A.

January, August & Final Reports

Part 1 - Summary Project Details

REPORTS

Please use your TAB key to complete parts1,2,4 & 5

CRDC Project Number: **CSE72C**

January Report: ☐ Due 29-Jan-01

August Report: ☐ Due 03-Aug-01

Final Report: ☒ Due within 3 months of project completion

Project Title: Resistance to Bt toxins in heliothine pests of cotton

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Part 3 – Final Report Format

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

1. **Outline the background to the project.**
   Bacillus thuringiensis (Bt) is the most widely used microbial insecticide and currently provides the only useful insecticidal genes for transgenic crops. The development of resistance to Bt detected in some lepidopterous pests (McGaughey, 1992, Moar et al. 1994, Müller-Cohn et al. 1994) is a major threat to strategies for protecting crops without contributing to the environmental damage resulting from the use of chemical insecticides. This project contributes to maintaining the value of Bt in crop protection by providing basic information and developing techniques that will be required for management of resistance to Bt in pest insects. The subject of the project is a major pest of cotton in Australia, Helicoverpa armigera, that has developed resistance to chemical insecticides. The use of a Bt toxin gene in transgenic cotton for the control of H. armigera in Australia has raised concerns about this pest developing resistance to Bt. This project sought to estimate the capacity of H. armigera for developing resistance to a Bt toxin, to examine the possibility of cross-resistance to other Bt toxins, to elucidate the mechanism(s) underlying resistance to Bt in H. armigera, and to identify methods for breaking or countering resistance.

   Bt is a Gram positive, spore-forming bacterium that produces large quantities of insecticidal toxin as a protein crystal. Formulations of the bacterial spore and toxin have been used for over 40 years to control lepidopteran pests and represent over 80% of the biological pesticides market. Bt formulations are specific for specific target pests and, as it is safe for beneficials, Bt does not lead to outbreaks of secondary pests (e.g. mites). Its lack of toxicity for mammals, fish and non-target invertebrates makes it a very useful substitute for synthetic chemical insecticides in insect pest control.

   The protein nature of the Bt insecticidal toxins provides an alternative to spraying Bt for controlling pests because plants can be genetically engineered to produce a Bt toxin within the plant tissue (Perlak et al. 1990, Voisey et al. 1994). The Australian cotton industry has embraced this technology in the form of INGARD™ which contains the cry1Ac gene (Peacock and Llewellyn, 1996). However, the loss of insecticidal activity in mid- to late season transgenic cotton (Ferguson, 1996) presents a serious problem as it provides the basis for selection for resistance to Cry1Ac.

   Toxin Specificity.
   There is a considerable variety of toxin genes among the >80 serotypes of Bt with more than 120 classes of toxin gene recognised. As host specificity is determined by part of the amino acid sequence, the toxin classes vary in their effectiveness for particular hosts; Cry1, Cry2 and Cry9 toxins are effective against Lepidoptera, Cry2 and Cry4 against Diptera, and Cry3 against Coleoptera. Within these major groupings there are also some significant differences. For example, although Cry1Ab and Cry1Ac have comparable activities against the tobacco hornworm, Manduca sexta, their activities against another caterpillar, Mamestra brassicae, vary by an order of magnitude (Milne et al., 1990). Helicoverpa species are generally less susceptible to Bt toxins than

most caterpillar pests, including Heliothis virescens; H. armigera is susceptible only to Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab at levels that are achievable in transgenic cotton (Akhurst and Liao, 1996).

Resistance to Bt

Despite expectations to the contrary, resistance to Bt has developed readily in field, as well as laboratory, strains of several important pests, including H. virescens and the diamondback moth, Plutella xylostella (McGaughey, 1992). The development of resistance has been both rapid and substantial. McGaughey and Beeman (1988) reported 2- to 29-fold resistance in 3 generations of selection of a laboratory colony of the Indian meal moth, Plodia interpunctella, with >250-fold increase in one colony over 36 generations. Laboratory selection of a resistant field population of Plu. xylostella produced a further 15- to 30-fold increase (LC50 was 430-820 times that for a susceptible strain) in 9 generations (Tabashnik et al., 1991). In H. virescens resistance in a lab-selected strain has reached 10,000-fold (Gould et al. 1995).

Studies of the mechanism of resistance to Bt show that resistance can develop by alterations at the binding site on the gut membrane. Resistance in a laboratory-selected population of Plo. interpunctella was shown to result from changes in the binding affinity of the toxin for the receptors on the brush border membrane (Van Rie et al., 1990). For some chemical insecticide resistance, laboratory-selection results in a different resistance mechanism than field-selection does (Roush and McKenzie, 1987). However, the Bt resistance mechanism detected in laboratory-selected Plo. interpunctella was the same as that in field-selected Plu. xylostella (Ferré et al., 1991).

