

Transgenic Cotton: Its Place in Integrated Pest Management

GARY P. FITT

CSIRO Cotton Research Unit, Narrabri

Introduction

Transgenic cottons expressing the crystal protein toxin genes from the bacterium, *Bacillus thuringiensis* (Bt) have progressed to the field testing stage and may soon become part of the Australian cotton production scene. Bt cottons offer the potential for highly efficacious use of the Bt proteins and for significant reductions in the requirement for pesticides to control Lepidopterous pests like *Helicoverpa*. These reductions should reduce environmental concerns associated with cotton growing, and from the viewpoint of IPM will allow the implementation of other novel management strategies which are not compatible with pesticide use (eg. food sprays, pheromones, various parasites and predators). In future these other methods may help in management of pests other than *Helicoverpa*. Bt cottons are thus compatible with many other IPM tools and will provide a new platform on which IPM systems can be based.

Environmental Concerns

Bacillus thuringiensis is a naturally occurring bacterium found commonly in soil. The Bt protein toxins being used in cotton are highly specific. They kill only certain caterpillar pests like *Helicoverpa* and have no effects on other insects or any vertebrates. Despite their specificity and the prospect of greatly reduced pesticide use, there are a number of hurdles to be overcome before Bt cottons are available commercially. Many scientists, regulators and the general public have concerns about the release of genetically engineered organisms. These concerns involve both ecological and philosophical uncertainties about the plants and how to use them wisely and must be addressed as part of the regulatory process before Bt cottons will become commercially available. Some of the questions related to environmental impact and answers available to date include:

- will the gene escape from cotton into other plants ? [very unlikely]
- will beneficial insects or non-target species be adversely affected ? [too early to say]
- will there be Bt protein in the lint, seed, oil or trash of Bt plants ? [no, yes, no, briefly]
- if there is, does this matter? [Bt toxins have no effects on animals other than caterpillars]

All these questions are being addressed as part of the current research program, though some of them (eg. questions about beneficials) cannot be answered until large areas of Bt cotton can be grown.

Regulatory Requirements

At present all work in the laboratory, glasshouse or field with transgenic organisms such as Bt cotton is regulated by GMAC, the Genetic Manipulation Advisory Committee. GMAC is broadly supportive of this work and no submissions for work with Bt cottons have been delayed to date. For the future the Federal government will establish a legislated authority to replace GMAC and handle the registration procedures for transgenic plants. This Gene Technology Authority (or whatever it is to be called) will have legislated powers, but when it will be in place is not clear. In addition to the GTA several other bodies will be involved in decisions about the release of transgenic cotton. These include the National Registration Authority (NRA) which registers pesticides, the National Food Authority (NFA), the Commonwealth Environmental Protection Authority (CEPA), as well as state EPA's in NSW and Queensland. Just how all these bodies will interact with the GTA has not been determined. Outside the regulatory bodies themselves there are the numerous environmental groups who will want to have some involvement or influence. Most environmental groups have a blanket opposition to release of genetically engineered organisms, but in the specific case of Bt cotton they are in a quandary because of the environmental benefits of reduced pesticides. The main requirement for the future is that the field trialing process and eventual release of Bt cottons must be done in strict compliance with the relevant regulations.

Progress with field Trials

The first small scale field trial (only 200 plants) of transgenic cottons in Australia was completed in the 1992/93 growing season using a number of CSIRO varieties transformed with the Cry IA(b) insecticidal protein gene from *B.thuringiensis* . From the 1993/94 season larger field trials (about 1 hectare in size) were conducted by both CSIRO and Deltapine each with genetically engineered varieties expressing Bt genes. The objectives of these field trials are to

quantify the efficacy of field grown Bt plants against *Helicoverpa* spp., and to commence studies of environmental impacts and management strategies in preparation for future commercial release.

