An Impact Assessment of CRDC Bt Technologies Investments: July 2010 to June 2018

Final Report

To

The Cotton Research and Development Corporation

Agtrans Research

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Acknowledgments

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Abbreviations

ABCA	Agricultural Biotechnology Council of Australia
ACRI	Australian Cotton Research Institute
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bt	*Bacillus thuringiensis* (bacterium)
BCR	Benefit-Cost Ratio
BMP	Best Management Practice
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CRC	Cooperative Research Centre
CRDC	Cotton Research and Development Corporation
CRRDC	Council of Rural Research and Development Corporations
CCA	Crop Consultants Australia
Cry proteins	Crystalline proteins (also known as Cry toxins) formed from the *Bacillus thuringiensis* bacterium
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GIS	Geographic Information Systems
RS	Heterozygotes
IPM	Integrated Pest Management
MIRR	Modified Internal Rate of Return
NSW	New South Wales
NDVI	Normalized Difference Vegetation Index
OGTR	Office of Gene Technology Regulator
PVB	Present Value of Benefits
QLD	Queensland
RDC	Research and Development Corporation
RD&E	Research, Development and Extension
RMP	Resistance Management Plan
RR	Resistant homozygotes
SS	Susceptible homozygotes
TIMS	Transgenic and Insect Management Strategies

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Executive Summary

The Investment

This report presents the results of an impact assessment of a cluster of six Bt Technologies projects funded by the Cotton Research and Development Corporation (CRDC) over the years ending June 2010 to 2018. In addition to CRDC funding (a combination of statutory levies paid by industry participants and matching Commonwealth funding), other resources were provided by research organisation contributions.

Methods

The six individual projects were first analysed qualitatively within a logical framework that considered project rationale, objectives, activities/outputs, outcomes, and impacts. Project Principal Investigators made comments on, and further inputs to, these logical frameworks. Some of the impacts identified through this process were then valued in financial terms. Benefits were estimated for a range of time frames up to 30 years from the year of last investment (2017/18). Past and future cash flows, expressed in 2016/17 dollar terms, were discounted to the year 2016/17 using a discount rate of 5% to estimate investment criteria. Investment criteria reported included Present Value of Benefits, Present Value of Costs, Net Present Value, Benefit-Cost Ratio, Internal Rate of Return, and the Modified Internal Rate of Return.

Impacts

The primary impacts identified were economic in nature, however social and environmental impacts also were identified. One impact was valued in monetary terms. The decision not to value other impacts was made based on a range of factors including the difficulty linking some project outcomes to impacts, a shortage of evidence to fully support the impact, or a high degree of uncertainty limiting reasonably accurate valuation. The impact valued is deemed to represent a conservative estimate of the value of the principal benefits derived from the cluster investment.

It is expected that the Australian cotton growing industry will be a primary beneficiary of the investment. Spill-over benefits to regional communities and to other cropping industries may occur.

Investment Criteria

Total funding from all sources for all six projects totalled $12.19 million (present value terms). The benefits from the investment were valued at $103.72 million (present value terms). This gave a Net Present Value of $91.54 million, a Benefit-Cost Ratio of 8.5 to 1, an Internal Rate of Return of 38.8% and a Modified Internal Rate of Return of 12.8%.

1. Introduction

Background to Impact Assessment

In calendar 2016 and 2017 the Cotton Research and Development Corporation (CRDC) has been carrying out a series of impact assessments of some of their principal Research, Development and Extension (RD&E) investments. The primary purpose of these impact assessments is to assist with portfolio management and provide accountability to the CRDC Board, its levy paying industry and the Australian Government. The results of the impact assessments can also can be used as inputs into the development and/or assessments of further research investments.

A further purpose of the CRDC impact assessments is potentially to contribute to a process being undertaken by the Council of Rural Research & Development Corporations (CRRDC). This process aims to demonstrate the impacts (and their value) that have emerged or are likely to emerge from the 15 Rural Research and Development Corporations (RDCs) including industry-owned companies. Valuation of these impacts, along with identification of investment expenditure, is required to demonstrate the RDCs' contribution to Australian rural industry as well as environmental and social impacts to Australia.

The following impact assessment addresses investment by CRDC in a cluster six *Bacillus thuringiensis* (Bt) technologies projects.

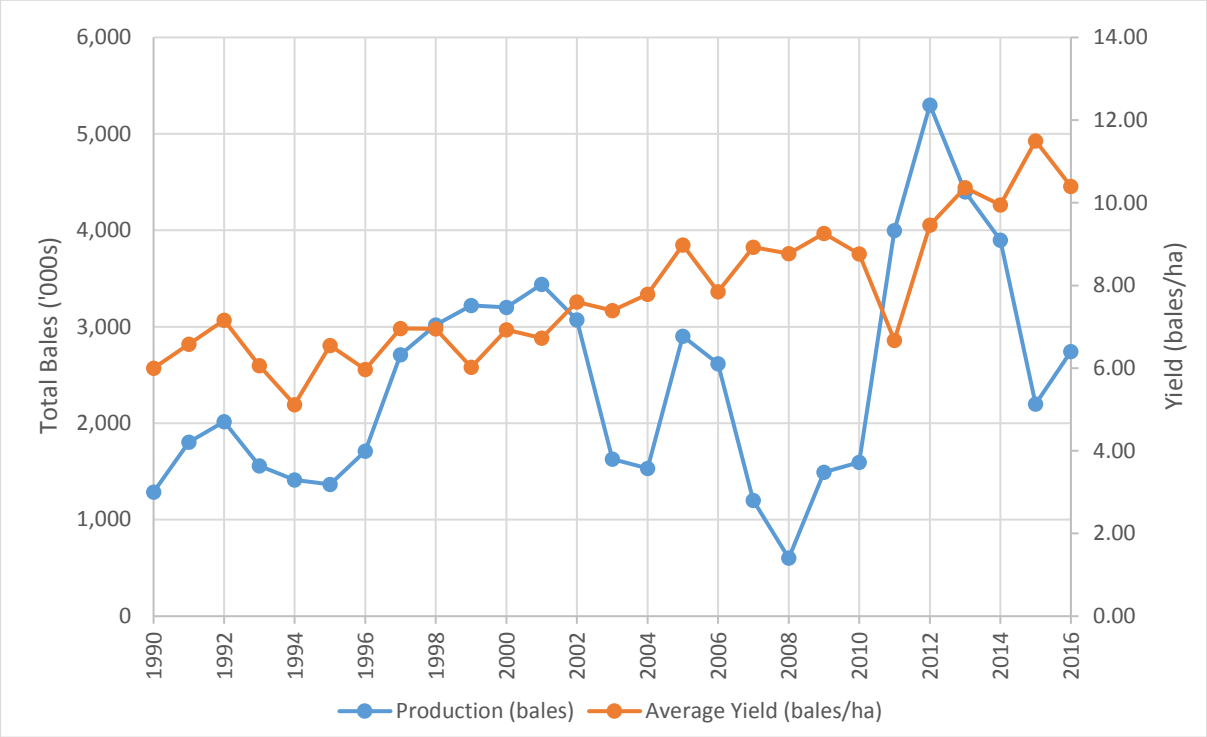
Overview of the Australian Cotton Industry

The Australian cotton industry is one of Australia's largest rural export earners and has an estimated annual average gross value of production of $2 billion. Australia produces both cotton lint and cottonseed. Cotton lint (fibre) makes up approximately 42% of picked cotton by weight and contributes about 85% of the total income from a cotton crop. The other 15% comes from cottonseed which is mostly used to make cottonseed oil and stock feed (Cotton Australia, 2016b).

Cotton is a summer crop and approximately 95% of the total area of cotton grown in Australia is grown under irrigation (Cotton Australia, 2016c). Australian irrigated lint yields are the highest of any major cotton producing country in the world and Australian cotton growers have produced, on average, an estimated 2.74 million bales of cotton lint per annum (where a standard Australian bale contains 227 kilograms of cotton lint) from an average cropping area of approximately 300,000 hectares (Cotton Australia, 2017).

Figure 1 shows annual production of cotton (in bales) and average yields (bales/ha) for the Australian cotton industry over time.

Figure 1: Australian Cotton Production for the years ended June 1990 to June 2016



Source: based on data from http://cottonaustralia.com.au/cotton-library/statistics

There are, approximately 1,200 cotton growers in Australia with about 60% of farms in New South Wales (NSW) and 40% in Queensland (QLD). The major production area in NSW stretches south from the Macintyre River on the QLD border and covers the Gwydir, Namoi and Macquarie valleys. In NSW, cotton is also grown along the Barwon and Darling rivers in the west and the Lachlan and Murrumbidgee rivers in the south.

Figure 2 shows the Australian cotton production regions on a map of eastern Australia.

Figure 2: Map of Key Cotton Production Regions in Australia

Source: Cotton Australia, 2017 URL: http://cottonaustralia.com.au/australian-cotton/basics/where-is-it-grown

Background to Bt Cotton

Bacillus thuringiensis

Bacillus thuringiensis (Bt) is a bacterium that forms characteristic, crystalline proteins. These proteins (known as Cry proteins or Cry toxins) are toxic for certain invertebrates, particularly insect larvae belonging to beetles (*Coleoptera*), flies (*Diptera*), and moths and butterflies (*Lepidoptera*). Each insecticidal Cry protein has a different physical structure and unique domain, and each protein is the product of a single gene (Monsanto Australia Ltd, 2011).

Bt cotton in Australia

In Australian, Bt genes are being used to develop genetically modified (GM), insect resistant cotton varieties. The first Australian cotton variety containing a Bt gene, known as Ingard®, was first grown commercially in 1996.

Ingard® contained a single Bt gene (Cry1Ac) and the variety was used to control two of Australian cotton's major pests, *Helicoverpa armigera* (*H. armigera*) and *Helicoverpa punctigera* (*H. punctigera*) or the cotton bollworm (ABCA, 2012).

In 2004, Bollgard II® replaced Ingard® as the transgenic variety of cotton available to Australian growers. Bollgard II® improved on Ingard® by incorporating an additional Bt

protein (Cry2Ab). The two genes (Cry1Ac and Cry2Ab) are distantly related but the toxins they encode do not share a common binding site meaning that it was unlikely that *Helicoverpa* species would have a single mechanism that could confer resistance to both toxins.