Change in binding affinity may not be the only determinant of resistance. Wolfersberger (1990) found that the binding affinities of Cry1Ab and Cry1Ac were inversely related to their toxicities for gypsy moth, Lymantria dispar. Gould et al. (1991) found alterations in the feeding behaviour of H. virescens that could contribute to the development of resistance. Resistance to Cry1A and to Cry1C in other lepidopteran species have also been shown to arise independently of changes in binding site (Estada and Ferré, 1994; Moar et al. 1994). Since the Bt protoxin is activated by proteases in the insect gut, the possibility of resistance arising from changes in gut proteases has also been proposed (Johnson et al., 1990). Oppert et al. (1996) has demonstrated that resistance in Plo. interpunctella can be associated with a change in gut proteases.

Managing Resistance

Cross-resistance can be a significant problem in managing insecticide resistance. When Plo. interpunctella was selected for resistance to DiPel, a commercial formulation of Bt toxins and spores, it did not increase resistance (it actually became less resistant) to Cry1C, which is not a component of DiPel and which recognises a different binding site (Van Rie et al., 1990). However, when H. virescens was selected against a single Cry1A toxin (Cry1Ac), it also developed resistance to the Cry1B, Cry1C and also to Cry2A toxins (Gould et al., 1991). It is uncertain whether this broader cross-resistance arises from the insect species selected or from selection against a single toxin rather than the multiple toxins used in the selection of Plo. interpunctella.

Although the lack of cross-resistance in Plu. xylostella might allow management of resistance by variation of the toxins, the slow restoration of susceptibility in Plu. xylostella after discontinuation of selection pressure indicates that rotation of toxins may not be effective for managing resistance in this pest (Tabashnik et al., 1991). Stability of resistance after relaxation of selection pressure was also recorded in H. virescens (Stone et al., 1989).

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2. **List the project objectives and the extent to which these have been achieved.**

This project aimed to assist the protection of cotton from heliothine pests through its contribution to the development of a resistance management strategy. Specifically, it sought to determine the mechanisms of resistance that develop in H. armigera and to determine the potential for cross-resistance to other Bt toxins. It also aimed to test for resistance breaking synergists.

The project has been successful in establishing a strain of H. armigera (BX) that has a high level of resistance to the Cry1A toxins and to INGARD cotton. The resistance has been shown to be associated with the loss of a specific binding site for the Cry1Ac toxin. Evaluation of cross-resistance potential showed that the BX strain is resistant to other Cry1A toxins but not to Cry2Aa or Cry2Ab. Evidence of resistance breaking synergy has been found but the basis for the synergy is yet to be elucidated.

The BX strain has been provided to other CRDC projects for genetic analysis. It is also being used to identify new genes that can be used to complement Cry1Ac in transgenic cotton.

3. **How has your research addressed the Corporations three outputs: Sustainability of natural resources, profitability and competitiveness, and/or people and communities?**

The project primarily contributes to sustainability and to people/communities by providing data required to sustain transgenic cotton as the mainstay of insect pest management in cotton. Characterisation of the BX strain provided invaluable data on allele frequency, genetic dominance, and impact on insect fitness that contribute significantly to the refinement of the refuge model for resistance management.

4. **Detail the methodology and justify the methodology used.**

Selection

Neonates of the BX strain were reared for 7d on an artificial diet containing 1.4ug/ml of Cry1Ac in spore/crystal mixture prepared from the HD73 strain of Bt. The largest insects were them transferred to toxin-free diet to complete development. This dosage killed <10% in the 7d exposure but represented a significant challenge as many more died subsequently and many survivors did not reproduce. Bioassays were conducted every third generation to monitor resistance development. Initially, bioassays were conducted using a diet incorporation method. However, as resistance increased the high demand for Cry1Ac made this impractical and a surface contamination assay was used. All assays were conducted with 24 neonates for each of seven dosages and were scored at 7d; each bioassay was replicated three times. LC50 was estimated by probit analysis using POLO PC software. Resistance ratio was calculated by the formula:

$$RR = LC50\ BX\ strain / LC50\ ANGR\ strain$$

Once appreciable resistance was obtained (generation 16), bioassays were conducted every generation and the selection dosage was increased to 14ug/ml (g18) and then to 42ug/ml of Cry1Ac (g27). At generation 17 the population was split and half was maintained under selection and the other half without selection.

