*Biocontrol Science and Technology (1994) 4, 549–553*

# Resistance Management Options for Conventional *Bacillus thuringiensis* and Transgenic Plants in Australian Summer Field Crops

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*Conventional* Bacillus thuringiensis (Bt) *is being increasingly used for control of* Helicoverpa *spp. in Australian cotton. Five years ago, the annual useage of* Bt *in cotton was less than 10 000 l. It is now about 200 000 l but still represents only about 0.5 of a spray in cotton which normally receives 6–8 conventional insecticide sprays for* Helicoverpa *control each season.* Bt *use in other field crops has been minimal. The present risk for development of resistance to* Bt *in* Helicoverpa *spp. is, therefore, low. However, any increased use of conventional* Bt *on cotton or extended registrations to alternative* Helicoverpa *host crops could increase the risk significantly. Susceptible bioassay baselines and discriminating doses for conventional* Bt *on both* H. armigera *and* H. punctigera *are currently being established. The aim is to assess the natural variability in bioassay response (diet incorporation technique using early third instars) in a number of strains of both species from a number of geographical areas. This will allow monitoring of the impact of increased* Bt *use and/or the introduction of transgenic cotton on potential resistance problems. The ecology of* H. armigera *and* H. punctigera *is discussed in this paper, particularly in relation to the differential resistance risk of the two species where* H. punctigera *essentially 'manages its own resistance'. This is extended to the development of a possible resistance management strategy for* H. armigera *on transgenic cotton which will rely on continual dilution of rare resistant mutants by large numbers of unsprayed susceptibles allowed to breed in designated refugia areas. This high-dose/high-immigration resistance management approach will essentially maintain resistant* H. armigera *individuals as rare, hopefully functionally recessive heterozygotes. Two other factors will be critical for the success of this strategy: (1) strains of conventional* Bt *with different toxin profiles (e.g.* Bt *subsp.* aizawai*) should be used on non-transgenic alternative crop hosts of* H. armigera *(e.g. sorghum, sunflowers, grain legumes, maize, tomatoes and oilseeds), and (2) development of transgenic alternative crop hosts of* H. armigera *(especially sorghum and maize) should concentrate on toxins other than those from* Bt.

**Keywords:** Bacillus thuringiensis, Helicoverpa *spp., resistance management, transgenic plants*

## INTRODUCTION

It is expected that transgenic cotton plants will be available in Australia in commercially significant quantities by the year 2000. These first plants will more than likely contain only the CryIAc insecticidal crystal protein (ICP). In the meantime, the use of conventional *Bacillus thuringiensis (Bt)* sprays continues to increase steadily, fuelled by a declining effectiveness of conventional insecticides due to increasing resistance. This raises a number of questions which are discussed in this paper. (1) Will prior exposure of *Helicoverpa* spp. to conventional *Bt* prejudice the successful release of transgenic cotton? (2) What is the best resistance management option for both conventional *Bt* sprays and transgenic cotton? (3) What is the role of alternative host crops in the management of potential resistance to conventional *Bt* and transgenic cotton?

