Biotechnology risks and benefits: the Ingard cotton example

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Abstract

Biotechnology has great potential for enhancing the productivity and sustainability of Australian agriculture through the development of transgenic crops and pastures. The incentive is to have improved tolerance to pests and diseases and better control of weeds while reducing reliance on synthetic pesticides. For some products, there is potential for increased yield as well. After five years of development and testing, 1996 saw the first large-scale commercial release in Australia of a genetically modified cotton (Ingard) with high tolerance to damage by caterpillars of *Helicoverpa*, the most serious insect pest of cotton. Ingard field experiments were carefully regulated by the Genetic Manipulation Advisory Committee for three years prior to the first commercial areas limited to 10% by the National Registration Authority. Growers are obliged to comply with a management strategy which is aimed at preventing *H. armigera* developing resistance to Ingard. In the first commercial season Ingard required only 43% of the insecticides required on conventional cotton.The insecticide sprays required on Ingard generally occurred later in the season because of reduced efficacy of the Ingard plants. Many of the agronomic management practices that growers and consultants had previously fine-tuned to their own production systems may need to be re-adjusted for Ingard cotton and new strategies explored to capture both the environmental and economic benefits inherent in the transgenic varieties. Other novel traits such as herbicide tolerance are not far from release and many new technologies are under development. Because of its power to mould the attributes of our management, biotechnology will allow us to develop production systems that are more viable, responsible and sustainable. The risks with use of biotechnology are carefully regulated in Australia, with a thorough and conservative approach being taken. That policy should continue.

Key words

Biotechnology, cotton, productivity, sustainability, Helicoverpa.

The manipulation of our agricultural plants and animals to better meet the need to clothe and feed our modern society has long been the goal of traditional agronomy. These conventional disciplines have now been launched into the technological age with the advent of biotechnology - the precise physical manipulation of the genetic make-up of our traditional crops and animals. This technology has already had a major impact on industrial microbiology: some foods we eat and medicines we take contain the products of biotechnology. Similar impacts on agronomy are to be expected despite still being in the early stages of development with genetically modified cotton, soybeans, corn, canola, potatoes and tomatoes. If the technical challenges can be overcome, the biotechnological approach to crop improvement holds the promise of making substantial contributions to improving environmental responsibility and sustainability of our modern agricultural production systems.

This paper will explore the development of one particular genetically modified crop, Ingard cotton, as an example of the power of biotechnology to assist in the environmental imperative of minimising the depend-ence of the cropping system on synthetic pesticides. This aim is to be achieved by the development of varieties that have some resistance to the main insect pests of cotton. Such an objective would relieve the cropping system of a significant pollution potential and may help break the cycle of insecticide discovery, overuse and development of resistance by insect pests.

The cotton production system evolution

The cotton industry has grown dramatically in the past two decades in Australia. Production in 1998 will exceed 600,000 tonne of lint, having been at levels around 45,000 tonne in the 1970's. Although local processing capability has increased, about 90% of cotton production is exported - mainly to Asia. The Australian cotton production system is intensive, with amongst the highest yields in the world. About 90% of the crop is irrigated, with high inputs of fertilizer and pesticide.

Given that the cotton plant is very attractive to insect pests and that pest numbers are high in Australia, considerable pest protection practices are required for profitable cotton production. However the intensity of insect control measures has risks. Resistance of *H. armigera* to DDT was one key component of the WA cotton industry demise up to 1974. DDT resistance was also evident in NSW and Qld at that time. In surveys of cotton pest control practices during the 1980s, Forrester *et a.* (7) found the average insecticide program involved 11 sprays involving endosulfan, pyrethroids and organo-phosphates, used in that order through the season. The careful use of pyrethroids during this time only delayed resistance in *H. armigera* for awhile - that chemistry was not effective at the field level by 1995. Instances of possible chemical contamination of water (endosulfan) and livestock (helix) also highlight the need to dramatically revise the cotton pest protection system in Australia.

Over the past 30 years in Australia there has been a continual evolution of chemical and management strategies to combat the direct and indirect cost of insect control in cotton. Varying elements of Integrated Pest Management (IPM) have been introduced, such as chemical group rotation (7), economic damage thres-holds (1), host plant resistance (14), beneficial insects (15, 10) and cultural methods (5).

