



FINAL REPORT 2016

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: CSE1402

Project Title: Monitoring to manage resistance to Bt toxins

Project Start Date: 1.7.2013 **Project Completion Date:** 30.6.2016
Research Program: 2. Farming Systems

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Signature of Research Provider Representative:

Date Submitted:

20 September 2016

Background

1. Outline the background to the project.

The industry is entering a critical period in managing resistance to Bt-cotton. One technology provider is about to release their 3rd generation product which is based on the current two gene varieties (Cry1Ac, Cry2Ab) plus Vip3A. It is possible that in the near future other technology providers will enter the market. If more than one Bt-cotton product is commercial, at least in the short term, it is likely that larvae will be exposed to the same or different varieties of Bt toxins within the 3 classes that are currently included in the resistance monitoring program: Cry1A, Cry2A and Vip3A. Resistant insects are likely to tolerate different varieties of toxins within the same class, so for instance, resistance frequencies for Cry2Aa should be the same as those for Cry2Ab. The development of robust resistance management plans (RMPs) for these technologies depends critically on the frequencies of common resistances to these key classes of Bt toxins.

This proposal continues a program initiated in 1994 to monitor resistance in field populations of *H. armigera* and *H. punctigera* to the Bt toxins produced in transgenic cotton. Since CSIRO took the lead on the project in 2002/03 we have used F2 screens to show that resistant alleles are rare for the Cry1Ac protein (present in Bt-cotton since 1996), but in both *Helicoverpa* spp. frequencies of resistance for the Cry2Ab protein (present since 2004) and the Vip3A protein (not yet present) are unexpectedly high.

F2 screens are valuable for identifying new types of resistances, including those that are recessive. However, it is probable that the common recessive resistances that are likely to form the main threat to the cotton industry have already been identified. We therefore proposed that going forward, the resistance monitoring program intermittently performs F2 screens (every 4 or 5 years; which falls outside of the period of this project), and routinely focuses on more efficient F1 screens to detect any changes in frequencies that have already been identified. During this project we introduced a new component that adds value to the current F1 screens by challenging families not only against the toxin that is tolerated by the resistant parent used in the screen but also those from the other two classes. This approach allowed us to test for potential multi-resistances as well as novel dominant forms of resistance which will inform adaptive management of Bt resistance. Concurrently with this new approach, opportunities for improving the efficacy of existing day-to-day processes were explored by visiting mass rearing facilities to observe techniques.

In 2008 a survey of Crop Consultants Australia (CCA) members determined that from 2005-2008 on average 15% of the Bt-cotton area all cropping regions occasionally carried *Helicoverpa* spp. larvae at above threshold levels. Since then, the incidence and distribution of larvae has been gathered via the End of Season Survey of CCA members. We formally incorporated analysis of this data as a new component of this project to evaluate if it is necessary to reinstate testing survivors for Bt resistance.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

The key objectives outlined in the original proposal were achieved however in-house training of Narrabri based technical staff in molecular techniques was not conducted as part of this project since the related work proposed to CRDC was not funded (see Objective 4 in bold font).

TABLE 1: The objectives, milestones, and performance indicators of the project.