To date the trials have demonstrated that the Bt plants can work extremely well in the field. Although similar numbers of *Helicoverpa* eggs are laid on the Bt plants, no larvae survive during much of the season. However we have found that small numbers of *Helicoverpa* larvae can survive on the Bt plants late in the season. Laboratory bioassays have confirmed that the efficacy of the toxin in the plants changes as the plants get older. What is happening is not yet clear. While this low level of survival late season (maybe 5 % of larvae could survive) will not result in significant damage (indeed beneficials are likely to kill these larvae anyway), it is nevertheless concerning for the long term management of Bt cottons to avoid the development of resistance to them.

To date we have seen few differences between Bt plants and normal plants in the abundance of or damage from other pests, nor any reduction in the numbers of beneficial insects, although studies with larger areas are clearly needed to fully evaluate such changes.

Such large scale studies will commence from next season (1994/95), when a 10 hectare block of Bt cotton will be grown for experimental purposes. This block will be used to address some of the environmental and management questions. Since these are not specific to particular cotton varieties the work will be done in a co-ordinated way with financial support from Cotton Seed Distributors, Deltapine, Monsanto and the Cotton CRC. CSIRO and Deltapine will of course conduct their own trials with particular varieties where questions of varietal performance and efficacy of Bt genes will be explored. The co-ordinated approach will answer many of the critical questions required for eventual release and ensure that the whole industry gains access to the technology as early as possible.

Potential Ecological Impacts

There are a number of potential environmental impacts of the deployment of Bt transgenic cotton. For many organisms these impacts will be positive (through the removal of disruptive pesticides), while others may be negatively affected through the removal of prey (eg. beneficials which rely on *Helicoverpa* and other Bt susceptible Lepidoptera). The magnitude of these potential impacts depends largely on the significance of cotton as a habitat or food source for each species. Negative effects on beneficials are of concern to some environmentalists. However, even if some beneficials are less abundant in transgenic cotton, it must be remembered that beneficials are virtually absent from commercial cotton crops where pesticides are extensively used. The appropriate control for any comparison of the impact of transgenic cotton must be the current production system based on extensive pesticide use.

Reduced use of chemicals for *Helicoverpa* on transgenic varieties may also favour an increase in abundance of some pests previously considered of minor importance. Such pests may become so important that pesticides may again become an important management option, thus defeating the original value of the transgenic expression system. The sucking pests (mirids, aphids and thrips) are of most concern here, with the mirid bugs (*Creontiades dilutus*, *Campylomma livida*) the prime candidates to become more significant pests. Populations of mirids do not appear to be regulated by predators and parasites (M. Miles pers. comm.), but rather by climatic factors and host associations. This lack of biotic control coupled with relaxation of pesticide suppression could well see mirids emerge as a major problem in transgenic cotton. This possibility is further complicated if it is necessary to use highly disruptive pesticides (organophosphates or synthetic pyrethroids) for sucking pest control, thus potentially disrupting biological regulation of other pests. No selective chemicals are currently available for mirids. The development of new management techniques for mirids, whether by means of host plant resistance or specific non-disruptive chemicals, may be crucial for the successful deployment of transgenic cottons.

In evaluating Bt cotton we must also consider the minor caterpillar pests which feed on cotton. To date we have shown that the Bt plants effectively control rough bollworm, cotton looper,

tobacco looper, and the cotton leaf perforator. A number of other species wait to be tested. These include lesser armyworm, cotton tipworm and the pink bollworm.

Will *Helicoverpa* develop resistance to the Bt plants ?

Perhaps the major concern about Bt plants is the possibility that *Helicoverpa* may quickly develop resistance to them (McGaughey and Whalon 1992). Resistance to Bt toxins has already occurred in field populations of some insects (Tabashnik 1994a) due to excessive use of Bt sprays. The first Bt cottons released will contain a single Bt toxin gene. These will be followed very soon by plants with two different Bt genes. Because the Bt plants produce the toxic proteins continuously we will be imposing intense selection pressure on *Helicoverpa* populations to evolve resistance to the toxins. Each generation on cotton will be selected and the potential for resistance to occur quickly may be high. To avoid this happening it will be essential that the cotton industry implement a comprehensive resistance management strategy from the first release of Bt cottons. All the potential benefits of Bt cottons could be lost if *H. armigera* quickly develops resistance.