More recently, a new insect resistant variety, Bollgard 3®, with a third Bt protein (Vip3A) was released for commercial use for the 2016/17 cotton season. This third gene does not form the crystals characteristic of Cry toxins and has a different mode of action against *Lepidoptera* species.

Bt cotton and pesticide use

A review of pesticide use in Australia, carried out by the Australian Academy of Technological Sciences and Engineering (Radcliffe, 2002), estimated that the use of Ingard® had reduced the use of insecticides on Australian cotton by up to 57%. Today, more than 99% of planted cotton in Australia is transgenic and the GM cotton varieties form a key component of growers' integrated pest management (IPM) strategies. Over the last decade, growers have reduced insecticide use by 89% (Cotton Australia, 2016a).

Regulatory approval and resistance management planning for Bt cotton

In Australia, the introduction of each new genetic trait must be individually assessed on a case by case basis by the Office of Gene Technology Regulator (OGTR), Food Standards Australia New Zealand (FSANZ) and the Australian Pesticides and Veterinary Medicines Authority (APVMA).

As part of this process, Cotton Australia, through the Transgenic and Insect Management Strategies (TIMS) Committee, have developed a resistance management plan (RMP) for each proposed variety that must be reviewed and approved by the APVMA (Monsanto Australia, 2014).

The Australian cotton industry adopted a necessarily conservative RMP for Ingard® due to the critical importance of preserving the Cry1Ac gene and included restrictions on the area that could be planted. The RMP and associated area restrictions were relaxed for the introduction of Bollgard II® because of the perceived difficulty for insects to evolve resistance to both proteins within Bollgard II®.

Baseline data on the sensitivity of field populations of *H. armigera* and *H. punctigera* to Bt products (Cry1Ac) were collected prior to the widespread deployment of Ingard® in the mid-1990s and again prior to the release of Bollgard II® (for both Cry1Ac and Cry2Ab). Ongoing monitoring for resistance to Bt toxins is critical for resistance management and the sustainability of Bt technologies in Australian cotton, as well as for the planning and development of future GM cotton varieties.

The Importance of Bt Technologies Research

Resistance monitoring and other Bt related R&D provides input into the resistance management planning for Bt cotton. This research is an essential component in the regulatory approval process for each new transgenic variety in terms of data requirements as well as industry approval.

Robust Bt technologies research contributes to maintaining the efficacy and longevity of each new Bt cotton variety and therefore to the future productivity, profitability and sustainability of the Australian cotton industry.

2. Methods

The evaluation approach follows general impact assessment guidelines that are now well entrenched within the Australian primary industry research sector including RDCs, Cooperative Research Centres (CRCs) and some Universities. The impact assessment uses Cost-Benefit Analysis that entails both qualitative and quantitative approaches in accord with the CRRDC's impact assessment guidelines (CRRDC, 2014).

The assessment process commenced with the identification and a brief description of each of the six projects in terms of their objectives, activities and outputs, outcomes, and actual and/or potential impacts. The individual project outcomes and impacts were then integrated and described at the aggregate cluster level. The principal economic, environmental and social impacts at the cluster level were then summarised in a triple bottom line table.

Some, but not all, of the impacts identified were then valued in monetary terms. The decision not to value certain impacts was made based on a range of factors including the difficulty linking some project outcomes to impacts, a shortage of evidence to fully support the impact, or a high degree of uncertainty limiting reasonably accurate valuation. The impacts valued are deemed to represent a conservative estimate of the value of the principal benefits derived from investment in the cluster.

The benefits valued were then compared with the investment costs for all projects. This allowed aggregate investment criteria to be produced for the investment in the cluster of the six Bt technologies projects.

3. Description of the Projects

Table 1 provides a list of all six projects, including project codes, Principal Investigator (PI) and full project titles, defined in the population for the Bt Technologies Cluster.

Table 1: Projects Included in the Population of the Bt Technologies Cluster

Project Code	Principal Investigator	Project Title
CSE1103	Sharon Downes	Monitoring for resistance to Bt cotton
CSE1402	Sharon Downes	Monitoring to manage resistance to Bt toxins
CSE1304	Mary Whitehouse	Managing Bt resistance and induced tolerance with effective refuge crops in preparation for Bollgard III
CSE1601	Mary Whitehouse	Managing Bt resistance and induced tolerance in Bollgard 3 using refuge crops
CSE1404	Stuart Whitten	Economic risk assessment of resistance management strategies for Bt cotton
CLW1602	Tom Walsh	Travel Bursary: Nutritionally mediated susceptibility *of Helicoverpa armigera* and *Helicoverpa punctigera* to BT toxins (Cry1Ac, Cry2Ab, Vip3A)

A full description of each of the six projects is presented in Tables 2 to 7. The projects are summarised in a logical framework format (rationale, objectives, activities and outputs, outcomes and impacts).

Table 2: Logical Framework for Project CSE1103

CSE1103: Monitoring for resistance to Bt cotton	
Project details	Organisation: CSIRO Sustainable Agriculture Flagship Period: July 2010 to June 2013 Principal investigator: Sharon Downes
Background & Rationale	Cry toxins have been the primary source of proteins used to produce insect resistant cotton in Australia. Cry toxins in classes other than Cry1A and Cry 2A that have an impact of *Lepidoptera* have been identified, however only a limited number are toxic enough to be useful. Also, insects resistant to one Cry toxin may show cross-resistance to closely related toxins, further limiting the number of useful options. Vip3A is a non-Cry Bt toxin that is effective against *Lepidoptera* (including *Helicoverpa* species). This protein does not form the crystals characteristic of Cry toxins and has a different mode of action against the insects. Vip3A was stacked with Bollgard II® to create the third-generation product, Bollgard 3®. A critical component of the RMP for Bt cotton in Australia is ongoing monitoring to ensure early detection of resistance to the transgenic crop varieties in insect populations. To develop the RMP specific to Bollgard 3®, the frequency of Vip3A resistance genes in populations of *H. armigera* and *H. punctigera* needed to be determined. Project CSE1103 was funded as a continuation of the CRDC Bt Resistance Monitoring Program screening against Cry1Ac, Cry2Ab, and to establish baseline resistance data for Vip3A, for both *H. armigera* and *H. punctigera* in Australian cotton.
Objectives	The key objectives of the project were to:

	1. Provide early warning of the advent of Bt resistance in natural populations of *Helicoverpa* spp. 2. Attempt to capture any genes conferring resistance to Bt in *Helicoverpa* spp. 3. Develop sensitive bioassays for *Helicoverpa* spp. against Vip3A. 4. Establish baseline resistance in natural populations of *Helicoverpa* spp. to Vip3A. 5. In year 1 of the study, co-ordinate collection of Bollgard II® *Helicoverpa* survivors and test moths for resistance to Bt. 6. Develop sensitive bioassays for *S. litura* against Cry1Ac and Cry2Ab. 7. Screen field populations of *S. litura* for Bt resistance. 8. Improve industry confidence in, and understanding of, the program. 9. Streamline collection of material for several programs. 10. Verify that the outcome of the protocols used by CSIRO and Monsanto to set up and score these screens leads to the same scored outcomes.
Activities and Outputs	• Samples of *H. armigera* and *H. punctigera* eggs were collected from the Lower Namoi, Upper Namoi and Gwydir valleys as well as from Emerald, Darling Downs, Macquarie, MacIntyre, Lachlan and St George valleys with collaborators from Crop Consultants Australia (CCA). • Samples were collected from cotton, plus all other crops that are hosts to *Helicoverpa*. • Variation in abundances of *H. armigera* and *H. punctigera* dictated the relative proportion and number of each species sampled in each season and largely reflected differences in insect 'pressure'. • Field-collected eggs were raised individually to adults in a laboratory. Pairs (one male, one female) then were placed into containers and eggs were collected from these and subsequent generational pairings. • The project used F_2 screens against Cry1Ac (produced from Bt strain HD73), Cry2Ab (sourced from dried and ground corn leaf material) and Vip3A (cloned from *E. Coli*). • F_2 screens involved the testing of the grandchildren of the original pairs of moths (raised from the field-collected eggs) to detect heterozygote (RS) individuals and homozygous resistant individuals (RR). • The data showed that: o In both species of *Helicoverpa*, the first isolations of alleles conferring resistance were detected. However, the frequency of alleles conferring resistance to Cry1Ac remained low over time. o *H. armigera* exhibited a gradual increase over time in frequencies of Cry2Ab resistance alleles, but the increase was not statistically significant. o *H. punctigera* exhibited a gradual increase over time in frequencies of Cry2Ab resistance alleles and this increase was statistically significant. o Three cases of cross-resistance were identified during the project. One individual scored positive for Cry2Ab resistance and Cry1Ac resistance. Two individuals scored positive for Cry2Ab resistance and Vip3A resistance. o It was not possible to determine whether those families originated from individuals that carried resistance genes for both toxins or one individual that carried a resistance gene against one type of toxin mating with an individual that carried a resistance gene against another type of toxin.