No.	Objective	Milestone	Performance Indicator
1	Provide early warning in populations of <i>Helicoverpa</i> spp. of increased frequencies in previously isolated common recessive forms of Bt resistance	Liaison with CCA to establish collaborators for collection of <i>Helicoverpa</i> spp. material throughout the cotton regions	Effective communication with CCA collaborators as evidenced by excellent collections and positive feedback from participants on the process
		Screened <i>Helicoverpa</i> spp. populations using the F1 method against classes of Bt toxins deployed in current and imminent products	Shifts in frequencies of previously isolated common recessive forms of Bt resistance rigorously assessed in <i>Helicoverpa</i> field populations
2	Detect and attempt to isolate novel dominant forms of resistance to Bt toxins in <i>Helicoverpa</i> spp.	Screened <i>Helicoverpa</i> spp. populations using the F1 method against all classes of Bt toxins deployed in current and imminent products, not just the toxin that matches the known resistant parent	Any new dominant forms of resistance to Bt identified in <i>Helicoverpa</i> populations. Endeavoured to rear all suspected resistant strains of <i>Helicoverpa</i> spp. to F3 for further characterisation
3	Explore opportunities for improving the efficacy of existing day-to-day processes by visiting mass rearing facilities to observe techniques	Visit AgBiotech to learn about specific laboratory operations that could improve efficiencies of the monitoring project	Potential opportunities for improving efficiencies of the laboratory component of the monitoring project identified
4	Depending on progress with a proposed related project, key technician to begin in-house training to prepare for the potential future use of molecular tools	Principle Investigator and Technical Officer trained by experienced CSIRO staff at Black Mountain on use of appropriate molecular tools	Resistance monitoring team at Narrabri positioned to capitalize on potential opportunities for incorporating molecular diagnostic tools into future monitoring programs
5	Assess the ongoing incidence and control of surviving <i>Helicoverpa</i> spp. larvae on Bt-cotton	Appropriate questions included in the End of Season CCA Survey, and data analysed for evidence of increasing incidence of survivors over time	Rigorous assessment of the resistance risk posed by surviving <i>Helicoverpa</i> spp. larvae on Bt-cotton
6	Improve industry stewardship of Bt technologies	Regular short articles in relevant publications, and effective and prompt communication of results to industry	Excellent feedback from stakeholders and improved interest in the program
		Work with the National Cotton D & D Team's Target Lead in Resistance Management on initiatives outlined in CRDC 12-13PRP0003B	Improved stewardship of Bt technologies by stakeholders
7	Timely submission of final report	Completion of final report	Final report submitted by 30 September 2016

Methods, Results and Discussion

Objective 1.1 – Liaison with CCA to establish collaborators for collection of *Helicoverpa* spp. material throughout the cotton regions

In collaboration with Dr Lisa Bird (NSW DPI, conventional insecticide monitoring), I liaised with the CCA to establish collaborators for collection of *Helicoverpa* spp. material in Hillston, Emerald, St George, Darling Downs, Macquarie and Mungindi. We supplied collaborators with the protocols, tools and consumables required to perform the collections.

CSIRO and NSW DPI were responsible for collecting material from McIntyre, and (in part) Mungindi. At these sites collections were made once a month from November through to February; while this was a lower total number of collections per region relative to those scouted by the CCA, the increased number of samples collected by our team per visit resulted in a similar or superior overall effort.

A detailed account of this collaboration, including the collections made within this project by CSIRO and NSW DPI, is provided in reporting for that project by the CCA.

Objective 1.2 – Screened *Helicoverpa* spp. populations using the F₁ method against classes of Bt toxins deployed in current and imminent products

In the previous versions of this project we screened *Helicoverpa* spp. populations using F₂ screens and F₁ screens. In the current project we transitioned to screening using the F₁ method only. Consequently a greater effort is being invested in using F₁ screens not only against Cry2Ab (the toxin of greatest current interest) but also against Vip3A (in *H. punctigera* and *H. armigera*) and Cry1Ac (in *H. punctigera* only since we do not have a Cry1Ac resistant strain of *H. armigera*). These screens are designed to specifically examine changes in frequencies of the common resistances that we have already isolated.

Cry1Ac

In 2013/14, 2014/15 and 2015/16 we screened 498, 394 and 828 alleles from *H. punctigera* and isolated 2, 1 and 6 cases respectively conferring resistance to Cry1Ac (see Table 2); this frequency is higher than for 2014/15. Of these alleles, two were contributed from one individual that was homozygous (rr) for resistance. For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry1Ac since 2013/14 is 9/1720 (0.005), and analyses on this data show that there has not been a significant change in frequency over time (in all cases $P > 0.05$).

TABLE 2: Summary of results from F₁ screens of *H. punctigera* against Cry1Ac. Data are presented as the frequency for that testing season. For each entry we have indicated the number of homozygous (rr) resistant individuals that contributed to the frequency.

Species	Year	Cry1Ac F ₁ screen			No. rr
		alleles tested	scored positive	Freq. of r	
<i>H. punctigera</i>	2013/14	498	2	0.004	0
	2014/15	394	1	0.003	0
	2015/16	828	6	0.007	1
	Total	1720	9	0.005	1

Cry2Ab

In 2013/14, 2014/15 and 2015/16 we screened 684, 720, and 602 alleles from *H. armigera* and isolated 10, 15 and 12 cases respectively conferring resistance to Cry2Ab (see Table 3). Of these alleles isolated in each of 2013/14 and 2014/15, two were contributed from one individual that was homozygous for resistance. For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 261/9357, and analyses on this data show that there has not been a significant change in frequency over time (in all cases $P > 0.05$).