These elements of IPM have not been released or adopted at the same time, nor have they been used consistently. The industry has recently developed a code of Best Practice which is the new generation of discipline to adopt IPM. Regulatory and economic incentives are also needed to encourage responsible farming practices.

In-built Host Plant Resistance (HPR) to insects in cotton cultivars is an attractive concept in IPM, but it cannot be viewed as a stand-alone IPM strategy. In the past, HPR traits such as okra leaf (14), have made significant contributions to reducing pesticide usage in cotton, but have relied upon reducing the preference for cotton as a host for insect pests rather than killing the pests. The science of biotechnology has the potential to substantially increase this genotype component of IPM through the development of insect tolerant cultivars with very strong in-built insecticidal properties, comparable to those of chemical pesticides. However, for long-term sustainability, Ingard should be only one components of an integrated approach to insect control.

Ingard cotton

Bacillus thuringiensis, a soil bacterium long associated with the fermented biological pesticide products such as Dipel, produces a number of potent but highly specific insecticidal proteins packaged during fermentation into protein crystals that together with the bacterial spores are toxic to many of the agriculturally important insect pests. Certain of these insecticidal protein genes (Bt or Cry genes) now form the basis for a number of insect control strategies in transgenic crops including cotton, corn and potato. Several of these Cry genes have been isolated from bacteria and modified to function in plant. When expressed in the plant from an introduced transgene the toxic proteins are present whenever the plant is attacked by a susceptible pest. The introduced genes are inherited just as any other Mendelian trait and can be manipulated by conventional plant breeding into elite commercial backgrounds.

The Bt gene we have been using in transgenic cotton was developed by the Monsanto Company as a fully synthetic gene that allows high levels of expression in plant cells and that encodes a protein almost identical to the naturally occurring CryIA(c) protein produced by the *kurstaki* strain of *Bacillus thuringiensis* used to produce the spray formulation (11). The Bt gene has been introduced by Monsanto into cotton to control members of the *Heliothis-Helicoverpa* complex of caterpillars that are pests of cotton world wide (in Australia - *Helicoverpa armigera* and *Helicoverpa punctigera*; in the US - *Heliothis virescens* and *Helicoverpa zea*). In cotton the gene is expressed everywhere in the plant and under laboratory conditions will kill neonate larvae of all the major *Heliothis-Helicoverpa* species, although *Helicoverpa* are more difficult to kill, particularly as older larvae (Jenkins *et al.* 1998). The CryIA(c) protein has no effect on other pests, beneficials or animals, as the protein is specific to sites on the gut wall of a few Lepidopteran species.

After extensive laboratory and field evaluation by Monsanto, transgenic cotton lines were backcrossed into elite cotton germplasm in the US (sold as Bollgard in Deltapine and Paymaster varieties) and Australia (sold as Ingard in CSIRO and Deltapine varieties). These breeding programs culminated in the first commercial releases of Bt cotton in Australia in 1996, but only after widespread testing and evaluation in small-scale field tests.

Performance of Bt cotton in terms of benefit to the farmers and the environment has differed in the two countries due to the different pest complexes.? Overall Ingard has been less successful in Australia because of the higher tolerance to the insecticidal protein exhibited by *Helicoverpa* and the different pricing policies for the technology between Australia and the US.

Regulatory processes in Australia

Because Ingard cotton involved the use of biotechnology it, like other genetically modified organisms (GMOs) produced using gene technology, is subject to much stricter regulatory control than are conventionally developed varieties. Multiple tiers of regulatory assess-ment begin with the Genetic Manipulation Advisory Committee (GMAC) that regulates both small-scale laboratory work involving GMOs as well as the small-scale field testing and eventual general release of transgenic plants, animals and microbes. GMAC is primarily responsible for the scientific assessment of the risks associated with biotechnology and takes submiss-ions from both proponents and the public. It is a non-statutory body which makes expert recommendations to the relevant authorities and is responsible for notifying Common-wealth, State and regional authorities of the intention to release transgenic organisms that might fall under their statutory domain.

A recent government inquiry has recommended changes to the advisory nature of GMAC and the formation of an all embracing Federal authority, the Gene Technology Authority (GTA), to streamline the process of the regulatory assessment of products of biotechnology and Parliament is in the process of drawing up the necessary legislation. The GTA would consult with all the relevant authorities and be the final arbiter of the release of transgenic plants, animals and microbes into commercial use.

