RESISTANCE TO INGARD® COTTON BY THE COTTON BOLLWORM, *HELICOVERPA ARMIGERA*

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The increasing difficulty of controlling cotton bollworm, *Helicoverpa armigera*, in Australia due to its resistance to many chemical insecticides and the pressure to reduce the usage of chemicals led to the adoption of transgenic cotton as the key component of its pest control strategy by the Australian cotton industry. The commercial varieties available to date are based on the INGARD® technology and contain an insecticidal protein (Cry1Ac) which was obtained from the bacterium *Bacillus thuringiensis* (Bt).

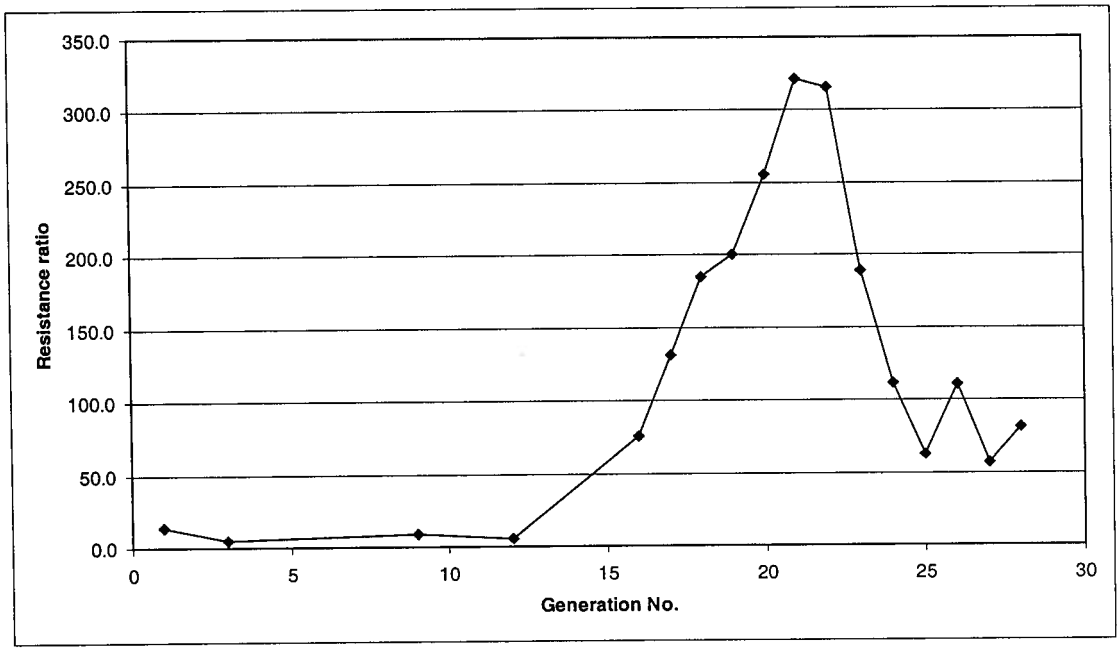
As INGARD® varieties contain only one insecticidal protein, growers and researchers were concerned that cotton bollworm might become resistant to the transgenic cotton. At the inception of the project, resistance to the insecticidal proteins had not yet been demonstrated for any cotton pests. However, we were aware that the diamondback moth had become highly resistant to Bt sprays in many parts of the world. Subsequently, some 26 species of insect pests, including *Heliothis virescens*, have shown the ability to develop substantial resistance to Bt proteins. The naturally high tolerance of *H. armigera* for the Cry1Ac protein produced by INGARD® and the decline in insecticidal activity through the latter part of the season (Fitt, 1998) suggest that inappropriate usage could well lead to INGARD® losing its ability to prevent *H. armigera* damage. Consequently, the CRDC and CSIRO funded a project to investigate the potential for cotton bollworm to develop resistance to transgenic cotton and evaluation of the consequences of such resistance.

Selection for Resistance to Cry1Ac

H. armigera survivors of Dr Neil Forrester's discriminating dosage screen of field populations (Forrester, 1998), insects collected by Dr David Murray, and an existing laboratory colony supplied by Dr Joanne Daly, were combined to establish a starter colony. Neonate insects from this colony were fed a mixture of Cry1Ac crystals and Bt spores for seven days. At that time, the largest survivors were transferred to a Cry1Ac-free diet to complete their development and produce the succeeding generation. Bioassays were conducted at each third generation to test for the development of resistance. After 16 generations of selection, we found that the cotton bollworms had become markedly less sensitive to the crystal/spore mixture. Continuing selection resulted in the insects becoming significantly more resistant (Fig. 1) so that increasing the selection dosage by 7-fold at generation 17 had no noticeable effect on growth.

Generation 21 was nearly 50-fold more resistant to Cry1Ac than generations 1-12 and over 300-fold more resistant than a susceptible laboratory colony. The resistance ratio declined after generation 23, with the selected colony being 50- to 100-fold more resistant than the susceptible laboratory colony and only 8- to 17-fold more than the founder colony.

Figure 1. Selection of *H. armigera* for resistance to Cry1Ac.



However even at the reduced level, the resistance was large enough to pose problems. First instar larvae from generation 25 placed on glasshouse grown INGARD® cotton (V15i) were able to feed and complete larval development whereas larvae from a susceptible laboratory population all died within three days and caused very little damage. The selected insects were able to complete their larval development on V15i and produce pupae from which emerged fertile adults. This experiment demonstrated that this relatively low level of resistance is sufficient for *H. armigera* to cause economically serious problems.