In 2013/14, 2014/15 and 2015/16 we screened 582, 488 and 940 alleles from *H. punctigera* and isolated 5, 5 and 10 cases conferring resistance to Cry2Ab (see Table 3). Of these alleles isolated in each of 2014/15 and 2015/16, four were contributed from two individuals that were homozygous for resistance. For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 108/5588, and analyses on this data show that there has not been a significant change in frequency over time (in all cases $P > 0.05$).

Table 3: Summary of results from F_1 screens of *H. armigera* and *H. punctigera* against Cry2Ab. Data are presented as the frequency for that testing season. For each entry we have indicated the number of homozygous (rr) resistant individuals that contributed to the frequency.

Species	Year	Cry2Ab F_1 screen			
		alleles tested	scored positive	Freq. of r	No. rr
<i>H. punctigera</i>	2007/08	194	2	0.010	0
	2008/09	640	30	0.047	1
	2009/10	1138	15	0.013	1
	2010/11	358	10	0.028	0
	2011/12	736	24	0.033	3
	2012/13	518	7	0.014	2
	2013/14	582	5	0.010	0
	2014/15	488	5	0.011	2
	2015/16	940	10	0.011	2
Total	5588	108	0.019	11	
<i>H. armigera</i>	2007/08	278	9	0.032	0
	2008/09	3104	69	0.022	1
	2009/10	1710	37	0.022	0
	2010/11	1810	80	0.044	3
	2011/12	832	33	0.040	1
	2012/13	770	18	0.023	2
	2013/14	684	10	0.015	1
	2014/15	720	15	0.021	1
	2015/16	602	12	0.020	0
Total	9357	261	0.028	9	

Vip3A

In 2013/14, 2014/15 and 2015/16 we screened 642, 626 and 542 alleles from *H. armigera* and isolated 6, 10 and 6 cases conferring resistance to Vip3A (see Table 4). Of these alleles isolated in each of 2013/14, 2014/15 and 2015/16, two were contributed from one individual that was homozygous (rr) for resistance. For *H. armigera* the cumulative frequency of alleles conferring resistance to Vip3A since 2013/14 is 22/1928, and analyses on this data show that there has not been a significant change in frequency over time (in all cases $P > 0.05$).

Since 2009/10 we began F_1 screens against Vip3A in *H. punctigera*. In 2011/12, the frequency was 0.095 (7/74); since it was obtained from a relatively small sample it is excluded from the overall summary results and in analyses. In 2013/14, 2014/15 and 2015/2016 we screened 588, 414 and 882 alleles from *H. punctigera* and isolated 6, 1, and 5 cases conferring resistance to Vip3A (see Table 4). Of these alleles, in 2013/14 four were contributed from two individuals that were homozygous for resistance, and in 2015/16 two were contributed from one individual that was homozygous for resistance. Of these alleles isolated in each of 2013/14, 2014/15 and 2015/16, two were contributed from one individual that was homozygous for resistance. For *H. punctigera* the cumulative frequency of alleles conferring resistance to Vip3A since the CSIRO program began (2007/08) is 36/3484, and analyses on this data show that there has not been a significant change in frequency over time (in all cases $P > 0.05$).

Table 4: Summary of results from F₁ screens of *H. armigera* and *H. punctigera* against Vip3A. Data are presented as the final frequency for that testing season. For each entry we have indicated the number of homozygous (rr) resistant individuals that contributed to the frequency. *Note the very small sample for 2011/12 has been excluded from the total estimates.

Species	Year	Vip3A F ₁ screen			
		alleles tested	scored positive	Freq. of r	No. rr
<i>H. punctigera</i>	2009/10	1144	16	0.014	0
	2010/11	172	3	0.017	0
	2011/12	74	7	0.095	0
	2012/13	284	5	0.018	2
	2013/14	588	6	0.011	2
	2014/15	414	1	0.002	0
	2015/16	882	5	0.006	1
Total	3484	36	0.012	5	
<i>H. armigera</i>	2013/14	642	6	0.009	1
	2014/15	626	10	0.016	1
	2015/16	542	6	0.011	1
	Total	1928	22	0.012	3

2.1 – Screened *Helicoverpa* spp. populations using the F₁ method against all classes of Bt toxins deployed in current and imminent products, not just the toxin that matches the known resistant parent

In addition to screening against the toxin of interest (i.e., the toxin that is ineffective against the known resistant parent used in the screen), we have introduced screens against all classes of Bt toxins in an effort to detect any novel forms of resistance that carry dominance. This allocation of insect material to resistance testing is changed somewhat from previous years and considerable time and attention was devoted to refining the new approach and identifying efficiencies in the way the data is collected and recorded.