Currently Ingard is viewed as a plant produced pesticide and therefore its commercial use is regulated by the National Registration Authority (NRA) for Agricultural and Veterinary Chemicals just like any pesticide spray. The NRA is responsible for assessing the safety and efficacy of pesticides and reviews applications from both proponents and the public, taking advice from relevant State and Federal agencies, including GMAC. Ingard, like a pesticide, has been conditionally approved for five years for use under specified label conditions and like a pesticide, growers must legally comply with the label conditions. Acting on advice from GMAC the NRA also imposed some geographical (exclude Central Qld) and area (10% of area) restrictions on the use of Ingard that were aimed at ensuring that its short-term commercial use would not compromise its potential long-term benefits to the cotton industry through the selection of resistant *Helicoverpa*. Therefore, a condition of the registration is that growers will adopt an industry approved management strategy for Ingard cotton. The relevant Industry body that advises the NRA and GMAC on the management issues is the Transgenic and Insecticide Management Strategy Committee (TIMS). This is a committee of the Australian Cotton Growers Research Association containing representatives from growers, industry, and scientists that discusses and proposes management strategies for both transgenics and conventional pesticide chemistries. In addition to the regulatory framework, growers also buy a single use license from Monsanto that constitutes a legal contract to follow the recommended management strategy and also to prevent growers from saving seed from one year to the next.

The regulatory history of Ingard in Australia

The first field study (1992/93) was very carefully contained. It had only 150 transgenic plants surrounded by a fence and then by a conventional (unsprayed) cotton buffer for 50 metres in all directions. This buffer was sampled very intensively to determine if there was any transfer of pollen from the transgenic plants to conventional cotton. The buffer and transgenic plants were destroyed by cultivation at the end of the season. The second season (1993/94) had a few thousand plants (0.03 ha) for similar studies; again the buffer was destroyed.

The third season (1994/95) had one 10 ha plot for very detailed measurements of the impact of Bt cotton plants on the environment, including any negative impacts on beneficial insects. The fourth season (1995/96) had four 10 ha blocks for similar measurements. This season also had some seed crops to provide for the following season.

The first commercial year (1996/97) was when NRA allowed up to 10% of the crop area to be sown to Ingard.? About 30,000 ha were grown - growers were obliged to comply with strict guidelines for managing the crop.? There was a reduction in insecticide use (see later). In the second year (1997/98) of commercial production, NRA allowed only 15% of the cotton area to be sown to Ingard. Again, growers were obliged to comply with strict management guidelines. The commercial area for 1998/99 is yet to be determined.

The scale of Bt cotton in the US by comparison, Bollgard has been planted on 600,000 ha in 1996 and 800,000 ha 1997, more than 25% of *Heliothis*-prone production areas. They have similar management requirements.

Efficacy in insect control by Ingard is of prime importance to the grower and field trials were conducted over a period of four years to assess efficacy under a variety of environments and management regimes and to convince both the growers and regulators of the value of the technology. All trials demonstrated that Ingard was a potent killer of *Helicoverpa* for most of the growing season but towards the end of the season efficacy declined as the plant vegetative growth ceased and boll maturation proceeded (6). This correlated with a reduction in plant protein production (including Bt). Spraying to control late season *Helicoverpa* was there-fore expected in some crops depending on the pest pressure at this time in the season. Overall, there were clearly large savings in pesticide usage; the trial crops generally required less than half of the pesticide needed to produce a conventional crop. By mid-1996 all regulatory agencies were convinced that the restricted general release of Ingard cotton posed no great threat to either the environment, society or the technology itself and it was cleared for commercial production.