	○ There was no statistically significant difference in the frequency of Vip3A resistance alleles detected over time for either *H. armigera* or *H. punctigera*. ○ In 2012/13, and prior to the release of Bollgard 3®, F_2 data indicated that 6% of *H. armigera* population are RS for Vip3A. • F_1 screens also were used against Cry2Ab (the toxin of greatest interest with respect to existing resistance risk) as F_1 screens have a greater reliability in returning precise frequencies. • F_1 screens involved the testing of the offspring of a single-pair mating between moths from a Cry2Ab resistant strain maintained in the lab. • Data from the F_1 screens indicated that: ○ There was no significant relationship between the frequency of Cry2Ab resistance alleles and time for either *H. armigera* or *H. punctigera*. ○ In 2012/13, 5% of *H. armigera* individuals in the population are RS for the Cry2Ab resistance gene which was lower than the 8% recorded for 2011/12. ○ Also, 3% of *H. punctigera* are RS for the Cry2Ab gene, which was lower than the 7% for 2011/12. ○ There was no statistically significant difference in the frequency of Vip3A resistance alleles detected over time for either *Helicoverpa* spp. ○ Data indicated that approximately 4% of the *H. punctigera* population were RS for Vip3A. However, only small samples were subjected to F_1 screening, therefore no reliable conclusions could be drawn from the F_1 data. • 912 surviving *Helicoverpa* larvae from Bollgard II® cotton fields were submitted for resistance screening in 2010/11 (first year of the project); 908 of these were *H. armigera*. • Data collected from the F_1 and F_2 larvae screens suggested that Bollgard II® survivors do occasionally carry Cry2Ab resistance genes. • In 2010/11 there was no statistically significant difference in the frequency of Cry2Ab resistance genes in *H. armigera* samples from Bollgard II® compared to those collected randomly. • As with the random sample, all but one of the Bollgard II® survivors that scored positive for Cry2Ab resistance had only one copy of the gene which means they should be killed by the toxin. • Most of the larvae that survive on Bollgard II® do not carry resistance genes for Cry1Ac or Cry2Ab and no evidence of physiological resistance was found. Hence, the active collection of larvae was discontinued as part of the Bt resistance monitoring project. • The incidence of surviving larvae in Bollgard II® crops now is monitored through an annual survey of crop consultants carried out by CCA. • Data collected from an annual survey of crop consultants (2005/06 to 2011/12) confirmed that the proportion of Bollgard II® with survivors was not increasing. • *Spodoptera litura* are known to tolerate high doses of Bt toxins and are therefore expected to survive on Bt cotton. It is possible that they will evolve to become even more resistant to the technology. • The project sought to develop sensitive bioassays for *S. litura* against Cry1Ac and Cry2Ab to establish a baseline from which any changes in response to Bt toxins could be measured. • The approach (initiated during the previous iteration of project CSE1103) was to determine a discriminating concentration for screening based on the development rates of larvae rather than mortality.

	• Screens were conducted in 2010/11 and data produced verified the preliminary concentrations (identified under the previous project) as being appropriate. • No samples were able to be obtained during 2011/12 and 2012/13, so the project team was unable to use the verified concentrations to screen field populations of *S. litura* for baseline levels of resistance. • Several tasks were undertaken to improve the Australian cotton industry's confidence in, and understanding of, the Bt resistance monitoring program. • Tasks included: contributions to science articles targeted at the cotton industry (e.g. Australian Cotton Grower Magazine, CRDC Spotlight Magazine), presentations at REFCOM and TIMS forums and various grower meetings. • The project also engaged in a side-by-side exercise with Monsanto to evaluate the compatibility in the common procedures used by both organisations to screen for Cry2Ab resistance. • The side-by-side process enabled both CSIRO and Monsanto to observe and to document the exact steps in all the lab processes used by each other to identify similarities and differences. • The exercise identified some differences that would affect lab operating efficiency and some that would affect the resistance frequencies recorded. • Differences included: (1) the threshold used to determine whether resistance is present based on the number of medium-sized larvae able to survive on the toxin, and (2) the method used to further test suspect families. • In order to pool data in the future, both labs would have to be using the same procedures and scoring method. However, procedural change from either party is unlikely. If either organisation modified their scoring procedures, they would effectively no long have a historical data set. • The Bt Technology Panel noted that it is unlikely that resistance monitoring data will be able to be pooled between the two labs in the future.
Outcomes	• The data, particularly with regard to trends in the frequencies of Bt resistance alleles (especially those conferring an advantage against Cry2Ab), were provided to TIMS to help inform the committee on the merit of recommending changes to the RMP for Bollgard II® that would enhance the longevity of the variety (and any other varieties that make use of Cry1Ac and Cry2Ab). • Information on the gradual increase over time of Cry2Ab resistance alleles in *H. punctigera* led to changes to the refuge options under the Bollgard II® RMP. This included removing maize and sorghum as refuge crop options as they are not good hosts for *H. punctigera* (S. Downes, pers. comm., 2017). • The frequency information for Cry1Ac, Cry2Ab and Vip3A were used as a guide for inputs into models that were used to assist with developing the Bollgard 3® RMP (S. Downes, pers. comm., 2017). • The Bt Resistance Monitoring Program is ongoing, activities from project CSE1103 were continued under a subsequent CRDC funded project, CSE1402: *Monitoring to manage resistance to Bt toxins*. • These ongoing resistance monitoring activities now occur every other year in line with the relatively low perceived risk by the cotton industry of resistance developing (S. Downes, pers. comm., 2017).
Impacts	• Contribution to maintained efficacy of Bollgard II® until Bollgard 3® technology became available. This would have resulted in maintained cost

	savings for Australian cotton growers through fewer insecticide sprays required, and maintained cotton average yields given seasonal insect pressure. • Contribution to a less conservative RMP for Bollgard 3® resulting in decreased refuge requirements, and more flexible pupae busting requirements leading to reduced costs and increased production for Australian cotton growers. • Contribution to the longevity of Bollgard 3®, and other current and future cotton varieties that make use of Bt toxins, through information used to develop and modify transgenic cotton RMPs. • Increased scientific and research capacity from process and knowledge sharing between CSIRO and Monsanto. • Increased grower confidence in, and understanding of, the Bt Resistance Monitoring Program leading to potentially greater industry participation in the monitoring process and faster adoption of recommended resistance management and integrated pest management strategies for Australian cotton.

Table 3: Logical Framework for Project CSE1402

CSE1402: Monitoring to manage resistance to Bt toxins	
Project details	Organisation: CSIRO Agriculture and Food Period: July 2013 to June 2016 Principal investigator: Sharon Downes
Rationale	Project CSE1402 follows on from project CSE1103 (see Table xx) and was funded as a continuation of the CRDC Bt Resistance Monitoring Program to provide ongoing screening against Bt toxins for both *H. armigera* and *H. punctigera* in Australian cotton.
Objectives	The key objectives of the project were to: 1. Provide early warning in populations of *Helicoverpa* spp. of increased frequencies in previously isolated common recessive forms of Bt resistance. 2. Detect and attempt to isolate novel dominant forms of resistance to Bt toxins in *Helicoverpa* spp. 3. Explore opportunities for improving the efficacy of existing day-to-day processes by visiting mass rearing facilities to observe techniques. 4. Depending on progress with a proposed related project, key technician to begin in-house training for the potential future use of molecular tools. 5. Assess the ongoing incidence of surviving *Helicoverpa* spp. larvae on Bt-cotton. 6. Improve industry stewardship of Bt technologies. 7. Timely submission of final report.
Activities and Outputs	• *Helicoverpa* spp. material was collected from various cotton growing areas including Hillston, Emerald, St George, the Darling Downs, Macquarie, MacIntyre, and Mungindi in collaboration with Lisa Bird (NSW Department of Primary Industries, convention insecticide monitoring) and CCA. • *Helicoverpa* spp. populations were then screened using the F_1 method only. The F_1 screens were designed to specifically examine changes in frequencies of the common resistances that had already been isolated previously. • The samples were screened against Cry1Ac (in *H. punctigera* only), Cry2Ab and Vip3A. • Analysis of data from the screening process showed:

	○ There had been no significant change in the frequency of alleles conferring Cry1Ac resistance over time for *H. punctigera*. ○ As of 2015/16, 2% of *H. punctigera* individuals in the population are RS for the Cry1Ac resistance gene. ○ For both *H. armigera* and *H. punctigera* there was no significant change in the frequency of alleles conferring Cry2Ab resistance over time. ○ 4% of *H. armigera* and 3% of *H. punctigera* individuals in the population are RS for the Cry2Ab resistance gene. ○ For both *H. armigera* and *H. punctigera* there was no significant change in the frequency of alleles conferring Vip3A resistance over time. ○ 2% of *H. armigera* and 2% of *H. punctigera* individuals in the population are RS for the Vip3A resistance gene. ○ Frequencies of individuals that are RR for the Cry1Ac, Cry2Ab, or Vip3A allele are not significantly different from what was expected based on the frequency of RS individuals. • *Helicoverpa* spp. also were screened against all classes of Bt toxins deployed in current and imminent products, rather than just the toxin that matched the known resistant parent. • This meant that, in addition to screening against the toxin of interest, screens against all classes of Bt toxins were conducted in an effort to detect any novel forms of resistance that carry dominance. • Screening was carried out in 2014/15 and 2015/16. No new forms of dominant resistances for Cry1Ac, Cry2Ab and Vip3A were identified in *H. armigera* and *H. punctigera.* • In April 2014, the project team, accompanied by a representative from Monsanto, toured the rearing facility at AgBiTech in Toowoomba. • The team examined details of the AgBiTech diet making and rearing processes to explore ways to improve efficiency and reduce health and safety issues in the Bt resistance monitoring research. • From this examination, the project team confirmed that it was most cost-effective to continue in-house production of diet. • In September 2015, the project team also visited the labs of Monsanto in Toowoomba to see operation of their automatic liquid dispenser for pouring diet. • As a result, the project team were successful with an internal bid that had CSIRO invest in similar infrastructure for its Narrabri facility. • The dispenser is not yet operation (as of September 2017) as the lab in which it will be housed is undergoing renovations. The investment will likely improve efficiency and potentially address health and safety concerns about overuse injuries in the lab (S. Downes, pers. comm., 2017). • Data from the CCA annual survey were analysed for evidence of increasing incidence of survivors for Bollgard II® over time. The analysis found that: ○ the proportion of Bollgard II® with survivors is not increasing, ○ there is no trend among seasons for one growing region to be more likely to have Bollgard II® with survivors, ○ most of the Bollgard II® with survivors was treated with a Heliocide, ○ thresholds were equally as likely to be driven by numbers of medium-large versus small larvae, and ○ Bollgard II® is sometimes sprayed for larvae below threshold. • The project also contributed regular articles in relevant cotton publications, a number of scientific journal article, as well as giving presentations at the Bt Tech Panel meeting in 2014 and the REFCOM meetings in 2015 and 2016.