In 2014/15 in *H. punctigera* we screened alleles to identify new dominant resistances for Cry1Ac (n=140), Cry2Ab (n=176) and Vip3A (n=126) and found none. In *H. armigera* we screened alleles to identify new dominant resistances for Cry1Ac (n=246), Cry2Ab (n=312) and Vip3A (n=240) and found none.

In 2015/16 in *H. punctigera* we screened alleles to identify new dominant resistances for Cry1Ac (n=294), Cry2Ab (n=322) and Vip3A (n=312) and found none. In *H. armigera* we screened alleles to identify new dominant resistances for Cry1Ac (n=166), Cry2Ab (n=240) and Vip3A (n=166) and found none.

3.1 – Visit AgBiTech to learn about specific laboratory operations that could improve efficiencies of the monitoring project

In April 2014 Sharon Downes, Tom Walsh and Tracey Parker visited AgBiTech; Kristen Knight (Monsanto) accompanied the group. We were unable to tour the main virus production facility (to protect IP) but were taken through the rearing facility. Our main interest was examining the detail of the diet making and rearing process to both improve efficiencies and alleviate potential HSE issues involved with, for example, the repetitive pouring of liquid diet. The key actions from this visit were:

1. To explore the cost effectiveness of having AgBiTech make and ship diet for general rearing to ACRI;
2. To explore the cost effectiveness and practicality of an automatic liquid dispenser for pouring diet; and
3. To explore the cost effectiveness and practicality of shifting our rearing process to use trays that would enable the entire larval phase to be reared to pupae without transfer.

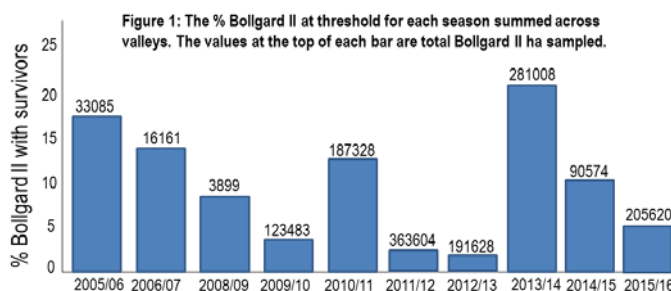
As a result of this analyses it became apparent that it was most efficient and cost-effective to continue in-house production of diet. We trialled shifting our rearing process to using trays that would enable the entire larval phase to be reared to pupae without transfer but for several reasons this method proved impractical.

In September 2015 Tracey Parker and Sharon Downes visited the laboratories of Monsanto in Toowoomba to see the operations of their automatic liquid dispenser for pouring diet. We were successful at an internal bid for CSIRO to invest \$45,000 in this infrastructure for its Narrabri facility.

5.1 – Appropriate questions included in the End of Season CCA Survey, and data analysed for evidence of increasing incidence of survivors over time

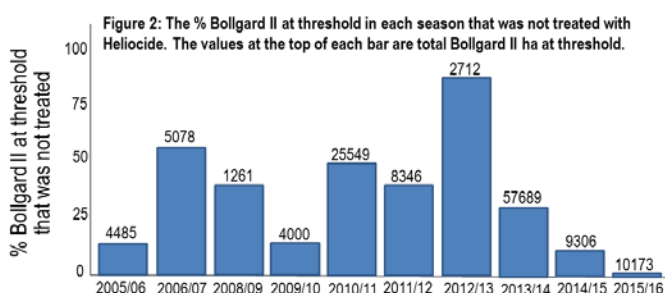
We included a modified series of questions on survivors in Bollgard II in the end of season CCA surveys in seasons 2009 to 2016. Across those years the survey included 28 to 63 CCA members and covered 50-75% of the total licensed Bollgard II[®] area. All valleys were represented. In this report the data are analysed according to climatic region – Tropical (Central Qld), Middle (Gwydir, Mungindi, Macintyre, Namoi, St George, Dirranbandi), Darling Downs, and Cool (Upper Namoi, Macquarie, Southern NSW). Bollgard II[®] was considered to carry survivors if it was at threshold: at least 2 larvae 3-8 mm/m in at least 2 consecutive checks or 1 larvae > 8mm/m.