There appears to be a fitness cost associated with this resistance. Resistant *H. armigera* developed more slowly on conventional cotton (V15) than the susceptible strain and even more slowly on transgenic cotton. However, when selection pressure was no longer applied to a sub-sample of the resistant colony, these insects did not revert to a susceptible line. Both the slower development on conventional cotton and the stability of resistance need to be considered in deciding the detail of a resistance management strategy. Care must be taken to ensure that adult *H. armigera* produced from refugia are not so far out of synchrony with the survivors on transgenic cotton that the two sub-populations are unable to mate. The stability of resistance increases the desirability of preventing the occurrence of homozygous resistant individuals in field populations.

The small number of insects involved in these selections indicates that it is most unlikely that this resistance resulted from a mutation. It is more likely that at least one of less than one thousand insects used to start the colony carried a gene for resistance to Cry1Ac. This is consistent with data obtained for other species, including *Helicoverpa zea* and *Heliothis virescens*, in the USA where the frequency of individuals carrying a resistance gene in untreated populations has been estimated to be approximately 0.1 – 0.2% (Gould *et al.*,

1997; Tabashnik *et al.*, 1997). If this estimate of resistance frequency is accurate, we could expect resistance to become a significant problem in the field in much less than 16 generations (4-5 years) if no effective resistance management strategy was implemented.

Characterisation of Resistance

The nature of the resistance in the BX strain was explored to assess the possibility of its occurrence in field populations of cotton bollworm and the consequences of this resistance for the use of alternative Bt products.

Preliminary studies on the resistance mechanism indicate that broad cross-resistance in this strain is very unlikely. The complexity of the mode of action of the Bt proteins means that a resistance mechanism can act in many places (Table 1). Some of these mechanisms (e.g. change in binding characteristics) would produce resistance to only one or a few insecticidal proteins whereas others (e.g. gut proteases) could result in resistance to a broader range. Our investigation of the mode of action in this resistant strain of *H. armigera* indicates that one of the two Cry1Ac binding sites (the higher affinity site) detected in several susceptible strains is absent from the resistant strain. This indicates that the resistance will be narrow spectrum and that not only will the Cry1Ac-resistant strain be susceptible to Cry2A proteins but also to new Bt proteins (except perhaps new Cry1A types) capable of killing *H. armigera*. The association of the loss of a binding site with resistance in the only insect species that has developed resistance to Bt products in the field (diamondback moth) suggests that the laboratory-selected strain is a good model for further study.

Table 1. Mechanisms that could produce resistance to Bt insecticidal proteins

Steps in the mode of action of insecticidal proteins	**Potential resistance mechanism**
Ingestion	Avoidance behaviour
Solubilisation of the crystal[1]	Gut pH and/or reduction potential reduced
Activation of the protoxin[1]	Proteases fail to cleave protoxin
Translocation across the peritrophic membrane	Proteases digest activated toxin; Increased toxin binding to peritrophic membrane or other tissues
Binding to midgut epithelium	Concentration of specific binding site reduced; Binding affinity reduced
Pore formation	Post-binding structural alteration inhibited
Cell lysis	Ionic compensation mechanism
Breakdown of gut integrity, leading to death	Increased rate of gut cell replacement

[1] Little or no relevance to Bt proteins in transgenic cotton

The use of two (or more) insecticidal agents with different modes of action is considered to be one of the major options for resistance management. In our earlier study of the toxicity of Bt for *H. armigera*, we found that this insect was susceptible to only four insecticidal Bt proteins (Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab; Akhurst and Liao, 1996). The resistant strain was not cross-resistant to the sprayable Bt formulation, DiPel, which contains the Cry1Ab, Cry1Ac and Cry2Aa proteins. It was resistant to the sprayable formulation MVP® which contains only Cry1Ac, although to a lesser extent than to the pure Cry1Ac crystal (Table 2). Tests with the purified individual proteins showed that the resistant strain was resistant to both Cry1Ab and Cry1Ac but not to Cry2Aa (Table 2). It is evident from these results that there is at least one alternative insecticidal protein that can be used, either in transgenic plants or in sprayable formulations, for managing resistance to INGARD™ cotton.

Table 2. Susceptibility of the Cry1Ac-resistant strain of cotton bollworm to Bt products

Bt Product	Generation No.	Resistance ratio[1]
DiPel® MVP Cry1Ac	18	4.9 69.3 187.6
Cry1Ab Cry1Ac	28	157.3 81.6
Cry2Aa Cry1Ac	19	2.3 203.8

[1] Comparison of selected strain with a susceptible laboratory colony

Conclusion

The development of resistance to the insecticidal component of INGARD™ cotton in *H. armigera* strain demonstrates that the caution taken in introducing the transgenic technology was well-justified. Presently, there is no indication of resistance to INGARD® cotton in field populations of *H. armigera* and no indication that the resistance to Cry1Ac in *H. armigera* will extend to other insecticidal proteins. However, *H. armigera* is capable of resistance of a magnitude that would result in significant, perhaps severe, damage to INGARD® cotton. Moreover, the relatively small number of field-collected insects used to establish the selection colony indicates that genes for resistance already exist in Australia and perhaps at a threateningly high level. The evidence from this study of Bt resistance in cotton bollworm shows that the transgenic cotton technology has to be used with discretion and vigilance if it is to continue to serve the industry.

The demonstration that the resistance is linked to the loss of a specific binding protein can be combined with the results of a PhD project funded by the CRDC to enable the

identification of the binding protein. With this information, it will be feasible to develop a test for resistance alleles in field-collected insects that will enable sensitive monitoring of the resistance status of field-populations. The monitoring results can be used to enhance the quality of decision-making for the resistance management strategy.

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