## PRIOR EXPOSURE OF *HELICOVERPA* SPP. TO CONVENTIONAL *BT*

Due to increasing resistance problems with *Helicoverpa armigera* (Hübner) in Australian summer field crops and an increasing community awareness of environmental issues, there has been a steady increase in the use of conventional *Bt* sprays in the Australian cotton industry. Even as recently as 5 years ago, annual useage of *Bt* in cotton was less than 10 000 l (Forrester, 1994). This has now increased to about 200 000 l but this still represents only about 0.5 of a spray in cotton which would normally receive from six to eight conventional insecticide sprays for *Helicoverpa* control each season. In addition, *Bt* use in alternative host field crops has been minimal. Thus, for the moment, the risk for development of resistance to *Bt* in *Helicoverpa* spp. remains low, and resistance management recommendations for *Bt* are only briefly referred to in one of the key guidelines accompanying the Australian Insecticide Resistance Management (IRM) strategy (Shaw, 1993): "Avoid continuous sprays of any one chemical group, including *Bts*". However, the potential use of *Bt* has been estimated at up to 2–3 million l (used in mixtures with conventional insecticides) and at least double this if used alone, representing about six sprays in cotton (Forrester, 1994). Obviously at these useage levels there would be a serious resistance risk. The present indications are that these levels will not be reached at least in the near future due to the high cost of conventional *Bt* sprays but the situation could easily change with de-registration of, or increasing resistance to, certain conventional insecticides and increasing commercial competition reducing the price of *Bt*. These factors are under constant surveillance and a research project has been recently funded by the Australian Cotton Research & Development Corporation to establish susceptible bioassay baselines and discriminating doses for conventional *Bt* on both *H. armigera* and *H. punctigera* Wallengren. The aim of this project is to assess the natural variability in bioassay response (diet incorporation technique using early third instars) in a number of strains of both species from a number of geographical areas. So far, 20 *H. armigera* and 23 *H. punctigera* colonies have been tested, and a number of potential discriminating doses have been calibrated this season on field populations of both *H. armigera* and *H. punctigera*. The most likely discriminating dose so far seems to be 1.0 mg of Dipel 2X (32 000 IU $mg^{-1}$) $ml^{-1}$ of diet which has given 96.5% ($n = 3271$) and 99.0% ($n = 312$) mortality for *H. punctigera* and *H. armigera* respectively (Forrester & Forsell, 1994). Later, it is planned to also develop baseline data for the individual ICPs. The successful development of a discriminating dose for *Bt* will allow us to monitor the impact of increased *Bt* use and/or the introduction of transgenic cotton on potential resistance problems and to react accordingly. This will be critical for the successful adoption of any resistance management strategy (Forrester, 1990).

Although the resistance risk to conventional *Bt* is low at present in Australian *H. armigera*, there are other areas of the world where the risk is acute. For example in China, the intensive and increasing use of *Bt* against insecticide-resistant *H. armigera* (an average of 2–3 sprays in cotton in Shandong province in the 1993 season with up to 10 sprays or more in the worst affected areas) may well result in development of resistance to conventional *Bt* in *H. armigera* and thus nullify any potential benefit from the later introduction of transgenic cottons expressing

*Bt* ICPs (IOPRM, 1993). If this happens, then one might ask how far these *Bt* resistant *H. armigera* could migrate, and could successful IRM in one country be negated by ineffective or non-existent strategies in neighbouring countries?

## THE BEST RESISTANCE MANAGEMENT OPTION FOR BOTH CONVENTIONAL *BT* SPRAYS AND TRANSGENIC COTTON

The successful Australian IRM strategy for summer field crops has taught us a lot about the resistance management of *Helicoverpa* spp. (Forrester *et al.*, 1993), encompassing the biology and ecology of both *H. armigera* and *H. punctigera*, particularly in relation to their differential resistance risk, and the importance of the human element in implementing and servicing an IRM strategy. The success of this strategy for conventional insecticides has left a legacy of confidence and credibility which can be tapped for any future resistance management efforts centred on *Bt* and/or transgenic cotton.