On the premise that the decline in RR observed in the BX strain might be the result of inbreeding and loss of overall fitness, this strain was outcrossed to broaden its genetic base. The IS strain was established by crossing BX that completed larval development on INGARD plants (see below) with ANGR. This new strain was selected at ever increasing dosages. The IS strain was outcrossed to ANGR, re-selected and outcrossed again several times to establish nearly isogenic resistant and susceptible strains for better genetic and molecular analyses. Changes in resistance in IS were monitored by bioassay using the method described above for the BX strain.

After 10 generations of selection, the IS strain was tested for homozygosity. Single pair matings were conducted and the F1 generation exposed to a dose of Cry1Ac (4000ng/cm2) that kills all ANGR and very few IS individuals. It should therefore discriminate between the homozygous susceptible and homozygous resistant individuals.

The VicRATS and RATS populations selected by Dr Neil Forrester were crossed with ANGR, a susceptible laboratory strain to establish two other strains (VX and RX, respectively). The VX strain was selected against a spore/crystal mixture of strain HD73 and RX was selected against MVP I, a commercial product containing encapsulated Cry1Ac. These strains were difficult to maintain and were outcrossed to ANGR several times in efforts to stabilise the cultures.

Characterisation

Cross-resistance was tested by bioassay of the BX and a susceptible strain (ANGR) with Cry1Ab, Cry2Aa and Cry2Ab as well as the commercial products DiPel, XenTari and MVP I. A surface contamination method was used for these bioassays. For each Bt toxin preparation, 24 neonates were exposed to each of seven dosages and the results were scored at 7d; each bioassay was replicated three times. LC50 was estimated by probit analysis using POLO PC software. The susceptibility of IS to Cry1Ab, Cry2Aa, Cry2Ab, DiPel and Xentari was similarly tested.

We tested the hypothesis that the mechanism of resistance of action was a change in the specific binding site for Cry1Ac on the brush border membrane of H. armigera. Brush border membrane vesicles were prepared by the differential magnesium precipitation method of Wolfersberger et al. (1987). Cry1Ac crystals were purified on a sucrose gradient, dissolved and activated by trypsin cleavage. The activated toxin was radio-labelled with iodine and used to assess binding of the Cry1Ac toxin to brush border membrane vesicles of the BX strain at generations 0 (susceptible; RR = 7) and 26 (resistant; RR = 110). Competitive binding assays were conducted and the binding constant (Kcom) and binding site concentration (Bmax) were calculated using LIGAND software.

To assess the significance of the resistance level, BX larvae were placed on INGARD plants. Three first instar BX larvae were placed on each of 20 INGARD and parental glasshouse-grown plants (V15i and V15) and their survival and development monitored until pupation. Pupae were collected and the emerging adults placed with ANGR insects of the opposite gender. Fertilisation and oviposition were monitored.

The surprising results obtained from bioassays of the BX strain with Dipel and Xentari indicated synergy. To investigate that further, we commenced cloning the cry genes expressed in these strains (cry1Aa, cry1Ab, cry2Aa, cry1C, cry1D) for bioassays with mixtures of these toxins.

We also tested a potential synergistic interaction between chitinase and Cry1Ac. Dr Helen McFadden, CSIRO Plant Industry, provided leaves from transgenic cotton expressing chitinase. The leaves were frozen in liquid nitrogen and ground to a fine powder. Bioassay of neonate H. armigera on the cotton powder mixed with artificial diet showed that, at 350ug chitinase/ml, there was 30-50% mortality and about 80% reduction in weight of survivors at 7d.

Genetics

The BX strain was passed on to Dr Joanne Daly and Dr David Heckel for genetic analysis (CSE73C, CSE89C) and to Dr Robin Gunning for evaluation of the esterase profile.

5. **Detail results including the statistical analysis of results.**

Selection

No resistance was detected until 16 generations of selection, at which time the resistance ratio (measured against the ANGR strain) was 76. Under continuing selection, resistance increased to RR=321 at g21 (Figure 1). Resistance declined sharply in RR at g23, falling to RR=62 at g25. Despite increased selection pressure at g27, the resistance remained at 40-110 to g34.

After selection of a portion of the BX population was discontinued at g17, there was no significant change in RR over eight generations.

The IS strain reached an RR of ~100 in three generations of selection (Figure 2). Under increasing selection pressure, RR rose to 800 by g11. The IS strain was backcrossed to ANGR at g3 and the new strain, ISOC2, reached an RR of ~100 in three generations of selection at dosages <40ug/ml. After four generations of selection, the ISOC2 line was backcrossed to ANGR once more. In one generation of hard selection (400ug/ml), the new line, ISOC3, reached an RR of ~100.