The development, evaluation and implementation of resistance management strategies for transgenic cottons is a major component of our research leading to commercialisation. The cotton industry has been proactive in addressing concerns about resistance management and the experience with management of pyrethroid resistance suggests there is a real opportunity to develop and implement pre-emptive resistance management for the Bt cottons.

The issue of resistance to transgenic plants has been dealt with exhaustively elsewhere (Caprio, 1994; Daly, 1994; Fitt et al 1994; Forrester, 1994; Gould, 1994; Tabashnik, 1994b, Roush, 1994ab). Simulation studies (which make a number of assumptions) suggest that an effective management strategy for transgenic cotton could be built around effective refuges for susceptibles within the cotton cropping system (Roush, 1994b). Refugia are simply areas (crops or natural habitat) in the cropping system which produce *Helicoverpa* moths which have not been exposed to Bt toxins, thus maintaining susceptible individuals in the local population. Refuges for resistance management will work best when combined with a consistent and high

expression of the Bt toxin in the transgenic cotton. While no strategy can guarantee that resistance will never occur, simulation and laboratory/greenhouse studies suggest that a refugia/optimal dose strategy implemented pre-emptively could avoid the development of Bt resistance for many decades (Gould, 1994; Roush, 1994ab). These conclusions are based on certain assumptions about the genetic basis of resistance to Bt in *H. armigera* (ie. that Bt resistance is recessive), but at present we have no knowledge of the type of resistance which might arise. The optimal dose of Bt toxin in the plants is one which will kill not only susceptibles but also a very high proportion (>95%) of heterozygous resistant individuals. Hence, only very small numbers of homozygous resistant individuals should survive on the plants. Random mating between these survivors and susceptible insects generated from the refugia will ensure that all progeny of any resistant survivors will be heterozygotes of which <5% might survive on the Bt plants. Achieving high expression of the Bt toxin in cotton is a job for the genetic engineers and breeders. Implementing refugia will be where growers and their consultants must play a key role.

The major criteria defining effective refuges are that they produce adult *Helicoverpa* and are not exposed to Bt sprays. Refuges must generate enough susceptible adult *Helicoverpa* to ensure that matings between two resistant survivors from a transgenic crop are extremely unlikely events. Since resistant survivors would likely be extremely scarce, the refuge need not necessarily occupy a significant area relative to the transgenic crop, though there are distinct advantages in having refuges in close proximity to transgenic crops to ensure random mating. In this context the potential use of Bt sprays on non-cotton crops becomes an issue. At present Bt sprays are used sparingly on cotton (Forrester, 1994) and not at all on other field crops. The introduction of Bt cotton may see Bt sprays targeted to these other crops, potentially compromising their value as a refuge for cotton. Thus, it will probably be necessary for the cotton industry to adopt a conservative position and implement refugia within the cotton system itself.

There are several options for providing refugia in cotton cropping systems. Refugia could be provided by other existing crop or non-crop host plants normally present in the cropping

system, by refuge crops specially planted by cotton growers, or by areas of non-transgenic cotton managed to allow some *Helicoverpa* to survive. Each of these options has advantages and disadvantages and the practical and economic consequences of each needs to be researched. Existing non-cotton crops, mostly dryland, may be highly variable in abundance depending on seasonal weather conditions and may not generate sufficient moths at the right times; other crops sown by cotton growers may be disruptive to farming practices and require substantial inputs. Normal (non-transgenic) cotton is a potentially favoured refuge since it should produce cohorts of susceptible moths in synchrony with any potentially resistant survivors which might emerge from the transgenic crops. Areas of normal cotton could be deployed as refuges in several ways - as seed mixtures of normal and transgenic plants in the same field, as strips of normal cotton in transgenic fields or as separate fields of normal cotton on each farm. A difficulty with this last option is that some damage to the crop would have to be sustained to ensure that sufficient moths are produced to provide an effective refuge. So inevitably refuges will impose some cost on growers. How much of a cost remains to be determined. Many of the options for using refuges are being actively researched now. At present all we can say is that refuges to maintain susceptibles will be important.