	• An article published in Current Opinions in Insect Science (Downes, Walsh, & Tay, 2016) recommended a shift in the focus of the Bt resistance monitoring research toward known resistances and suggested that screening continues for new, dominant resistances for all known Bt toxins. • Also, it was recommended that the Program should move to intermittent screening for F_2 screens, performing them every half decade so that any new recessive resistances can be isolated and studied. • Finally, it was envisaged that there would be a shift towards using molecular tools to assist bioassays. By using genetic tools researchers will be able to tell whether a test insect carries a known resistance gene, so that it will be possible to identify new resistances.
Outcomes	• The data, particularly with regard to trends in the frequencies of Bt resistance alleles (especially those conferring an advantage against Cry2Ab), were regularly provided to TIMS to help inform the committee on the merit of recommending changes to the RMP for Bollgard II® that would enhance the longevity of the variety (and any other varieties that make use of Cry1Ac and Cry2Ab). • The data were also used to inform the development of a robust RMP for Bollgard 3® which was subsequently approved. Bollgard 3® was successfully released, and first grown commercially in the 2016/17 season with a less conservative RMP than was used for Bollgard II®. • The Bt Resistance Monitoring Program is ongoing, activities from project CSE1402 were continued under a subsequent and still current CRDC funded project, CSE1701: *Resistance research and monitoring to enhance stewardship of Bt cotton and management of Helicoverpa spp*.
Impacts	• Contribution to maintained efficacy of Bollgard II® until Bollgard 3® technology became available. This would have resulted in maintained cost savings for Australian cotton growers through fewer insecticide sprays required, and maintained cotton average yields given seasonal insect pressure. • Contribution to a less conservative RMP for Bollgard 3® resulting in decreased refuge requirements, and more flexible pupae busting requirements leading to reduced costs and increased production for Australian cotton growers. • Contribution to the longevity of Bollgard 3®, and other current and future cotton varieties that make use of Bt toxins, through information used to develop and modify transgenic cotton RMPs. • Increased scientific and research capacity from process and knowledge sharing between CSIRO and Monsanto. • Increased grower confidence in, and understanding of, the Bt Resistance Monitoring Program leading to potentially greater industry participation in the monitoring process and faster adoption of recommended resistance management and integrated pest management strategies for Australian cotton.

Table 4: Logical Framework for Project CSE1304

CSE1304: Managing Bt resistance and induced tolerance with effective refuge crops in preparation for Bollgard III	
Project details	Organisation: CSIRO Agriculture Period: July 2012 to June 2015 Principal investigator: Mary Whitehouse

Rationale	Moths of the genus *Helicoverpa* are known as the most destructive pests in Australian cotton. In particular, the species *H. armigera* had been observed to quickly develop resistance (within five to eight years) to almost every insecticide used in its control. To slow *H. armigera* developing resistance to Bt cotton, an RMP has been put in place for each Bt cotton variety prior to its commercial release. A key tool of the RMPs is the use of refuges that help maintain the efficacy of Bt cotton by producing unselected, susceptible *Helicoverpa* moths that mate with potentially resistant moths emerging from the Bt crops, thereby slowing the development of resistance to the Bt toxins. For refuges to counter genetic resistance and potential inducible tolerance to Bt toxins they must work optimally on farms and produce as many susceptible moths as possible. Project CSE1304 was funded to test the likelihood that inducible tolerance could occur in field crops of Bt cotton (and, if so, if refuges could reduce that risk), and also to provide information on best management practices (BMPs) to improve refuge governance and develop better monitoring techniques.
Objectives	The overall aim of the project was: • To improve the ability of refuges to counter the threat of *Helicoverpa* by: ensuring refuge models assumptions are robust; testing that if inducible tolerance can flourish in the field, it can be countered by refuges; & encouraging on-farm refuges to perform optimally by improving refuge management. The project was made up of three interconnected parts. The project's objectives were to answer the following questions within the three component parts. <u>1. Testing genetic assumptions of underlying refuge models.</u> (i) Are the same number of RR moths/m found in Bt crops as in refuges, & is the ratio of RS to SS moths emerging from Bt crops & refuges the same? (ii) Do moths mix between nearby Bt crops & refuges, but less over larger distances? <u>2. Inducible tolerance & its potential mitigation by refuges</u>. (i) Can Helicoverpa develop tolerance to Cry2ab, Cry1Ac & VIP3A concurrently? (ii) Can Helicoverpa develop tolerance on glasshouse grown Bollgard II plants producing low levels of Bt toxin? (iii) Can tolerance be arrested by stopping exposure by some individuals to Bt toxin for one or more generations? (iv) Do Helicoverpa field survivors have inducible tolerance to Bt toxins? (v) Do the offspring of moths emerging from Bt crops & refuges have different levels of tolerance to Bt toxin? (vi) Are there genetic differences between laboratory & field tolerant Helicoverpa? <u>3. Optimizing on-farm refuge governance.</u> (i) Develop refuge crop agronomy to enhance moth productivity. (ii) Benchmark the ability of refuges to attract and produce moths. (iii) Review and test the minimum optimum width for refuges.

	(iv) Promote refuge governance. (v) Produce a simple refuge effectiveness calculator within the myBMP system.
Activities and Outputs	***Comparing Field Moth Emergences*** • In the three seasons of the project (2012/13 to 2014/15), moths were gathered from two sites, the Australian Cotton Research Institute (ACRI) at Narrabri and a commercial farm in the Namoi. In the third season (2014/15) samples were also collected on a commercial farm at Emerald. • Emerging moths were collected using White Cages from Bt cotton and its associated refuges across the sites. • For each moth, the project team recorded its species and the location in the field where it emerged. • Over the course of the project 472 *Helicoverpa* moths (alive and deceased) were collected (106 *H. armigera*, 365 *H. punctigera* and 1 other species) from the two primary sites. • Analysis of the project data indicated that 39 of the 472 *Helicoverpa* sampled had emerged from Bt cotton at the ACRI and Namoi sites and that there was no pattern to when *H. armigera* and *H. punctigera* are more likely to emerge out of cotton crops. • It also was found that, while there is no difference in the likelihood that a *H. armigera* or *H. punctigera* will emerge out of Bt cotton in any given year, the numbers of *Helicoverpa* found in Bt cotton were much higher than expected based on existing resistance estimates. • The project also sampled 1,257 other moths. Four species were identified and analysed from this collection: *Earias hueyeliara* (rough bollworm), *Mythimna loreyimima* (surgarcane army worm), *Endotricha puncticotalis*, and *Athetis tenuis*. • It was found that rough bollworm was controlled to a similar extent as *Helicoverpa*. The sugarcane army worm is less susceptible to the Bt toxins and had similar numbers in the Bt as the non-Bt cotton. The other moths were in significantly higher proportions in Bt cotton. ***Screening for Resistance and Induced Tolerance*** • All live moths collected in the field (about half of the 472 sampled) were taken back to the lab to form families. • 69 families were developed following the Bt Resistance Monitoring Program's standard protocol. • The resulting neonates were then either subjected to F_2 bioassays to test for resistance to the Cry1Ac and Cry2Ab genes, or sent to Adelaide for tolerance testing on full-dose response toxin bioassays. • The F_2 screens of larval families found two *H. armigera* and two *H. punctigera* tested positive for Cry2Ab resistance. Similar screens of the moth families found one *H. armigera* family tested positive for Cry1Ac and six families (four *H. armigera*, two *H. punctigera*) tested positive for Cry2Ab resistance. • Analysis of the screening results indicated that there was no significant difference in the ratio of RS moths on Bt cotton and non-Bt refuges for either species. • The tolerance testing showed that *Helicoverpa* developed tolerance to Cry1Ac within 10 generations. Tolerance to Cry2Ab developed in a similar manner but populations exposed to Cry1Ac gained higher levels of tolerance.

	• *H. punctigera* from the field had higher levels of tolerance to Cry1Ac than Cry2Ab. In the *H. armigera* bioassays there was no difference in levels of tolerance to either toxin. • The project also found that, irrespective of strains and toxins, tolerance levels dropped gradually to about half to one quarter of that of parental strains when subsequent generations were raised toxin-free. This suggests that the induced tolerance is reversible. • PhD Scholarship student, Sharna Holman, also demonstrated that tolerance to Bt cotton was significantly higher in the offspring of larvae exposed to Cry1Ac toxin as late instar larvae (3rd instar onwards) but not smaller larvae (neonates to 3rd instar). • Genetic testing was carried out on the majority of the collected moths and larvae (that reached maturity). Specimens were tested for HaR01 and HpR01 (the known Cry2Ab resistance alleles) using specific primers to sequence the particular gene containing the allele. • Results indicated that only one moth (*H. punctigera*) was likely to be carrying the resistance R01 gene. ***Testing the Relatedness of* Helicoverpa *moths*** • Further genetic analysis was undertaken to estimate relatedness between *H. armigera* and *H. punctigera* populations from separate field sites (ACRI and the commercial farm in the Namoi, 36km apart) during the first two seasons of the project. • The population program 'Genepop' (http://genepop.curtin.edu.au) was used to infer population differentiation between ACRI and the commercial farm populations based on allele frequencies from EPIC-PCR[1] markers. • A total of 21 *H. armigera* and 27 *H. puctigera* were genotyped using EPIC-PCR markers. • Findings suggested that moths from Bt cotton and its associated refuges across both sites were highly related (more than full-sibling). • This supports the view that moths from Bt cotton and non-Bt cotton readily mate, and that families mix over at least 40km. This may have ramifications for the spread of resistance genes as the mating of closely related *Helicoverpa* could result in a concentration of resistance genes. However, the cause of the high degree of relatedness was unclear. ***Larval Movement Between Bt Crops and Refuges*** • Comparative analysis of the toxin concentrations of different parts of Bt cotton plants was undertaken. • Results showed that, at the end of January (towards end of season), all structures tested (pollen and anthers, petals, boll skins, and boll internals) had lower concentrations of Cry1Ac than leaf samples. • The finding was also true for Cry2Ab toxin except for petals, which were found to have a higher concentration of Cry2Ab toxin. • Several experiments were conducted to examine the behaviour (including egg laying and movement) of *Helicoverpa* larvae. • Bt cotton plots were set up in between pigeon pea and non-Bt cotton plots • The project found that the moths showed a clear preference for laying eggs on pigeon pea and that, within the pigeon pea crop, there was no preference to lay either at the field edge, middle or near the Bt cotton edge. • The data indicated that the presence of an attractive pigeon pea crop did not increase the likelihood that the Bt plot would have a higher egg lay.