Single pair matings with generation 10 of the IS strain showed that the strain was not homozygous for resistance at the level tested.

Neither the VX nor the RX strains developed sustainable resistance. The VX strain reached an RR of nearly 90 but subsequently this declined to <5, which is not considered to be resistance. The VX strain was difficult to maintain and had

Figure 1. Response of *H. armigera* BX to selection against Cry1Ac.

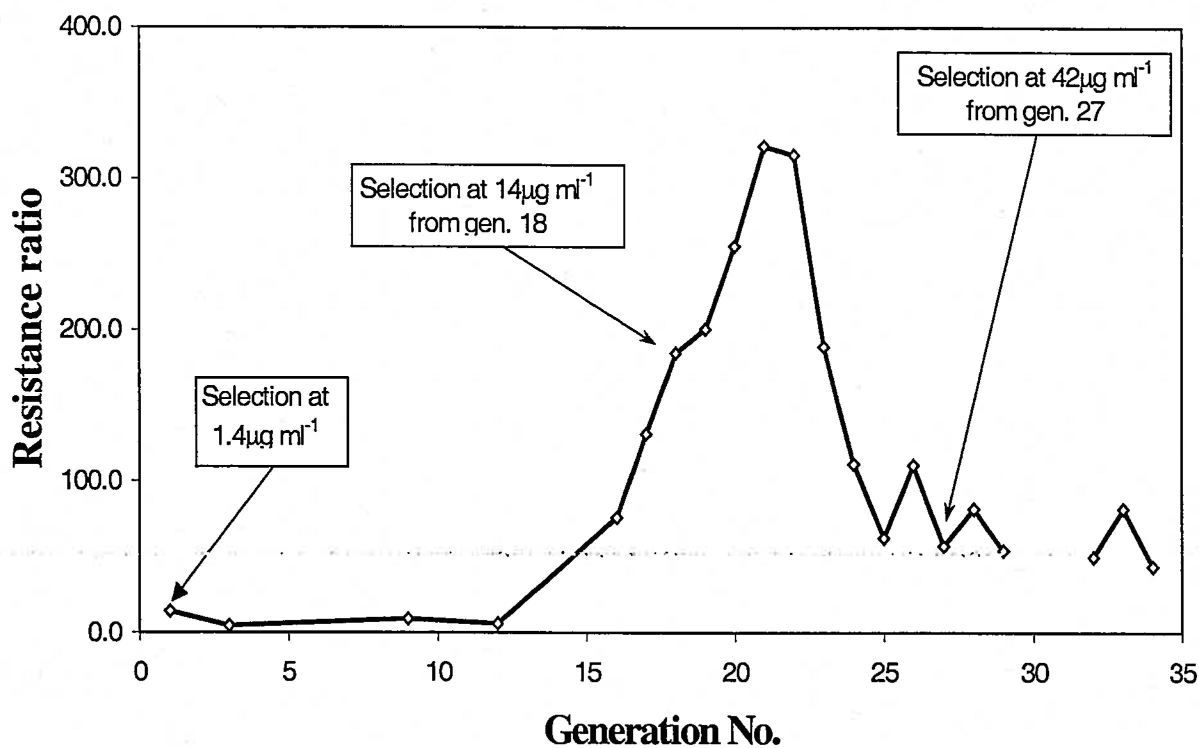
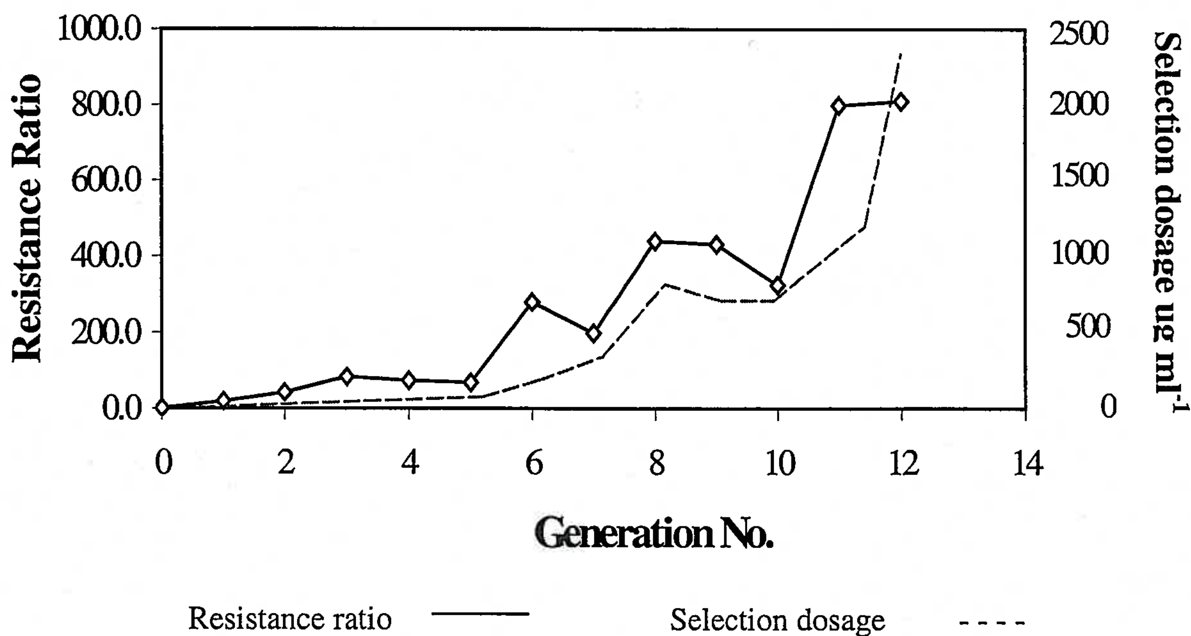