The % Bollgard II[®] with survivors is not increasing: Across years the proportion of the Bollgard II[®] area that reached threshold ranged 3 to 21 and did not increase (Fig 1). From 2005 until 2015 the average proportion of the Bollgard II[®] area that reached threshold was 10%.



There is no trend among seasons for one region to be more likely to have Bollgard II[®] with survivors: For instance, in 2009/10 the Downs region had a percentage of Bollgard II[®] with larvae that was above the average, while in 2013/14 the Tropical region had a percentage of Bollgard II[®] with larvae that was above the average. The data across all years fit with the dataset for 2005/08 which shows variation among seasons in the proportion of Bollgard II[®] at threshold for each valley.

Most of the Bollgard II[®] with survivors was treated: The percentage of Bollgard II at threshold that was not treated with a Helicoides varies among seasons ranging 12 to 85 (Fig 2). Since 2012/13 there has been a consistent trend for increased treatment of Bollgard II at threshold.



Thresholds were equally as likely to be driven by numbers of medium-large versus small larvae: We introduced a question to distinguish if thresholds were mostly driven by the component concerning medium-large larvae vs. small larvae. This is important because past work demonstrates that a proportion of medium-large larvae can survive and pupate under Bollgard II. If surviving larvae are challenged with an insecticide, a greater proportion of medium-large larvae are likely to “escape” this application. When the data were pooled across all regions, the proportional area of Bollgard II at threshold that was determined by small larvae alone ranged 30 to 86 and there was no pattern among years or regions.

Bollgard II is sometimes sprayed for larvae below threshold: From 2009 until 2015 the proportion of the Bollgard II[®] area that did not reach the Helicoverpa threshold but was sprayed once or more for Helicoverpa larvae ranged 6 to 25.

6.1 – Regular short articles in relevant publications, and effective and prompt communication of results to industry

Please see response for Section 9.

6.2 – Work with the National Cotton D & D Team’s Target Lead in Resistance Management on initiatives outlined in CRDC 12-13PRP0003B

I worked with the National Cotton D & D Team’s Lead in Resistance Management to write several of the articles outlined in Section 9.

Outcomes

2. Describe how the project’s outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

During the project we used F₁ screens to challenge 2,010 and 1,810 alleles of *H. armigera* against Cry2Ab and Vip3A respectively and 1,720, 2,006, and 1,884 alleles of *H. punctigera* to Cfy1Ac, Cry2Ab and Vip3A respectively. This data enabled us to achieve our main planned outcome of rigorously assessing the sensitivity of field populations of Helicoverpa to Cry1Ac, Cry2Ab and Vip3A toxins to detect early signs of the development of resistance to genetically modified cotton. It was utilised in mathematical models that evaluated the risk of resistance evolving to three toxin (Cry1Ac, Cry2Ab, Vip3A) cotton and ultimately to the development of a Resistance Management Plan for Bollgard III cotton.

3. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

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

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

This project follows from previous CRDC funded projects in which we developed sensitive bioassay protocols for monitoring resistance in *Helicoverpa* spp. to Cry1Ac, Cry2Ab and Vip3A; these refined methods were utilised in this project. We did not develop any methodology or equipment specifically for the work performed herein.

c) required changes to the Intellectual Property register.

No changes to the IP register are required.

Conclusion

4. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

- Currently in *H. punctigera* 2% of individuals in the population are heterozygous (rS) for the Cry1Ac resistance gene (based on F₁ data).
- Cry2Ab resistance genes and Vip3A resistance genes were present at detectable levels before Bt cotton expressing these traits was widespread.
- F₁ data demonstrate that currently in *H. armigera* 4% of individuals in the population are heterozygous (rS) for the Cry2Ab resistance gene and there is no indication of changes in resistance frequency since testing started in 2007/08.
- F₁ data demonstrate that currently in *H. punctigera* 3% of individuals in the population are heterozygous (rS) for the Cry2Ab resistance gene and there is no indication of changes in resistance frequency since testing started in 2007/08.
- Currently in *H. armigera* 2% of individuals in the population are heterozygous (rS) for the Vip3A resistance gene (based on F₁ data).
- Currently in *H. punctigera* 2% of individuals in the population are heterozygous (rS) for the Vip3A resistance gene (based on combined F₂ and F₁ data).
- Individuals that are homozygous for the Cry1Ac allele (*H. punctigera*), Cry2Ab allele (*H. armigera* and *H. punctigera*) or Vip3A allele (*H. armigera* and *H. punctigera*) have been detected since F₁ screens commenced; these frequencies are not significantly different from what is expected based on the frequencies of heterozygotes.
- No new dominant resistances to Cry1Ac, Cry2Ab or Vip3A have been detected.
- 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.
- A survey of crop consultants suggests that the presence of medium-large larvae in Bollgard II[®] is (1) not increasing (from 2005/06 to 2015/16); (2) widespread among valleys and climatic regions; and (3) usually controlled especially recently. Thresholds are equally likely to be driven by numbers of medium-large versus small larvae. Bollgard II is sometimes sprayed for larvae below threshold.