The differential resistance risk of *H. armigera* and *H. punctigera* in Australia has clearly indicated the critical importance of ecology in resistance management. These two sibling species have similar biochemical capabilities for metabolic detoxification of xenobiotics (see appendix 4 in Forrester *et al.*, 1993) but there has been no recorded resistance to any insecticide in *H. punctigera* despite *H. armigera* having developed resistance to virtually every insecticide used against it in any quantity (Section 1, Forrester *et al.*, 1993). Evidence in Appendix 4 of Forrester *et al.* (1993) indicated that this difference is probably due to the highly migratory, polyphagous nature of *H. punctigera* compared with the relatively oligophagous and facultatively migratory *H. armigera*. It is suggested that the large pool of unsprayed susceptible *H. punctigera* is so vast that it effectively swamps any resistance which develops in the intensively sprayed cropping areas. In other words, *H. punctigera* effectively manages its own resistance. This is a critical point as it is practical evidence for Benson's (1971) hypothesis for the management of resistance through the large-scale release of susceptible insects into the pest population even if it meant "sacrificing some of our food to the right insects, those with susceptible genotypes". Benson (1971) suggested that this 'genetic infusion' technique would be the only ultimate long-term solution to IRM as it "controlled the evolution of pest species". It is important to note that Benson's hypothesis and the example of nature's own highly successful IRM strategy for *H. punctigera* have remarkable parallels with Roush's (1994) suggestion for a resistance management strategy for transgenic cotton. He suggests a high-dose/refugia resistance management approach which essentially maintains resistant *H. armigera* individuals as rare, hopefully functionally recessive heterozygotes, and that the small refugia area would act as a dilution source for any resistant individuals which survive on the transgenic cotton. For this strategy to work, the concentration of toxin in the plant should be high enough to kill most (hopefully all) heterozygotes and the refugia should be both temporally and spatially contiguous with the transgenic crop. Cotton would be the ideal refugium crop as it would remain an attractive host for the same period as the transgenic cotton crop and every grower would need to leave a small refugium area on each farm. Discrete blocks of conventional cotton would be preferable to seed mixtures so as to avoid the possibility of inter-plant larval migration. This refugia area need not be totally unsprayed (just not protected with any *Bt* product) but should be allowed to produce some *H. armigera* to dilute any resistant homozygotes which may be produced in the transgenic crop. This could be easily achieved by setting a higher threshold in the refugia area for any *H. armigera* present in the crop but maintaining normal thresholds for *H. punctigera*. Such a strategy would most likely be acceptable to most growers as it would result in lower control costs, little (if any) loss in production overall and the possibility of achieving a successful and environmentally desirable sustainable crop protection technology. A high level of toxin expression in the plant will be a critical component for the long-term viability of this technique. Ideally, this level should be able to kill all heterozygote neonate larvae. As there are as yet no known *Bt*-resistant *H. armigera* strains, this level should be estimated to be 10 times the level needed to kill all susceptible neonates. If this level of expression is not being achieved, then serious thought should be given to delaying release of the technology until either the level of expression is increased or other genes are incorporated to elevate the total level of ICPs. The critical role of alternative *H. armigera* host crops in this strategy is discussed below.

## THE ROLE OF ALTERNATIVE HOST CROPS IN THE MANAGEMENT OF RESISTANCE

*H. armigera* is a major pest of summer field crops other than cotton and *Bt* is being seriously considered as a control option in these crops (e.g. sorghum, sunflowers, grain legumes, maize, tomatoes and oilseeds), particularly those catering for the increasing demand for 'organically grown' produce. If the CryIAc ICP is to be used in transgenic cotton (and this seems very likely) then strains with different toxin profiles to *Bt* subsp. *kurstaki* should be used on these alternative host crops (e.g. *Bt* subsp. *aizawai*). It is arguable whether transgenic plants or conventional *Bt*s should have access to a particular ICP, but it must be remembered that the lead time for incorporating an ICP into transgenic plants is far longer than that needed for developing, producing and registering new strains of conventional *Bt*s.

The other major impact of alternative host crops will be through the potential development of the transgenic crop technology for crops such as sorghum and maize, which are the key alternative host crops for *H. armigera*. If the transgenic crop technology is to develop in these crops, it should ideally concentrate on toxins other than those from *Bt*s. Once again, it is arguable whether transgenic cotton should receive any priority over transgenic sorghum or maize (or other alternative host crops) but it must be remembered that the majority of Australian society's environmental complaints against conventional insecticides emanate from the intensive crop protection methods currently employed in the cotton industry. In this regard, society as a whole would benefit more from the initial deployment of this new technology into the intensively protected cotton crop. The needs of these other alternative host crop growers should not be dismissed, but these crops should wait until (and receive priority when) the second generation of genetically engineered toxins become available.

## CONCLUSION

The transgenic crop technology will soon prove to be the most important development in crop protection since the discovery of synthetic insecticides. However, as with synthetic insecticides, the greatest risk to its continued success will be the threat of the development of resistance. There is a plethora of literature on the theory of resistance management options but the time is soon approaching for the actual implementation and verification of these theories in the field. In this regard, the background and experience gained from the successful Australian IRM strategy for conventional insecticides will prove invaluable for any attempt at a resistance management strategy for transgenic cotton and other summer field crops in Australia.

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