[1] EPIC-PCR: emulsion, paired isolation, and concentration polymerase chain reaction

	• The results of the larval movement experiments showed that 3rd to 4th instar larvae are unlikely to move within the crop if they don't need to. Also, larvae move more readily on pigeon pea as opposed to Bt cotton. • The results suggested that refuges need to maintain the 24-row width originally set out in the existing Bollgard II® RMP. • Further, refuges should be in a different field, or separated by a clear gap (e.g. road or track), to Bt cotton to discourage movement of larvae between the refuge and the Bt cotton. ***Comparing the Attractiveness of Bt Cotton and its Refuges*** • The project continued the work of previous projects, comparing the relative attractiveness of Bt cotton and its refuges, thereby benchmarking actual performance of refuges on commercial farms. • Each season, for the duration of the project, commercial fields were tested in both January and February. Crops (Bt cotton and associated refuges) were sampled and data were recorded including: plant height, number of plants, bud/squares, flowers, and pods/bolls. The sample was then visually searched for *Helicoverpa* eggs and grubs. • It was found that pigeon pea crops tended to attract more egg lays than cotton refuges or Bt cotton crops and that pigeon pea became more attractive later in the season. • However, pigeon pea was not consistently more attractive to *Helicoverpa* and therefore it was not performing its role within the RMP as well as expected. • The project suggested that pigeon pea are not diluting resistance genes as expected, but in their existing form, could be acting more like a trap crop. • The existing Bollgard II® RMP suggested growers maintain their refuge crop until the next season following a Genetic Dilution model. However, the project findings suggest that ploughing in the refuges may be the best approach (known as Seasonal Quarantining). ***Testing the Ability of Satellite Imagery to Identify Bt Cotton and Refuge Attractiveness Characteristics*** • During the 2013/14 and 2014/15 growing seasons, experiments were conducted to test the ability of remote sensing (using satellite, GIS image capture) to identify characteristics of Bt cotton and its refuges. • The studies were carried out on 100 square kilometres of a cotton growing region near Narrabri which included ACRI. • Images were captured and analysed in collaboration with Peter Verwey (NSW Department of Primary Industries) using the programs ArgGIS desktop and ArgGIS Pro. • Three types of analyses of cotton field crops were undertaken: 1. The relationship between high resolution satellite imagery and nutrients, water stress, and yield; 2. The relationship between high resolution satellite imagery and egg lays and vegetative measurements; and 3. Comparing fine-scaled to course scale imagery. • Analyses 2 and 3 above were also undertaken for pigeon pea crops. • The analyses included calculating the Normalised Difference Vegetation Index (NDVI), which is a measure of photosynthetic activity. • Results of the analyses support the findings of other studies that showed that, while NDVIs are useful for assessing quality and productivity in small uniform plots, they are less reliable for comparisons at the large scale.

	• The results showed that satellite imagery may not be useful for short-term analysis (whether, at any point in time, a refuge is underperforming) but may be useful for the long-term assessment of a crop's health. ***Extension/Communication*** • The project included several extension/communication activities such as reports to growers, conference presentations and talks/presentations to cotton groups and the TIMS committee. • The project produced a large number of scientific articles and other publications to support dissemination of the project findings. • The project team found it was difficult to attract growers to listen to the messages promoting refuge governance and that, while growers acknowledge the need to produce refuges to maintain the efficacy of Bt cotton, they see the threat as long-term, and the success of the RMPs to date may have made them complacent. • The project team worked with members of the myBMP team to improve the information in myBMP on refuge maintenance. However, results from project CSE1304 indicated that refuges may be operating in a manner different to that expected. • It was suggested that the preliminary findings be further tested for validation before any changes are made to myBMP.
Outcomes	• Findings from the project, particularly with regard to row spacing and location/separation of Bt refuge crops, were provided to TIMS to help inform the committee on the merit of recommending changes to the RMP for Bollgard II® that would enhance the longevity of the variety. • The data were also used to inform the development of a robust RMP for Bollgard 3®. • The Bt Resistance Monitoring Program, including R&D related to Bt toxin tolerance and refuge performance, is ongoing. Activities from project CSE1304 were continued under a subsequent CRDC funded project, CSE1601: *Managing Bt resistance and induced tolerance in Bollgard 3 using refuge crops*.
Impacts	• Contribution to maintained efficacy of Bollgard II® until Bollgard 3® technology became available. This would have resulted in maintained cost savings for Australian cotton growers through fewer insecticide sprays required, and maintained cotton average yields given seasonal insect pressure. • Contribution to a less conservative RMP for Bollgard 3® resulting in decreased refuge requirements, and more flexible pupae busting requirements leading to reduced costs and increased production for Australian cotton growers. • Contribution to the longevity of Bollgard 3®, and other current and future cotton varieties that make use of Bt toxins, through information used to develop and modify transgenic cotton RMPs. • Increased scientific and research capacity.

Table 5: Logical Framework for Project CSE1601

CSE1601: Managing Bt resistance and induced tolerance in Bollgard 3 using refuge crops	
Project details	Organisation: CSIRO Agriculture Period: July 2015 to June 2018 Principal investigator: Mary Whitehouse

Rationale	Project CSE1601 follows on from project CSE1304 (see Table 4) and was funded to continue key research on the effectiveness of refuges for managing resistance to Bt toxins in *Helicoverpa* spp. and to continue investigations on induced tolerance to Bt toxins given the release of Bollgard 3®.
Objectives	The aim of the project was to expand on the work from CSE1304 from Bollgard II® to Bollgard 3®. In particular, the project's objectives were to: 1. Record moth emergence from Bollgard III crops & associated refuges in the Lower Namoi and Emerald to quantify the proportion of moths in this system that have been exposed to Bt toxins. 2. Quantify differences in moth emergences from Bollgard II and Bollgard III crops. 3. Record moth emergence from unstructured refuges to better estimate the proportion of moths from these refuges in cotton. 4. Test if the influx of C4 moths occurs throughout the industry and is consistent over several seasons. 5. Promote and quantify good refuge management practices by quantifying the output of well managed refuges and poorly managed refuges. 6. Continue identifying the level of resistance and tolerance to Cry1Ac and Cry2Ab toxins in the F_2 generation of *Helicoverpa* moths caught emerging from Bollgard II, Bollgard III and pigeon pea.
Activities and Outputs	• During the first season of the project (2015/16), emerging *Helicoverpa* were collected, counted and analysed using White Cages set up in six different crops in the Lower Namoi and Emerald cotton regions. • Crop types included: Bollgard 3®, Bollgard II®, non-Bt cotton, irrigated pigeon pea, un-irrigated pigeon pea, and an unstructured refuge crop. • A stock route that runs past the ACRI facility was selected as the unstructured refuge for sampling in each season of the project. • Information on the herbaceous coverage present for the samples taken from the unstructured refuge also was obtained. • Six pheromone traps were used to collect additional *Helicoverpa* samples from various sites that were then prepared for future C3/C4 analysis[2]. • Results from the 2015/16 season indicate that there is no clear difference in the distribution of *H. armigera* and *H. punctigera* over the season. • Findings to date showed that moths emerged from the unstructured refuge early in the season. It was thought that the productivity of this type of refuge is linked to rainfall, and therefore it was more productive early in the season. • The project also found that there was little difference in the number of moths recorded emerging from the unmanaged pigeon pea crop versus the irrigated crop (however, it was noted that the area benefited from heavy rain in January and therefore the unmanaged pigeon pea was not under water stress). • The work to date indicated that, while refuges should be as close to the cotton crop as possible, they should not be so close that larvae can walk from pigeon pea directly into cotton. • Also, findings suggest that refuge crops (pigeon pea and non-Bt cotton) should not be destroyed when the Bt cotton crop is destroyed, but maintained as a trap crop at the end of the season.

[2] The plants that moths consumed as larvae can be classified using stable isotope analysis as either C3 plants (e.g. cotton, pigeon pea) or C4 plants (e.g. sorghum, maize, daises).

	• To increase the profile of refuges, the project is preparing a submission in support of a “Bt stewardship” award for each major cotton growing region. • Several conference presentations, articles in grower publications and scientific papers have been produced.
Outcomes	• Project CSE1601 is ongoing, however, the project team were in the process of finalising papers that present the data to support an argument to the TIMS committee for new recommendations for best practice in terms of refuge management. • The suggested changes include the location and distance of a refuge with respect to the Bt cotton crop and the maintenance of refuges at the end of the season as trap crops.
Impacts	• Contribution to the maintained efficacy and longevity of Bollgard 3®, and other current and future cotton varieties that make use of Bt toxins, through information used to develop and modify transgenic cotton RMPs. • Increased scientific and research capacity.

Table 6: Logical Framework for Project CSE1404

CSE1404: Economic risk assessment of resistance management strategies for Bt cotton	
Project details	Organisation: CSIRO Land and Water Period: May 2014 to June 2016 Principal investigator: Russell Gorddard
Rationale	CRDC had decided that, as Bt cotton and its RMP approached two decades of use, it would be useful to assess the costs and benefits of the Bt RMP to provide input into a strategic review of the Bt Resistance Management Program and to rekindle discussion on the economic merits of the RMP within the cotton industry. Project CSE1404 was funded to conduct the economic assessment.
Objectives	The project had the following objectives: 1. Estimate the economics of the RMP for Bt cotton to inform the TIMS committee and wider cotton industry in the lead up to full commercial uptake of BG3. 2. Help develop a shared understanding among industry of the implications for the RMP of uncertainties in the development of resistance, future gene technologies, and variation across the industry. 3. Assess the economic implications of uncertainties on the value of the RMP and therefore the robustness of the economics of risk management. 4. Identify the key drivers of values (scenarios, model parameters, RMP choices) to inform research priorities, extension/communication and RMP policy development. 5. Develop the capacity for integrated bio-economic analysis of new pest management issues and strategies. 6. Demonstrate the novelty and quality of the research and results by scientific publication.
Activities and Outputs	• A simple framework for evaluating and communicating economic trade-offs with regard to the Bt cotton RMP was developed. • The framework was used to structure grower workshops and formed the basis for a more detailed bio-economic model of resistance management. • The workshops were used to estimate the costs and benefits of the RMP and to calculate the delay in resistance required for the RMP to break even.