In thinking about resistance management we must also remember the minor caterpillar pests referred to earlier, some of which may also be candidates to develop resistance to the plants. Refugia might also be used to avoid resistance in these species, but the refuge might have to be a weed. For example Bladder ketmia is the main alternative host for rough bollworm. So another complication to consider. These issues highlight our poor understanding of the ecology and behaviour of these minor pests, which probably rightly have received little attention in the past. It will be useful for the future to commence some specific studies of species like rough bollworm so that we can better design resistance management strategies.

Components of a Management Strategy for Bt Cotton in Australia.

Over the next couple of years the details of a management strategy must be worked out through discussions among researchers, consultants and grower association.. Important components for such a strategy will include:

Regular crop checking -

other pests will remain and consultants will be essential for the management of transgenic crops and the implementation of resistance strategies

Effective refugia for susceptibles - within the cotton industry, preferably on farm to ensure the responsibility is shared among all growers

Strict crop hygiene - cultivation of the residues of transgenic cotton crops will be essential to ensure that no potentially resistant overwintering pupae survive

Gene pyramiding - hopefully other insecticidal genes besides Bt will become available. Pyramiding two genes in one variety will provide a much more stable system

Late season pesticide application - if some larvae do survive in transgenic crops late in the season a single application of HELIX could be used (if needed)

Monitoring of Bt resistance levels - underpinning the whole strategy will be regular monitoring of levels of Bt resistance in *H. armigera* and *H. punctigera*. At present we appear to have no resistance to Bt - we need to keep it that way.

Acknowledgments

I am grateful to Rick Roush (Cornell University) and Joanne Daly (CSIRO) for valuable discussions on the topics covered here, to the CRC for Sustainable Cotton Production and the Cotton Research and Development Corporation for research support and to Cheryl Mares, Vicki Wales, Judy Nobilo, Tracy Parker and Colin Tann for excellent technical assistance with the field and laboratory studies.

References

- CAPRIO, M.A. (1994) The interaction of Bt gene deployment and gene flow in insect populations. *Biocontrol Science and Technology* (in press)
- DALY, J.C. (1994) Resistance management strategies - don't ignore ecology. *Biocontrol Science and Technology* (in press)
- FITT, G.P., MARES, C.L., LLEWELLYN, D.J. (1994) Field evaluation and potential ecological impact of transgenic cottons (*Gossypium hirsutum*) in Australia. *Biocontrol Science and Technology* (in press).
- FORRESTER, N.W. (1994) Resistance management options for conventional Bt and transgenic plants in Australian summer field crops. *Biocontrol Science and Technology* (in press).
- GOULD, F. (1994) Potential and problems with multi-gene, high dose strategies for managing resistance to Bt toxins. *Biocontrol Science and Technology* (in press)

- MCGAUGHEY, W.H. & WHALON, M.E. (1992) Managing insect resistance to *Bacillus thuringiensis* toxins. *Science* **258**, 1451-1455.
- ROUSH, R. (1994a) Managing resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? *Biocontrol Science and Technology* (in press).
- ROUSH, R. T. (1994b) Can we slow adaptation by pests to insect-resistant transgenic crops?, in *Biotechnology for Integrated Pest Management* (PERSLEY, G. & MACINTYRE, R., Eds) CAB International, London, in press.
- TABASHNIK, B. E. (1994a) Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* **39**, 47-79.
- TABASHNIK, B. E. (1994b) Delaying insect adaptation to transgenic crops: seed mixtures and refugia reconsidered. *Proceedings of the Royal Society of London, Series B* **255**, 7-12.

