann C, Downes SJ, 2006, Research comments: Entomology, Cotton Seed Distributors Trial Book, pp. 54-56

Downes SJ, Wilson L, Kauter G, Farrell T, 2006, Preamble to the Resistance Management Plan (RMP) for Bollgard II® for 2006-2007, Cotton Pest Management Guide (Ed T Farrell), pp. 37-45

Rossiter L, Downes SJ, Mahon R, 2005, *Helicoverpa*: species mix, parasitism and resistance monitoring. The Australian Cottongrower Dec-Jan:66-69

Downes SJ, Rossiter L, Farrell T, Wilson L, Kauter G, 2005, Managing resistance: your IRMS and RMP questions answered. The Australian Cottongrower Oct-Nov:10-14

Refereed articles in scientific journals

Downes SJ, Parker T, Mahon R, 2008, Frequency of alleles conferring resistance to the Bt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae). *Journal of Economic Entomology* (in review)

Mahon R, Olsen K, Downes SJ, 2008, Isolations of Cry2Ab resistance in Australian populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) are allelic. *Journal of Economic Entomology* 101:909-914

Mahon R, Olsen K, Downes SJ, Addison S, 2007, Frequency of alleles conferring resistance to theBt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 100(6):1844-1853

Downes SJ, Mahon R, Olsen K, 2005, Monitoring and adaptive resistance management in Australia for Bt-cotton: current status and future challenges. *Journal of Invertebrate Pathology* 95:208-213

Presentations at scientific meetings and industry forums and seminars

Downes SJ, Mahon R, Parker T, Lu B, 2008, The changing Bt resistance landscape. Australian Cotton Conference, Gold Coast [Invited Plenary Lecture]

Downes SJ, Parker T, Mahon R, 2008, Frequency and characteristics of alleles conferring resistance to the Bt toxin Cry2Ab in Australian populations of the low risk target *Helicoverpa punctigera*. XXIII International Congress of Entomology, South Africa, July

Mahon R, Olsen K, Downes SJ, 2008, Cry2Ab resistance in *Helicoverpa armigera* and factors influencing the evolution of resistance to transgenic cotton in Australia. XXIII International Congress of Entomology, South Africa, July

Downes S, Mahon R, 2008, Bt resistance update, Cotton Consultants Australia Cotton Production Seminar, Narrabri, May

Mahon R, Downes SJ, 2008, Resistance in *Helicoverpa armigera* to Cry2Ab, Integrated Pest Management Forum, Toowoomba, June

Downes SJ, 2008, Is Helicoverpa developing resistance to Bt-cotton?, *Australian Cotton Research Institute*, May

Downes S, Mahon R, 2008, Survivors on Bollgard II cotton, Transgenic and Insect Management Strategy Resistance Roadshow, Emerald, Dalby, St George, Goondiwindi, Moree, Narrabri, Hillston, Warren, 26-30th May

Downes SJ, 2008, Bt resistance update and survivors on Bollgard II, Lower Balonne Field Day, 12th March

Mahon R, Olsen K, Young S, Downes SJ, 2007, Alleles conferring resistance to the Bt toxin Cry2Ab in Australian populations of *Helicoverpa armigera*, 4th World Cotton Research Conference, Lubock, Texas (Poster)

Lu B, Downes SJ, Wilson L, Gregg P, Kauter G, Knight K, 2007, Spray thresholds and mechanisms of survival for Bt-susceptible *Helicoverpa* living on Bollgard II® cotton. Cotton Catchment Communities Conference, Narrabri, August

Downes SJ, 2007, Adaptive resistance management of GM cotton, University of Melbourne, Department of Zoology, May

Downes S, Mahon R, 2007, Bt resistance update, Cotton Consultants Australia Cotton Production Seminar, Narrabri, June

Downes SJ, Mahon R, 2006, Monitoring for resistance to Bt-cotton in Australia: current status and future challenges. Combined meeting of the IX International Colloquium on Invertebrate Pathology and Microbial Control, XXXIX Annual Meeting of the Society of Invertebrate Pathologists and VIII International Conference on *Bacillus thuringiensis*, Wuhan, China [Invited presentation in the symposium on Monitoring and Managing for Bt-Resistance: The Challenges for the Next Decade]

Downes SJ, Mahon R, 2006, Resistance and refuge. Australian Cotton Conference, Gold Coast [Invited Plenary Lecture]