Risks: concerns raised by GMAC for the commercial use of Ingard

Outcrossing

The consequences of an unregulated flow of novel genes from a crop to a native species or weed could be quite catastrophic both environmentally and in agricultural production systems, if the fitness of the native or weed were affected. Fortunately, in Australia, none of the species related to cotton are weeds and although we have quite a diverse flora of native *Gossypium* species their distribution is relatively sparse and contact with cultivated cotton is rare. Extensive genetic and cytological studies have been carried out on the Australian native *Gossypium* species and particularly in the Eastern cotton cropping areas the risk of genes escaping into the endemic species is considered negligible as any hybrids with cultivated cotton that can be artificially produced are completely sterile (3). Cotton has been grown in Australia for up to 200 years and no natural hybrids with native species have ever been reported. Furthermore, in most of the current cropping areas cotton will only grow as a annual plant and is usually killed by frosts in winter so this provides an additional safeguard against transgenic cotton establishing feral populations in the wild that could increase the likelihood for contact with the native species. This was one reason for the initial restriction of Ingard by the NRA to below latitude 26?S, as above this latitude winter conditions would allow cotton to grow as a perennial.

The escape of transgenes to wild species or weeds is not a trivial issue since in some cases gene flow into weedy species has been documented. Transgenic herbicide tolerance genes introduced into canola, for example, have been recovered in wild turnip, a related *Brassica* species that is a wide-spread weed in Europe, even several kilometres away from small-scale field trials (2).

Resistance management

There is a history of *H. armigera* developing resistance to chemical pesticides applied to agricultural crops in Australia, including cotton (7). Management to prevent or delay the development of resistance to Bt by the target insect pests is therefore considered the key to the successful and long-term commercial use of Ingard. Neither growers, scientists or Monsanto would like to see Bt technology lost through resistance and all recognise that management of the technology can play an important part in this process. Considerable research has been put to developing management strategies to minimise the likelihood of *H. armigera* developing resistance. The current strategy is described below.

Other

Other issues were raised at the time of requesting general release of Ingard but these were all satisfactorily resolved by the research that was carried out during the pre-commercial phase of the assessment of Ingard. These included a number of ecological issues related to the potential impact of the Ingard on non-target insect species that are either neutral or of some benefit in a cotton crop. The large body of information already available on the insecticidal proteins of *B. thuringiensis* suggested that the effects would be very specific as they are only toxic to a narrow array of insect species. Large ecological impact studies failed to find any significant effects on the several hundred insect species found in cotton fields, other than the expected reductions in the numbers of the very few insect species that exclusively predate on or parasitise *Helicoverpa*, since the numbers of caterpillars, their food source, in the Ingard crop was significantly reduced (L.J.Wilson *pers. comm.*). Overall these insects are not at risk as *Helicoverpa* has many other crop and native species hosts that can support the survival of these dedicated predators and parasites.

Since the products of the Ingard crop were destined for both human and animal use it was essential to assess the impacts on these end-users. Both the lint and oil derived from Ingard can be shown to contain no detectable Bt protein and were therefore exempted from the need to specify minimum residue limits by both the NRA and the Australian and New Zealand Food Authority. The seed does contain the Bt protein, but being a protein it is degraded in the stomach of both ruminant and non-ruminant animals and so poses no risks to these animals or to the consumers of the food products derived from them.

The Ingard management strategy

Management is the key to the sustainability of Ingard as a crop protection technology and the success of the management strategies will depend largely on the compliance by growers. The cotton industry is very close-knit and forward thinking, so it is expected that com- pliance will be very high. Grower participation in the TIMS Committee has been mentioned. The key components to the management of Ingard by the grower to minimise resistance development and the loss of the technology are:

- Planting of refuges. This can either be of large areas of conventionally sprayed cotton, small areas of unsprayed cotton or small areas of other crops that are hosts for *H. armigera*. The important element is that the refuge must generate sufficient numbers of susceptible moths that are synchronous with moths that might emerge from an Ingard crop (13). Any resistance genes in *H. armigera* moths emerging from Ingard will be diluted by mating with moths from the refuge. Compliance with refuge sizes will be relatively easy in the first few years when the total area of Ingard cotton is still relatively small, but will challenge growers in later years when Ingard cotton will be the dominant crop.
- Destruction of overwintering *Helicoverpa* pupae by cultivating soon after harvest.
- Compliance with planting windows and plough-down dates. It is important that crops are not left in the field to regrow after defoliation and harvest as this will extend the period of selection imposed on the insects.

- Compliance with recommended spray thresholds. Ingard crops require just as much pest monitoring as conventional crops. Growers need to be vigilant in their insect scouting to ensure that they are aware of what is going on in their fields and spray with pesticides if insect numbers exceed pre-determined thresholds. These control strategies are being slowly refined by experience.