Extension Opportunities

5. 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 Section 9).

(c) for future research.

We recently published articles in Current Opinions in Insect Science and The Australian Cottongrower (see Section 9) on our recommendations for future research around resistance monitoring.

Briefly, we first suggest continuing with the shift in focus toward known resistances. CSIRO showed that there is a single gene responsible for resistance to Cry2Ab and to Vip3A and which is unique in the two *Helicoverpa* pests of cotton, *H. armigera* and *H. punctigera*, so it makes sense to monitor frequency changes in just these specific resistances. This can be done using the more efficient F₁ screens and doing away with labour-intensive F₂ screens.

Secondly we also suggest continuing to make sure that any new dominant resistances are detected by screening F₁ families not only for known resistances but also screening them against other relevant Bt toxins. If families originate from a field parent with a new dominant resistance there would be survival in the screens with the non-focal toxin.

Thirdly, a new change is to move to intermittent screening. F₂ screens have the advantage over F₁ screens of being able to detect all types of new resistances even those that are recessive. Based on CSIRO's data it is unlikely that any new recessive resistances would rapidly increase in frequency in the current Australian landscape, at least while growers adhere to mandated management tactics and stacked toxins are used. Rather than do away completely with F₂ screens, the future monitoring program will perform them every half decade so that any new recessive resistances can be isolated and studied. There is also scope for the F₁ screens of known resistances to be performed intensely only on every alternate year.

Fourthly, we envisage a shift towards using molecular tools to assist bioassays. CSIRO has identified the gene for Cry2Ab resistance in *H. armigera* and *H. punctigera*. Although the developed markers will not identify new resistances, in conjunction with bioassays they can identify cases where the resistance is not caused by known genes. For example, a potential problem with the standard F₁ screen is that it will give the same bioassay result if one of the test insects carries two copies of a known recessive resistance or one copy of a new dominant resistance. By using molecular genetic tools CSIRO will be able to tell whether the test insect carries a known resistance gene, so that by a process of elimination it will be possible to identify any new resistances.

9. A. List the publications arising from the research project and/or a publication plan.

(NB: Where possible, please provide a copy of any publication/s)

This project is part of an ongoing monitoring effort that is integral to decision making around the development of Resistance Management Plan's for Bt cotton. This long term effort has been extended along with related information on RMP's for Bt cotton, in the following forums. Note that PDF's of most of these files have been provided previously; the exceptions are underlined and provided as part of the metadata file.

Industry

Downes S, 2013, Bt Resistance Monitoring Update. *CRDC Spotlight Magazine* Spring: 14-16

Ceeney S, Baker G, Whitehouse M, Gregg P, Tann C, Leven T, **Downes SJ**, Wilson L, 2013, Refuge Crops: investing in cotton's future. *Australian Cottongrower* Dec-Jan: 14-16

Downes SJ, Mahon R, Walsh R, James B, Ceeney S, 2013, Vip3A resistance genes exist in *Helicoverpa* before release of Bollgard III. *Australian Cottongrower* Dec-Jan 17-19

Tann C, **Downes S**, Baker G, 2014, 2013 CSD Variety Trial Book: Entomology, May, 79-81

Downes S, Worm turns for cotton pest as Australia breeds in resistance. *The Conversation* (Blog) July 2013

Baker G, Ceeney S, **Downes S**, Tann C, 2013, Pigeon pea refuges - a smart choice for resistance management. *Australian Cottongrower* Aug-Sep: 12-14

Downes SJ, 2013, Bt resistance: measuring success one season at a time. *CRDC Spotlight Magazine* Spring 2013: 18-19