Mahon R, Downes SJ, 2006, Bt resistance management for Bollgard II®. Are there significant threats? Australian Cotton Trade Show, Moree

Downes SJ, Mahon R, 2005, What is the current situation for Bt resistance in field populations of *Helicoverpa armigera* in Australia? Australian Entomological Society Meeting, Canberra

Olsen KM, Mahon RJ, Downes SJ, 2005, Resistance to Cry2Ab toxin in the cotton bollworm *Helicoverpa armigera* in Australia: implications for the cotton industry. Australian Entomological Society Meeting, Canberra (Poster)

Downes SJ, Mahon R, Olsen K, Young S, 2005, Resistance to Cry2Ab toxin in the cotton bollworm *Helicoverpa armigera* in Australia. Pacific Rim Conference on the impacts of *Bacillus thuringiensis* on the environment, Victoria, Canada.

Online resources

No online resources have been developed from this project, although all end of month and end of season reports appear on the CRC web site under "publications".

http://www.cottoncrc.org.au/content/Industry/Publications/Pests_and_Beneficials/Insect_Resistance_Management.aspx

In the 2004/05 season Bollgard II® replaced Ingard® as the transgenic variety of cotton available to Australian growers. It improves on Ingard® by incorporating an additional insecticide protein (Cry2Ab) to combat *H. armigera.* Sequence information indicates that these genes are distantly related and the toxins they encode do not share a common binding site. Consequently it is thought unlikely that a single mechanism could confer resistance to both toxins. Due to the perceived difficulty for *H. armigera* to evolve resistance to both proteins within Bollgard II®, the RMP for transgenic cotton was relaxed to allow growers to plant up to around 95% of the total area to this product. Bollgard II® was well adopted, with >70% planted area throughout the industry.

The cotton industry has sought to acquire early warnings of changes in sensitivity of insect populations to toxins that may signal the presence of resistance to transgenic varieties of cotton. The sensitivity of field-collected populations of *H. armigera* and *H. punctigera* to Bt products was assayed before and subsequent to the widespread deployment of Ingard® cotton expressing Cry1Ac in the mid-1990's. During CSE102C, baseline levels of susceptibility to Cry2Ab were established in preparation for replacement in the 2004/05 season of Ingard® with Bollgard II®. Preserving the efficacy of Cry1Ac and Cry2Ab is critical for the future of the industry, not only for the efficacy of the Bollgard II® varieties of cotton, but also for the long-term future of cotton varieties expressing Cry1Ac or Cry2Ab in combination with other effective toxins.

In this project we achieved our main aim of rigorously assessing the sensitivity of field populations of Helicoverpa to both Cry1Ac and Cry2Ab to detect early signs of the development of resistance to genetically modified cotton. Through the introduction of a new screening technique (F_1 tests) we found that for *H. armigera* the assumed frequency of Cry2Ab resistance alleles in populations may be substantially (up to 6 times) higher than previously thought. In 2007/08 there was a significant increase in the frequency of Cry2Ab resistance alleles obtained using F_1 screens compared to previous seasons for *H. armigera*. Since the introduction of Bollgard II the frequency of Cry2Ab resistance alleles obtained using F_2 screens has also increased in *H. punctigera*. Despite these findings, Bollgard II should continue to provide excellent protection against Helicoverpa provided that the industry manages its stewardship responsibilities.

We recommend that the industry improve its compliance with the RMP particularly in terms of producing high quality refuges. Also, because late in the season Helicoverpa may be exposed to cotton that only expresses Cry2Ab, it is important to implement an effective pupae busting procedure to kill that last generation which may be enriched with Cry2Ab resistance genes.

There have been no reported field failures of Bollgard II and the occasional occurrence of threshold levels of Helicoverpa in some Bollgard II fields is not due to Bt resistance. Although survivors on Bollgard II are not currently resistant, it would be useful to control them so that they are not exposed to low doses of toxin which can select for resistance in the future. We need to verify the extent and distribution of fields with Helicoverpa survivors, and determine whether it's possible to predict if a particular field will have a problem. Gavin Whitburn is currently working with CCA members to collect this data.

Despite a poor history in developing resistance to conventional insecticide, the industry needs to regard *H. punctigera* as a potential risk of developing resistance to Bt.

For more information contact: Sharon Downes (Sharon.Downes@csiro.au) or Rod Mahon (Rod.Mahon@csiro.au), CSIRO Entomology