Figure 2. Response of *H. armigera* IS to selection against Cry1Ac.



Resistance ratio ———— Selection dosage - - - -

to be outcrossed on several occasions. The RX strain was rarely vigorous enough to allow selection. Both strains were eventually discarded.

Cross-resistance

The BX strain was also resistant to Cry1Ab but not to Cry2Aa or Cry2Ab (Table 1). Resistance to MVP I was evident but less than resistance to the Cry1Ac spore/crystal mix. However, resistance to either DiPel or XenTari was less than 10-fold and not considered significant. Similar results for individual Cry toxins were obtained with the IS strain but there was some significant resistanace to DiPel and XenTari (Table 2)

Table 1. Susceptibility of H. armigera BX strain to Bt toxin preparations

Toxin	Generation	Resistance ratio
Cry1Ac	18	188
DiPel		5
XenTari		7
MVP I		69
Cry1Ac	28	82
Cry1Ab		157
Cry2Aa		1
Cry1Ac	34	34
Cry2Ab		1

Table 2. Susceptibility of H. armigera IS strain to Bt toxin preparations

Toxin	Generation	Resistance ratio
Cry1Ac	10	581
Cry1Ab		870
Cry2Aa		1
Cry2Ab		2
Cry1Ac	12	809
DiPel		24
XenTari		14

Resistance Mechanism

The binding experiments showed that resistance in the BX strain is associated with the loss of a high affinity binding site for Cry1Ac. For generation 0, Kcom (affinity) and Bmax (concentration) were estimated to be 0.335nM and

0.93pmoles/mg, respectively, whereas no specific binding could be identified for generation 26.

Tolerance of INGARD Cotton

None of the susceptible H. armigera larvae were able to survive on the glasshouse grown V15i plants. However, the BX strain was able to complete larval development on 18/20 V15i plants, compared with 19/20 V15 plants (Table 3). While some of the survival might be attributable to cannibalism, the survival of the BX strain was less on V15i than on V15. The BX strain was also less able to complete its development on V15 than was the susceptible ANGR strain.

Table 3. Survival of BX generation 25 (RR = 63) on glasshouse grown INGARD and parental plants

Cotton line	H. armigera strain	No. plants with pupae	No. pupae (from 60)
V15	ANGR	20	50
	BX	19	35
V15i	ANGR	0	0
	BX	18	21

In addition to the poorer survival of the resistant strain on the parental cotton line, BX also took longer than the susceptible ANGR to complete its development (Figure 3). Development of the BX strain was similarly delayed on artificial diet.

No significant difference in the size of BX and ANGR pupae or in the fecundity of BX mated with ANGR compared to ANGR crosses could be detected. However, this experiment will have to be repeated with larger numbers to obtain reliable data.

Synergy

The cry1Aa, cry1Ab, cry1C and cry1D genes were cloned from the B. thuringiensis aizawai strain in XenTari. Each gene was amplified by PCR using proof-reading polymerase and the accuracy of amplification was confirmed by sequencing. A suitable expression vector has been obtained and each of the these genes will soon be expressed for evaluation of interactions.

The was no evident synergism between chitinase and Cry1Ac after 7d in terms of mortality. However, the weight of survivors from the chitinase/Cry1Ac mixtures was less than that of those on Cry1Ac or chitinase alone (Figure 4).