Baker G, **Downes SJ**, Tann C, Ceeney S, 2013, Pigeon pea refuges: a smart choice for delaying Bt resistance. *CRDC Spotlight Magazine* Spring 2013: 21-22

Bt Tech Panel Meeting July 2014 – CSIRO Starting Frequencies

Bt Tech Panel Meeting July 2014 – Survival of SS, rS and rr

REFCOM Meeting September 2015 – CSIRO Resistance Monitoring Update

REFCOM Meeting August 2016 – CSIRO Resistance Monitoring Update

Downes SJ, Walsh R, Tay Wee Tek, 2016, The future of the industry's Bt resistance monitoring program. *Australian Cottongrower* In press

The scientific community

Wilson L, **Downes S**, Baker G, Khan M, Whitehouse M, Mass S, 2013, IPM in the transgenic era: A review of challenges from emerging pests. *Crop & Pasture Science* 64:737-749

T. K. Walsh*, **S. J. Downes***, J. Gascoyne, W. James, T. Parker, J. Armstrong, R. J. Mahon, 2014, Dual Cry2Ab and Vip3A resistant strains of *Helicoverpa armigera* and *H. punctigera*; testing linkage between loci and monitoring of allele frequencies. *Journal of Economic Entomology* 107(4). DOI: 10.1603/EC13558 [*equal first author]

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B. Have you developed any online resources and what is the website address?

No online resources have been developed from this project.

Part 4 – Final Report Executive Summary

The industry is entering a critical period in managing resistance to Bt-cotton. One technology provider is about to release their 3rd generation product which is based on the current two gene varieties (Cry1Ac, Cry2Ab) plus Vip3A. It is possible that in the near future other technology providers will enter the market. If more than one Bt-cotton product is commercial, at least in the short term, it is likely that larvae will be exposed to the same or different varieties of Bt toxins within the 3 classes that are currently included in the resistance monitoring program: Cry1A, Cry2A and Vip3A. Resistant insects are likely to tolerate different varieties of toxins within the same class, so for instance, resistance frequencies for Cry2Aa should be the same as those for Cry2Ab. The development of robust resistance management plans (RMPs) for these technologies depends critically on the frequencies of common resistances to these key classes of Bt toxins.

In this project we achieved our main planned outcome of rigorously assessing the sensitivity of field populations of *Helicoverpa* to Cry1Ac, Cry2Ab and Vip3A toxins to detect early signs of the development of resistance to genetically modified cotton. It was utilised in mathematical models that evaluated the risk of resistance evolving to three toxin (Cry1Ac, Cry2Ab, Vip3A) cotton and ultimately to the development of a Resistance Management Plan for Bollgard III cotton.

In *H. punctigera* the proportion of individuals in the population that are heterozygous (rS) for the *cry1Ac* resistance gene is 2% (based on F₁ data: 1% of alleles is equivalent to 2% of individuals). Cry2Ab resistance genes were present at detectable levels before Bollgard II[®] was widespread. In *H. armigera* the proportion of individuals in the population that are heterozygous for the *cry2Ab* resistance gene is 4%. In *H. punctigera* the proportion of individuals in the population that are heterozygous for the *cry2Ab* resistance gene is 3%. Vip3A resistance genes are present at detectable levels before Bollgard III[®] is due to be released (in 2016/17). In *H. armigera* and *H. punctigera* the proportion of individuals in the population that are heterozygous for the *vip3A* resistance gene is 2%.

Individuals that are homozygous for the Cry1Ac allele (*H. punctigera*), Cry2Ab allele (*H. armigera* and *H. punctigera*) or Vip3A allele (*H. armigera* and *H. punctigera*) have been detected since F₁ screens commenced; these frequencies are not significantly different from what is expected based on the frequencies of heterozygotes.

No new dominant resistances to Cry1Ac, Cry2Ab or Vip3A have been detected.

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 a physiological resistance to Bt toxins. A survey of crop consultants suggests that the presence of medium-large larvae in Bollgard II[®] is (1) not increasing (from 2005/06 to 2015/16); (2) widespread among valleys and climatic regions; and (3) usually controlled especially recently. Thresholds are equally likely to be driven by numbers of medium-large versus small larvae. Bollgard II is sometimes sprayed for larvae below threshold.

Moving forward we suggest continuing with the shift in focus toward known resistances and screening for any new dominant resistances. New changes include screening every other year and shift towards using molecular tools to assist bioassays.

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