Results - Commercial performance of Ingard

Ingard performance can be assessed from both an environmental stand-point in terms of the reduction in chemical pesticide usage that it has achieved and from a commercial stand-point in terms of the benefit to the growers in producing high yield and quality with acceptable costs.

The yield potential of Ingard varieties developed by backcrossing with elite conventional varieties was evaluated in two seasons of multisite experiments. The results showed that Ingard varieties had identical yield potential and fibre properties to their recurrent parent. Yields of sprayed and unsprayed experiments from 1995/96 are shown in Fig. 1.

There has been variation in field yield performance of Ingard. Fig. 2 shows the relative yield of Ingard at 13 sites. Although on average Ingard had higher yield than conventional, there were a few locations where Ingard yielded slightly less than conventional. Higher yields were obtained where Ingard offered better pest protection than was achieved by sprayed conventional cotton. Cotton has an indeterminate growth habit; maximum yields are obtained from the best balance between vegetative and reproductive growth. Where there were lower yields we believe there was a physiological effect in either (a) rapid fruit setting in Ingard (from better insect control), creating an earlier termination in vegetative growth and reducing relative yield potential, and/or (b) cool, cloudy and waterlogged conditions in January which was a penalty on Ingard when it had a greater fruit load than conventional cotton.

A survey in 1996/97 showed a 50% reduction in total insecticide applications to Ingard when compared with conventional cotton crops (9). For *Helicoverpa* sprays the reduction was greater than 50%. These data are summarised in Fig. 3. The saved insecticides were from the endosulfan and pyrethroid groups which are cheaper and are applied in the first half of the growing season. The use of late season organophosphate sprays was similar because of the reduction in efficacy of Ingard plants towards the end of the season (6).

The average cost of insect control was $263/ha on Ingard and $467/ha on conventional. The $204/ha difference was marginally less than the average cost incurred by growers for the technology fee charged by Monsanto. There was considerable variation in the insecticide programs to cotton crops. Frequency distributions are shown in Fig. 4.

There was low efficacy in some Ingard crops necessitating insecticide use on those crops. The causes of low efficacy (*ie.* reduced Bt protein production) are the subject of ongoing research. We suspect variable conditions of waterlogging, temperature extremes, cloud and the variable growth created by those factors to play a part in affecting Bt protein concentrations - and hence insect efficacy. The shapes of the two frequency distributions in Fig. 4 are actually very similar, demonstrating that variation in insecticide program also exists for conventional cotton. For Ingard, the frequency distributions were affected by a number of management factors: some managers were not aware of possible efficacy reductions and did not spray when necessary; other managers were spraying Ingard on fear of low efficacy. The management of Ingard will be refined as managers gain experience and confidence with the technology.

The future

The development of resistance by *H. armigera* is a concern for Ingard. To combat that threat, a second, but different Bt gene is being developed. This protein has a different site of action in the larvae gut wall, so any insect possessing resistance to one Bt protein will be killed by the other. Ecological models demonstrate that this approach will delay resistance by a factor of ten; eg if resistance were to occur in five years with the single gene, then resistance would not occur for 50 years with the two gene (12).

Breeding lines with two separate Bt genes have been field tested in 1997/98, with further small trials planned for 1998/99.

Other genes currently being evaluated for cotton include herbicide tolerance (Glyphosate, Bromoxynil). The real environmental benefits of the potential weed management system needs to be evaluated; it is possible that there could be a significant reduction in the amount of residual herbicide applied (4). Management trials with Roundup Ready cotton have begun in the past two years, with up to 5,000 ha possible in 1998/99.

Conclusions

For risks, a key message about the introduction of biotechnology in this Ingard example is that regulatory bodies have been thorough and conservative. This policy should be maintained as both opponents and proponents of a product should be satisfied. The research community is confident there will be no outcrossing or environmental concerns, but there needs to be vigilance about preventing resistance of *H. armigera* to Bt. There is still opposition to biotechnology among the environmental community and growers are disappointed by the pricing of Ingard.

For benefits, Ingard is the biggest step achieved to date in reducing reliance on pesticides to cotton. Although commercial performance of Ingard has been less than hoped, on a realistic time scale, biotechnology is still very new, so future products derived from these techniques can only improve.

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