Figure 3. Larval development time of resistant and susceptible H. armigera on INGARD (V15I) and parental (V15) lines

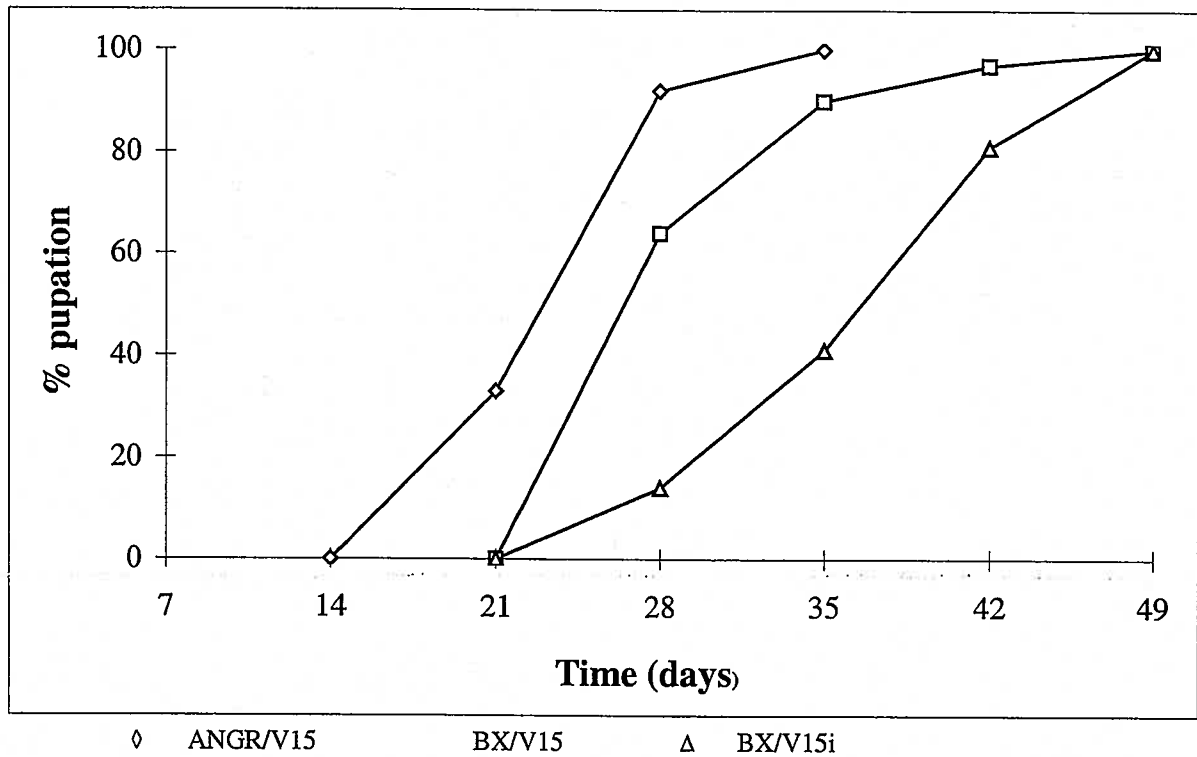
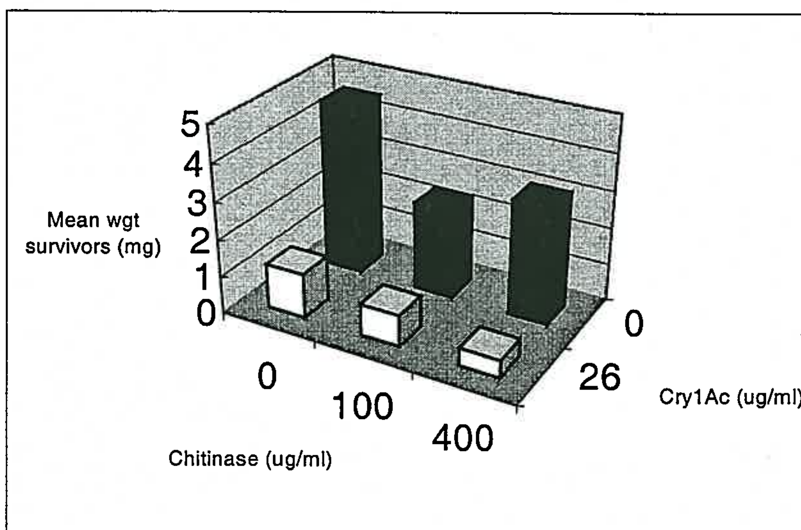


Figure 4. Effect of the interaction between Cry1Ac and chitinase on growth rate at 7d



6. **Discuss the results, and include an analysis of research outcomes compared with objectives.**

The development of the Cry1Ac-resistant strains of H. armigera (BX and IS) provides valuable models for evaluating the risks and consequences of field populations of this pest becoming resistant to INGARD cotton.