	• The framework then was used to explore how different economic, ecological and agronomic factors affect optimal resistance management. • Spreadsheet based simulation models were used to investigate how the 'time to failure' for Bt cotton varieties responded to assumed changes in refuge area and pupae busting. • The simulation models showed that the optimal management strategy for Bt cotton resistance management was constant for a wide variation in some of the key uncertainties (e.g. refuge area, level of resistance, and the value of Bt cotton). • The analysis considered the marginal revenue from growing extra refuge (taken as the profit from the extra area of cotton that could be grown because resistance build up is delayed) and marginal cost of refuge. • Using the developed modelling framework, the project found that the profit-maximising strategy for management of resistance in Bt cotton involves providing enough refuge to maintain susceptible populations, and including extra refuge as a safety margin. • The project findings supported the results of a previous study that concluded that pest populations should be monitored in both Bt and refuge fields, and that the proportion of non-Bt plants should be increased immediately if pest populations become small, regardless of whether resistance is detected. • The project results indicated that, if the annual cost of delaying resistance by an extra year is constant or increasing with increasing refuge size, economical management is likely to involve adjusting refuge area to balance rising marginal costs and decreasing marginal benefits. • Two different models were used to examine the effectiveness of additional refuge (safety margin) in delaying resistance build up. • Findings from a simulation model suggested that growing an extra hectare of refuge delays resistance enough to permit an extra hectare of cotton to be grown. That is, adding refuge results in cotton production being delayed but does not change the total area that can be grown before resistance build up. • A simple mathematical model, on the other hand, found that the benefit-cost ratio (BCR) of additional refuge is always less than one. • Findings to date were presented at the 2016 conference for the Australian Agricultural and Resource Economics Society.
Outcomes	• The project provided a simple and robust analysis of the optimal resistance management strategy for Bt cotton. The results are being developed into simple messages to help inform discussion about the RMP strategy. • Project findings regarding the marginal value of additional refuge areas (safety margin) were provided to the TIMS committee as part of the review process for the Bollgard 3® RMP approval.
Impacts	• Contribution to a less conservative RMP for Bollgard 3® resulting in decreased refuge requirements, and more flexible pupae busting requirements leading to reduced costs and increased production for Australian cotton growers and other current and future cotton varieties that make use of Bt toxins. • Increased scientific and research capacity.

Table 7: Logical Framework for Project CLW1602

CLW1602: Nutritionally mediated susceptibility of *Helicoverpa armigera* and *Helicoverpa punctigera* to 3 BT toxins (Cry1Ac, Cry1Ab, Vip3A)	
Project details	Organisation: CSIRO Land and Water Flagship Period: June 2016 to August 2016 Principal investigator: Tom Walsh
Rationale	Project CLW1602 was a travel bursary for a student exchange. The purpose of the travel was for Ashley Tessnow (PhD student from Texas, U.S.A.) to work with Tom Walsh to conduct experiments at CSIRO to determine if patterns of nutritionally mediated susceptibility to Bt toxins that had been reported in previous studies for *Helicoverpa zea* were also present in *H. armigera* and *H. punctigera*.
Objectives	The aim of the project was to enable CSIRO to replicate work conducted at Texas A&M in the U.S.A. on *Helicoverpa zea*.
Activities and Outputs	• Ashely Tessnow flew to Australia to conduct experiments with Tom Walsh at CSIRO's Black Mountain site. • The student conducted bioassays on *H. armigera* and *H. punctigera* insect lines using Bt toxins to compare the toxicity of the insecticidal proteins with different ratios of protein and carbohydrate. • The study found that there was no difference in nutritional regulation between resistant and susceptible genotypes of either *H. armigera* or *H. punctigera*. • The results of the experiments also showed that, when both *H. armigera* and *H. punctigera* are allowed to feed at their self-selected intake target, both species are less susceptible to the Cry1Ac and Cry2Ab toxins. • In contrast, it was found that macronutrient intake had no effect on either species' susceptibility to the Vip2A toxin. • A paper was produced reporting the project findings.
Outcomes	• The project findings have potential implications for monitoring resistance in Australia but further research was considered necessary. • The work contributed to building a collaborative relationship with Dr Greg Sword (Professor at Texas A&M, U.S.A. – A. Tessnow's supervisor) and his lab to facilitate international research on Bt resistance.
Impacts	• Minor contribution to the maintained efficacy and longevity of Bollgard 3®, and other current and future cotton varieties that make use of Bt toxins, through information used to improve resistance monitoring in Australian cotton. • Increased scientific and research capacity.

4. Cluster Investment

The Investment

The following tables show the annual investment by project for both CRDC (Table 8) and for researchers and any other investors (Table 9). Table 10 provides the total investment by year from both sources.

Table 8: Investment by CRDC for Years Ending June 2011 to June 2018
(nominal $)

Project ID	2011	2012	2013	2014	2015	2016	2017	2018	Total
CSE1103	496,791	300,643	320,761	0	0	0	0	0	1,118,195
CSE1402	0	0	0	428,101	450,390	462,353	0	0	1,340,844
CSE1304	0	0	450,883	462,657	474,341	0	0	0	1,387,881
CSE1601	0	0	0	0	0	230,481	253,916	259,921	744,318
CSE1404	0	0	0	48,695	146,331	144,581	0	0	339,607
CLW1602	0	0	0	0	0	6,000	1,000	0	7,000
Totals	496,791	300,643	771,644	939,453	1,071,062	843,415	254,916	259,921	4,937,845

Table 9: Investment by Researchers and Others for Years Ending June 2011 to June 2018
(nominal $)

Project ID	2011	2012	2013	2014	2015	2016	2017	2018	Total
CSE1103	406,022	334,753	341,771	0	0	0	0	0	1,082,546
CSE1402	0	0	0	395,690	406,433	417,519	0	0	1,219,642
CSE1304	0	0	403,468	528,633	552,505	0	0	0	1,484,606
CSE1601	0	0	0	0	0	206,689	254,362	261,993	723,044
CSE1404	0	0	0	35,781	97,889	94,515	0	0	228,185
CLW1602	0	0	0	0	0	0	0	0	0
Totals	406,022	334,753	745,239	960,104	1,056,827	718,723	254,362	261,993	4,738,023

Table 10: Total Investment in the Cluster of Six Projects
(nominal $)

Year ending 30 June	CRDC	Researchers and Others	Total
2011	496,791	406,022	902,813
2012	300,643	334,753	635,396
2013	771,644	745,239	1,516,883
2014	939,453	960,104	1,899,557
2015	1,071,062	1,056,827	2,127,889
2016	843,415	718,723	1,562,138
2017	254,916	254,362	509,278
2018	259,921	261,993	521,914
Totals	4,937,845	4,738,023	9,675,868

Program Management and Extension Costs

For CRDC investment, the cost of managing the CRDC funding was added to the CRDC contribution via a management cost multiplier (1.1325); this was estimated based on the average reported share of ‘employee benefits’ & ‘supplier’ expenses in total CRDC expenditure for 2014/15 and 2015/16 (CRDC, 2016).

No additional costs of extension were included as most projects were either largely extension-focussed, already included an extension component or provided input to the TIMS committee (which includes a high level of grower representation and involvement).

5. Impacts

Five potential impacts for the Bt Technologies cluster were assembled from the logical frameworks developed for the individual projects. Some projects delivered multiple impacts. Table 11 summarises the key potential impacts identified and signifies whether a contribution was made to each potential impact by each of the six projects.

Table 11: Contribution by Project to Principal Bt Technologies Cluster Impacts

Project Code	Increased cotton production from a less conservative RMP for Bollgard 3®	Maintained efficacy of Bollgard II® and/ or Bollgard 3®	Enhanced resistance management for Bt Cotton	Increased scientific research capacity	Regional community income spill-overs
CSE1103	✓	✓	✓	✓	✓
CSE1402	✓	✓	✓	✓	✓
CSE1304	✓	✓		✓	✓
CSE1601	✓	✓		✓	✓
CSE1404	✓			✓	✓
CLW1602			✓	✓	

From Table 11, the potential impacts then were condensed and described in a triple bottom line context. Table 12 provides a summary of the principal types of impacts divided into economic, environmental and social categories.

Table 12: Triple Bottom Line Categories of Principal Impacts from the Bt Technologies Cluster Investment

Economic	• Increased profits from increased cotton production as a result of a less conservative RMP for Bollgard 3® allowing less refuge area to be planted. • Delayed development of resistance to Bt toxins thereby maintaining the efficacy of Bollgard II® and Bollgard 3® through robust resistance monitoring and RMP development.
Environmental	• Maintained environmental outcomes from the maintained efficacy of Bt cotton varieties allowing growers to use less insecticides.
Social	• Increased scientific research capacity. • Increased regional community well-being from the spill-over effects to the community of increased farm productivity and profitability.

Public versus Private Benefits
Many of the benefits identified in this evaluation are cotton industry related and therefore are considered private benefits. Also, one environmental benefit and two indirect social benefits have been delivered including increased spill-overs to local communities.

Distribution of Impacts along the Supply Chain
Most impacts (economic, environmental and social) are concentrated at the cotton farm or regional cotton community level. Some of the financial benefits at the farm level will likely be passed along the input and output supply chains in proportion to the elasticities of supply and demand at different stages along the chain.

Impacts on other Industries
Some project outputs (e.g. monitoring resistance of pests) are not necessarily specific to cotton and could be beneficial to other crop industries in cotton areas or on cotton producing farms.

Impacts Overseas
Overseas benefits are not expected to be significant, as most research outputs apply to Australian cotton production environments.

Match with National Priorities
The Australian Government's Science and Research Priorities and Rural RD&E priorities are reproduced in Table 13. The cluster contributes primarily to Rural RD&E Priorities 2 and 4, and to Science and Research Priority 1.