Although the resistance ratio for the BX is strain is low in comparison to data reported for other species, it is highly significant. H. armigera has a high natural tolerance for Bt toxins (about 30 times that of Heliothis virescens) and a low level of resistance on top of this tolerance is sufficient to allow survival on glasshouse-grown transgenic cotton. Some further evaluation, involving testing of the ability of heterozygotes to survive on INGARD cotton conducted with higher expressing varieties and field grown plants, is required to gauge the practical significance of this level of resistance.

As the BX strain was established from field collected insects that had survived Dr Neil Forrester's screening assays, it was not possible to determine the frequency of the resistance allele in field populations with a high degree of accuracy. However, Dr Forrester estimated that the BX strain was established from the survivors of perhaps 3000 field-collected insects. This suggests that one or more insects in 3000 was carrying the resistance allele, an estimate that is of the same order as estimates of Cry1A-resistance alleles in Heliothis virescens, Pectinophora gossypiella and Chilo suppressalis. This relatively high frequency of resistance alleles in natural populations strongly supports the need for effective resistance management strategies.

The very strong response of the IS strain to selection indicated that the decline in resistance ratio for the BX strain after generation 22 was due to inbreeding lowering the general vigour of the strain. Application of increasing selection pressure to the IS strain showed that high resistance levels (RR=800) can be attained by this species. The demonstration by Olsen and Daly (CSE89C) that heterozygotes of BX and a susceptible strain have some tolerance of INGARD cotton suggests that heterozygotes of IS and a susceptible strain could be very damaging.

The test for homozygosity in the IS strain at generation 10 indicates that there may be more than one resistance mechanism operating in this strain. Evaluation of homozygosity on a discriminating dosage test based on the level of resistance in the IS strain indicated that this strain was not homozygous for resistance. However, although there is clearly a fitness cost associated with resistance in BX, culture of the BX strain in absence of selection did not result in a decline in resistance. This suggests that the BX strain was homozygous for resistance. The implication is that the higher level of resistance in the IS strain is due to an additional factor or factors. Although greater vigour resulting from outcrossing might be a contributing factor, it does not appear to be an adequate explanation for the >8-fold difference in resistance between BX and IS. Further investigation of the basis of the high resistance in the IS strain is required.

The restricted extent of cross-resistance in BX and IS means that the two gene strategy using the Cry1Ac/Cry2Ab combination for resistance management

remains a viable option. The lack of cross-resistance to Cry2A toxins and the cross-resistance to Cry1Ab are consistent with the discovery that the high affinity binding site for Cry1Ac (which also binds Cry1Ab) is absent in the BX strain.

The glasshouse trial on INGARD cotton shows that there is a fitness cost associated with Cry1A resistance in H. armigera. The development of the BX strain was significantly longer than that of the susceptible ANGR strain when both were growing on conventional cotton; the difference was much greater with BX on INGARD and ANGR on conventional cotton. This does not quite mirror the refuge situation in the field because the issue will be the rate of development of the heterozygotes on INGARD. However, heterozygotes are likely to take even longer than homozygous resistant individuals. Further work with resistant and susceptible insects with similar genetic backgrounds (as we are generating from the backcrosses with the IS strain) and on varieties with the current commercial INGARD varieties is required to more fully evaluate the fitness cost.

The bioassay results obtained with DiPel and XenTari were unexpected and may provide new insights into the action of the Cry toxins. The BX and IS strains were far less resistant to DiPel and XenTari than they were to Cry1Ac. Although DiPel contains Cry2Aa, this protein comprises less than 30% of the recognised insecticidal protein and so we would expect that the RR for DiPel would be at least 50% that for Cry1Ac. Despite carrying the cry2Ab gene, XenTari does not even produce any Cry2 protein and so it was expected that the RR for XenTari would be the same as for Cry1Ac. These results imply some resistance breaking interaction between the known toxins or some unidentified toxin in these strains. The component cry genes from DiPel and XenTari have been cloned and can be used to test the interaction hypothesis.

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

This project has fed vital information into the resistance management strategy. The data on allele frequency, fitness cost, and the partial dominance of the resistance allele (CSE89C) have underlined the need for stringent resistance management measures.