Table 13: Australian Government Research Priorities

Australian Government	
Rural RD&E Priorities (est. 2015)	**Science and Research Priorities (est. 2015)**
1. Advanced technology 2. Biosecurity 3. Soil, water and managing natural resources 4. Adoption of R&D	1. Food 2. Soil and Water 3. Transport 4. Cybersecurity 5. Energy and Resources 6. Manufacturing 7. Environmental Change 8. Health

Sources: (DAWR, 2016) and (OCS, 2015)

6. Valuation of Impacts

Impacts Valued
Analyses were undertaken for total benefits that included future expected benefits. A degree of conservatism was used when finalising assumptions, particularly when a high degree of uncertainty was involved. Sensitivity analyses were undertaken for those variables where there was greatest uncertainty or for those that were identified as key drivers of the investment criteria.

One of the impacts identified in Table 12 was valued. Five of the six projects in the population were identified as contributing to this impact. The impact valued was:

- Increased profits from increased cotton production as a result of a less conservative RMP for Bollgard 3® allowing less refuge area to be planted.

Impacts Not Valued
Not all impacts identified in Table 12 could be valued in the assessment. The social impacts identified but not valued included:

- Increased scientific and research capacity
- Increased community well-being through the spill-over effects of increased farm productivity and profitability

The environmental impact identified but not valued was:

- Maintained environmental outcomes from the maintained efficacy of Bt cotton varieties allowing growers to use less insecticides.

The economic impact identified but not valued was:

- Delayed development of resistance to Bt toxins thereby maintaining the efficacy of Bollgard II® and Bollgard 3® through robust resistance monitoring and RMP development.

Reasons for choosing not to value these impacts included time and resources available, the availability of accurate baseline data and the uncertain relationships between the research outputs, outcomes and impacts.

In particular, the economic impact associated with delayed development of resistance to Bt toxins was not valued because of the difficulty in forming robust assumptions on the rate of future resistance development given the introduction of Bollgard 3® (including a third Bt toxin) and the uncertain relationships between (1) the Bt technologies cluster investment and any potential changes in resistance development, and (2) development of resistance and potential future cotton productivity impacts.

Benefit Valued: Increased profits to Australian Bt cotton growers
Refuge areas paired with Bt cotton crops are an essential component of the RMP for Bt varieties. However, the refuge crop areas are generally not productive as the crop is usually destroyed as part of the resistance management/ pest management process.

Refuge requirements for the first Bt variety Ingard® were necessarily conservative to protect the use of the original Cry1Ac protein. The RMP for Bollgard II® was less conservative but still required a refuge area of 5% for unsprayed pigeon pea and 10% for unsprayed non-Bt cotton relative to the area of Bt cotton grown.

The information/data produced by the Bt technologies cluster investment was used by the Insecticidal Transgenic Crops (Bt) Technical Panel to provide advice to the TIMS committee

on the scientific merit of the proposed resistance management strategies for the Bollgard 3® RMP including the refuge areas (Ceeney & Leven, 2014).

The resistance frequency information was just one of the key inputs into models used to develop the Bollgard 3® RMP. Other inputs include how well the Vip3A toxin kills insects and how that efficacy changes throughout a season, as well as whether there were costs associated with insects being resistant to each toxin (S. Downes, pers. comm., 2017).

Bollgard 3®'s RMP was approved by the TIMS committee and subsequently by the APVMA with smaller refuge requirements than Bollgard II®. Refuge requirements for Bollgard 3® are now 2.5% for unsprayed pigeon pea and 5% for non-Bt cotton relative to the area of Bt cotton grown. Bollgard 3® was first grown commercially with its accompanying RMP in the 2016/17 season.

The reduced refuge area requirements mean a greater area of productive, Bt cotton can be planted meaning increased productivity and profits for Australian Bt cotton growers.

Specific details of the assumptions for the estimated benefit are provided in Table 14.

Counterfactual
A considerable amount of industry and Monsanto-funded research is carried out under permit to generate data and respond to key objectives for the evaluation of the Bollgard 3® RMP (Cotton Australia, 2014). It was assumed that, without the investment in the Bt technologies cluster, only Monsanto generated data would have been available for the development of the RMP and therefore the Bollgard 3® RMP would likely have been more conservative, in line with the refuge requirements of Bollgard II®.

Summary of Assumptions
A summary of the specific assumptions made for valuation of the impacts is shown in Table 14.

Table 14: Summary of Assumptions

Variable	Assumption	Source
Average annual cotton area	341,087 ha	20-year average area planted 1997 to 2016, Cotton Australia, 2017
Proportion of cotton area planted to Bt cotton varieties	95% (approximately 324,032 ha)	Conservative estimate based on data from Cotton Australia, 2016b
Proportion of Bt cotton area paired with pigeon pea refuge	80% (approximately 259,226 ha)	Grundy, Chauhan, & Knight, 2017
Proportion of Bt cotton area paired with non-Bt cotton area	20% (approximately 64,806 ha)	
Average cotton yield	9.4 bales / ha	10-year average, 2007 to 2016, Cotton Australia, 2017
Average Australian cotton price	$511 per bale	Cotton Australia, 2016b
Without Investment: more conservative RMP		
Original refuge area requirement under the Bollgard II® RMP – assumed similar refuge areas to continue for Bollgard 3® under a more conservative RMP	pigeon pea: 5% non-Bt cotton: 10% (relative to the area of Bt-cotton grown)	Baker, Ceeney, Downes, & Tann, 2013

Total crop area planted (Bt-cotton plus required refuge)	Bt paired with pigeon pea: 272,869 ha	259,226 / (1 – 5%)
	Bt paired with non-Bt: 72,007 ha	64,806 / (1 – 10%)
Total non-productive crop area (refuge area planted)	Pigeon pea: 13,643 ha	272,869 ha – 259,226 ha
	Non-Bt cotton: 7,201 ha	72,007 ha – 64,806 ha
With Investment: less conservative RMP		
First year of impact	2017	First year of commercial planting for Bollgard 3® with accompanying RMP
Refuge area requirement under the Bollgard 3® RMP	pigeon pea: 2.5% non-Bt cotton: 5% (relative to the area of Bt-cotton grown)	(Monsanto Australia Ltd, n.d.)
Total crop area planted (Bt-cotton plus required refuge)	Bt paired with pigeon pea: 265,873 ha	259,226 / (1 – 2.5%)
	Bt paired with non-Bt: 68,217 ha	64,806 / (1 – 5%)
Total non-productive crop area (refuge area planted)	Pigeon pea: 6,647 ha	265,873 ha – 259,226 ha
	Non-Bt cotton: 3,411 ha	68,217 ha – 64,806 ha
Additional area of commercial cotton grown	10,786 ha per annum from 2018 onward	(13,643 – 6,647) + (7,201 – 3,411) (pigeon pea) (non-Bt cotton)
Risk Factors/Attribution		
Bt Technologies cluster investment input attribution factor given data and knowledge sharing with Monsanto	50%	Agtrans Research
Attribution of the contribution of cluster investment outputs to the decision making and development of the Bollgard 3® RMP	30%	
Future probability of impact given assumed release of further Bt cotton varieties	50% from 2029	Based on release of Bollgard II® 12 years after original Bt-variety Ingard® and assumed continued development of Bt cotton varieties by Monsanto

7. Results

All past costs and benefits were expressed in 2016/17 dollar terms using the Implicit Price Deflator for GDP (ABS, 2016). All benefits after 2016/17 were expressed in 2016/17 dollar terms. All costs and benefits were discounted to 2016/17 using a discount rate of 5%. A reinvestment rate of 5% was used for estimating the Modified Internal Rate of Return (MIRR). The base analysis used the best available estimates for each variable, notwithstanding a level of uncertainty for many of the estimates. All analyses ran for the length of the investment period plus 30 years from the last year of investment (2017/18) to the final year of benefits assumed.

Investment Criteria

Tables 15 and 16 show the investment criteria estimated for different periods of benefits for both the total investment and for the CRDC investment respectively. The present value of benefits (PVB) attributable to CRDC investment only, shown in Table 16, has been estimated by multiplying the total PVB ($103.72 million) by the CRDC proportion of real investment (54.1%).

Table 15: Investment Criteria for Total Investment in the Six Projects
(Discount rate 5%, Re-investment rate 5%)