Some further evaluation of the resistant H. armigera is required to refine the resistance management strategy. The ability of heterozygotes to survive on INGARD cotton conducted with higher expressing varieties and field grown plants needs to be quantified. The basis for the high level of resistance in the IS strain should be characterised and the reason why DiPel and XenTari are more toxic to the resistant strains than predicted should be determined. The frequency of the resistance allele in field populations should be estimated with greater accuracy by crossing field collected insects with the BX or IS strain. Fitness costs, including effects on mating efficiency and fecundity, should be evaluated in near isogenic susceptible and resistant strains to exclude extraneous genetic effects. We intend to submit a proposal to this effect for the 2002/2003 year.

The resistant strain is being used to test insecticidal proteins that might be used in conjunction with Cry1Ac.

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

The significant technology from this project is the development of the resistant strains. It feeds into several other cotton-oriented projects and is being used to test new insecticidal proteins for their complementarity with Cry1Ac in a resistance management program.

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

10. State the recommendations on the activities or other steps that may be taken to further develop, disseminate, or to exploit the project technology.

11. List the publications arising from the research project.

Akhurst, R.J. and James, W.J. (1999). Cry1Ac-resistant Helicoverpa armigera feed and grow on transgenic cotton. Abstr. XXXII Meeting, Soc. Invertebr. Pathol., Irvine. p.20.

Akhurst, R.J. and James, W.J. (1999). Cry 1Ac- resistant Helicoverpa armigera. Abstr. 30th Ann Meeting, Aust. Entomol. Soc., Canberra. p.1.

Akhurst, R.J. and James, W.J. (1999). Helicoverpa armigera resistance to transgenic cotton expressing the Cry1Ac d-endotoxin of B. thuringiensis. In: Biotechnology of Bacillus thuringiensis, Vol. 3. Yu, Z., Sun, M. and Liu, Z. (eds). Science Press, Beijing. p.200.

Akhurst, R., James, W. and Bird, L. (2000). Resistance to INGARD® cotton by the cotton bollworm Helicoverpa armigera. Proc. 10th Australian Cotton Conference, Brisbane (CD-ROM)

Akhurst, R., James, W., Bird, L. and Beard, C. (2001). Characterisation of a strain of cotton bollworm, H. armigera, resistant to the Cry1A toxins of Bacillus thuringiensis. Abstr. 34th Ann. Meeting, Soc. Invertebr. Pathol., Noordwijkerhout. p.22.

Part 4 – Final Report Plain English Summary

You must submit a half to one page Plain English Summary of your research proposal that is not commercial in confidence, and that can be published on the World Wide Web. An electronic copy of the Plain English Summary must also be forwarded by e-mail (angela@crdc.org.au).

Transgenic cotton was adopted by the Australian cotton industry as the core of its pest management strategy as the result of increasing problems associated with the usage of synthetic chemical insecticides, namely resistance in cotton bollworm and environmental damage. The INGARD varieties currently in use in Australia derive their resistance to caterpillars from the production of an insecticidal protein produced by a gene from the bacterium Bacillus thuringiensis (Bt). Although only one insect species, the diamondback moth, has developed resistance to Bt in natural populations, the capacity for developing Bt resistance by laboratory selection has been demonstrated in more than 20 species. One of these 20 is Heliothis virescens, a major pest of cotton in the USA. In consequence, the CRDC funded several projects, including the one reported here, to commence investigation of resistance to Bt by the cotton bollworm in Australia.

Obtaining Bt resistant cotton bollworm proved to be a difficult task because this insect does not take well to being cultured on Bt toxins under laboratory conditions. Nevertheless, by identifying suitable conditions and persisting in our efforts, we were able to generate a strain of cotton bollworm (the BX strain) that was significantly resistant to Cry1Ac, the insecticidal protein produced by INGARD cotton. We have used this strain and supplied it to other research groups to gain a better understanding of the potential Bt resistance problem in cotton bollworm.

Several major findings have arisen from the investigation of this strain. We have shown that the resistance is associated with the loss of a specific binding site for the Bt toxin. As this is the only Bt resistance mechanism found in field-collected insects of any species, we believe that the BX strain is a good model for studying resistance to Bt in cotton bollworm. We have estimated that the resistance allele occurs naturally in approximately one in 3000 cotton bollworms, an estimate that is in line with estimates for other species. We have also shown that there is a fitness cost associated with this resistance that results in the resistant strain maturing more slowly than a susceptible strain. In conducting their genetic analysis of this strain, Daly and Olsen (CSE89c) showed that the resistance allele is only partially recessive. These factors of allele frequency, fitness cost and partial recessiveness are important components of the model used in designing the refuge strategy for resistance management.

In summary, this project has shown cotton bollworm has the capability to develop resistance to INGARD cotton and that the risk of resistance development in field populations is high. The data obtained from the analysis of the resistance strain show that the refuge strategy is essential and must be managed with a great deal of care and attention.