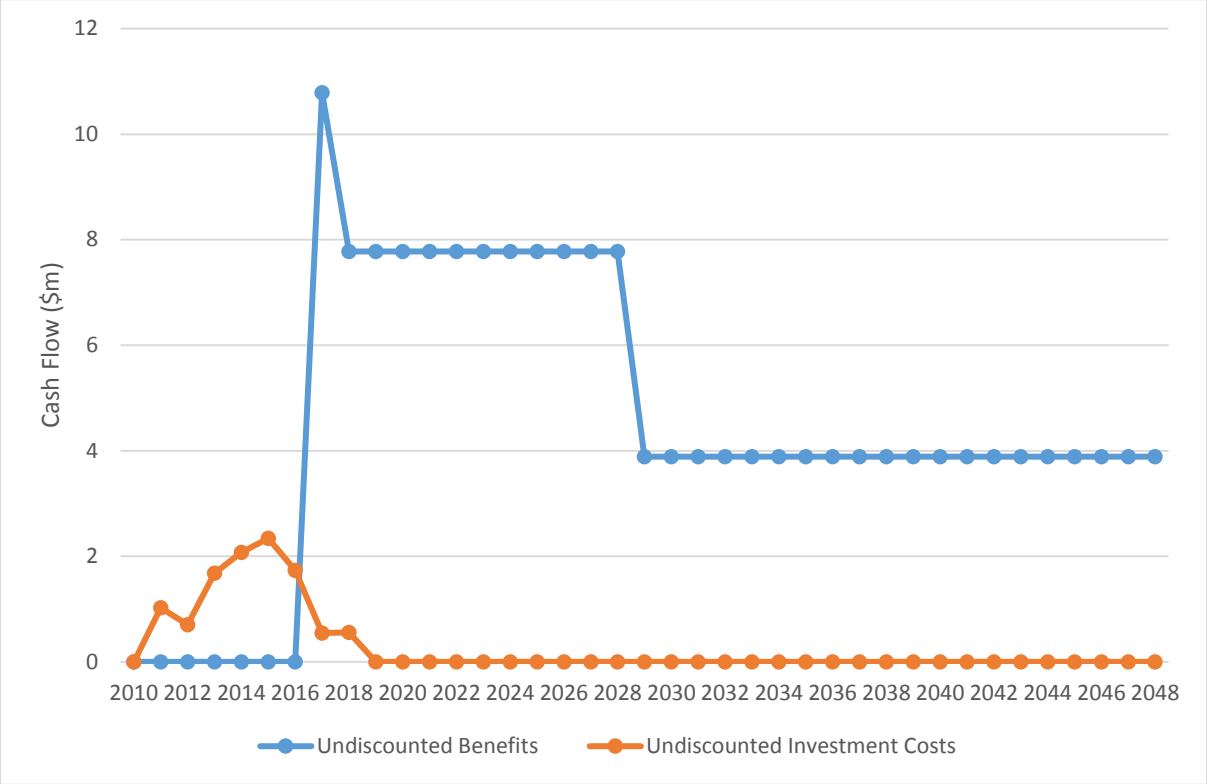
Investment Criteria	**Years from last year of investment**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Present Value of Benefits ($m)	18.19	50.26	75.39	85.23	92.94	98.99	103.72
Present Value of Costs ($m)	12.19	12.19	12.19	12.19	12.19	12.19	12.19
Net Present Value ($m)	6.01	38.07	63.20	73.05	80.76	86.80	91.54
Benefit-Cost Ratio	1.49	4.12	6.19	6.99	7.63	8.12	8.51
Internal Rate of Return (%)	18.04	36.55	38.57	38.74	38.77	38.78	38.78
Modified Internal Rate of Return (%)	61.74	34.54	24.80	19.16	16.11	14.17	12.81

Table 16: Investment Criteria for CRDC Investment in the Six Projects
(Discount rate 5%, Reinvestment rate 5%)

Investment Criteria	**Years from last year of investment**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Present Value of Benefits ($m)	9.85	27.22	40.82	46.15	50.33	53.60	56.17
Present Value of Costs ($m)	6.60	6.60	6.60	6.60	6.60	6.60	6.60
Net Present Value ($m)	3.25	20.62	34.22	39.55	43.73	47.00	49.57
Benefit-Cost Ratio	1.49	4.12	6.18	6.99	7.63	8.12	8.51
Internal Rate of Return (%)	17.98	36.43	38.45	38.62	38.65	38.66	38.66
Modified Internal Rate of Return (%)	61.58	34.50	24.78	19.15	16.10	14.16	12.80

The annual undiscounted benefit and cost cash flows for the total investment for the duration of investment period plus 30 years from the last year of investment are shown in Figure 3.

Figure 3: Annual Cash Flow of Undiscounted Total Benefits and Total Investment Costs in the Bt Technologies Project Cluster



Sensitivity Analyses

A sensitivity analysis was carried out on the discount rate. The analysis was performed for the total investment and with benefits taken over the life of the investment plus 30 years from the last year of investment. All other parameters were held at their base values. Table 17 presents the results. The results showed a moderate sensitivity to the discount rate.

Table 17: Sensitivity to Discount Rate
(Total investment, 30 years)

Investment Criteria	**Discount rate**		
	0%	**5% (base)**	**10%**
Present value of benefits ($m)	174.11	103.72	72.90
Present value of costs ($m)	10.65	12.19	13.95
Net present value ($m)	163.46	91.54	58.95
Benefit-cost ratio	16.34	8.51	5.22

An additional sensitivity analysis was conducted on the two key attribution factors as they were observed to be key drivers of the investment criteria. Investment criteria for various scenarios for the attribution factors are provided in Table 18. The investment criteria show a moderate to high sensitivity to the assumed attribution factors.

Table 18: Sensitivity to Change in Attribution of Benefits
(Total investment, 30 years, 5% discount rate)

Investment Criteria	**Attribution of Benefits to Bt Technologies R&D Investment**		
	1. 25% 2. 10%	**Base 1. Monsanto Input Attribution: 50% 2. Investment Input to RMP Attribution: 30%**	**1. 75% 2. 50%**
Present value of benefits ($m)	17.29	103.72	259.31
Present value of costs ($m)	12.19	12.19	12.19
Net present value ($m)	5.10	91.54	247.12
Benefit-cost ratio	1.42	8.51	21.28

Confidence Ratings

The results produced are highly dependent on the assumptions made, many of which are uncertain. There are two factors that warrant recognition. The first factor is the coverage of benefits. Where there are multiple types of benefits it is often not possible to quantify all the benefits that may be linked to the investment. The second factor involves uncertainty regarding the assumptions made, including the linkage between the research and the assumed outcomes.

A confidence rating based on these two factors has been given to the results of the investment analysis (Table 19). The rating categories used are High, Medium and Low, where:

High: denotes a good coverage of benefits or reasonable confidence in the assumptions made

Medium: denotes only a reasonable coverage of benefits or some uncertainties in assumptions made

Low: denotes a poor coverage of benefits or many uncertainties in assumptions made

Table 19: Confidence in Analysis of Cluster

Coverage of Benefits	**Confidence in Assumptions**
Medium-High	Medium

Coverage of benefits was assessed as medium-high as the impact valued was considered the most significant and direct impact of the investment. While some impacts were not valued, these were subjectively assessed as being minor relative to those valued.

Confidence in assumptions was rated as medium as some of the assumptions made, particularly those related to attribution and risk, were uncertain.

8. Conclusions

The six projects in the Bt Technologies Cluster were all either completed or substantially completed and all produced knowledge relevant to Bt resistance monitoring and management issues at both cotton farm and industry levels. Impacts were identified that addressed both farm productivity and maintained environmental outcomes.
Funding for the six projects in the cluster totalled $12.19 million (present value terms) and produced aggregate total expected benefits of $103.72 million (present value terms). This gave a net present value of $91.54 million, a benefit-cost ratio of 8.5 to 1, an internal rate of return of 38.8% and a modified internal rate of return of 12.8%.

References

Agricultural Biotechnology Council of Australia. (2012). *GM Cotton in Australia: a resource guide.* Melbourne: Agricultural Biotechnology Council of Australia.

Australian Bureau of Statistics. (2016, October 28). *5204.0 - Australian System of National Accounts 2015-16.* Retrieved from Australian Bureau of Statistics: http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/5204.02015-16?OpenDocument

Baker, G., Ceeney, S., Downes, S., & Tann, C. (2013, August-September). Pigeon pea refuges - a smart choice for resistance management. *The Australian Cotton Grower*, pp. 12-14.

Ceeney, S., & Leven, T. (2014, June-July). World's-Best Science: The basis of the Bollgard III RMP. *The Australian Cotton Grower*, pp. 18-20.

Cotton Australia. (2016a). *Biotechnology and Cotton*. Retrieved from Cotton Australia: http://cottonaustralia.com.au/cotton-library/fact-sheets/cotton-fact-file-biotechnology

Cotton Australia. (2016b). *Economics of Cotton in Australia*. Retrieved March 2017, from Cotton Australia: http://cottonaustralia.com.au/cotton-library/fact-sheets/cotton-fact-file-the-economics-of-cotton-in-australia

Cotton Australia. (2016c). *Cotton Annual 2016: Australian Cotton Industry Statistics.* Mascot, NSW: Cotton Australia.

Cotton Australia. (2017). *Statistics*. Retrieved March 2017, from Cotton Australia: http://cottonaustralia.com.au/cotton-library/statistics

Cotton Australia Ltd. (2014, September 2). *TIMS Committee Meets To Consider Bollgard III(R) Resistance Management Plan*. Retrieved from Cotton Australia - News/Media: http://cottonaustralia.com.au/news/article/tims-committee-meets-to-consider-bollgard-iii-resistance-management-plan

Cotton Research and Development Corporation. (2016). *CRDC Annual Report 2015-2016.* Narrabri: Cotton Research and Development Corporation. Retrieved from http://www.crdc.com.au/sites/default/files/pdf/CRDC%20Annual%20Report%202015-16.pdf

Council of Rural Research and Development Corporations. (2014). *Impact Assessment Guidelines.* Canberra: Council of Rural Research and Development Corporations. Retrieved from http://www.ruralrdc.com.au/wp-content/uploads/2016/04/CRRDC-Impact-Assessment-Guidelines-V.1-070514.pdf

Department of Agriculture and Water Resources. (2016, July). *Rural Research, Development and Extension Piorities*. Retrieved from Department of Agriculture and Water Resources - Research and Innovation: http://www.agriculture.gov.au/ag-farm-food/innovation/priorities

Downes, S., Walsh, T., & Tay, W. (2016). Bt resistance in Australian insect pests. *Current Opinions in Insect Science, 15*, 78-83.

Grundy, P., Chauhan, Y., & Knight, K. (2017, December 2016 - January 2017). A new era dawns for Bollgard 3 refuges. *The Australian Cotton Grower*, pp. 22-24.

Monsanto Australia Ltd. (2011). *Bollgard II(R) cotton - Technical Manual.* Melbourne: Monsanto Australia Ltd.

Monsanto Australia Ltd. (2014). *Bollgard 3 Industry Update.* Retrieved from Cotton Seed Distributors: http://www.csd.net.au/assets/bg3-0a3426b4e7e5e071f39e349a5e70d822.pdf

Monsanto Australia Ltd. (n.d.). *Bollgard 3(R) Refuge Planning Guide: Get The Most Out Of Your Refuge.* Melbourne: Monsanto Australia Ltd.

Office of the Chief Scientist. (2015). *Revised Queensland Science and Research Priorities.* Brisbane: Queensland Government. Retrieved from http://www.chiefscientist.gov.au/wp-content/uploads/STRATEGIC-SCIENCE-AND-RESEARCH-PRIORITIES_181214web.pdf

Radcliffe, J. C. (2002). *Pesticide use in Australia.* Melbourne: Australian Academy of Technological Sciences and Engineering.