Managing Resistance Risks in Biotechnology

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Introduction

Biotechnology has been practiced in agriculture for millennia, including through traditional selection for pest resistant crop varieties. Marker-assisted selection, where molecular genetic markers are used to accelerate the breeding process for key traits, is now widely used. Biotechnology is also being use to understand the basic genetic machinery of crops. The entire genome of the small weedy cabbage relative *Arabidopsis* has been sequenced, a major success which rivals the human genome project in scope and importance, and allows a much deeper understanding of plant function. Modern techniques in genetics and biochemistry have been applied to unravel everything from the effects of introduction practices on the genetic variability of insects and diseases brought from Europe for the biocontrol of weeds, to the characterisation of pesticide resistance mechanisms. The detection and diagnosis of pests and diseases has also been dramatically improved by molecular means, often through antibody systems, similar to home pregnancy test kits, and some can be used in the field. An example was the Lepton test kit to distinguish species of cotton bollworms (*Helicoverpa armigera* vs. *H. punctigera*).

The evolution of resistance in pests has been a concern for many pest management tools produced by biotechnology, including for microbial sprays such as those derived from *Bacillus thuringiensis,* and for classically bred pest and disease tolerant varieties. However, when it comes to managing resistance risks in biotechnology, the greatest concerns are directed at insect and herbicide resistant transgenic varieties, more commonly called genetically modified or "GM". This is partly because activist groups and the regulatory agencies that have had to react to them have been particularly concerned about any crops produced by the movement of genes between species. However, even among scientists already satisfied by overwhelming data on the human and environmental safety of transgenic crops (e.g., http://europa.eu.int/comm/research/quality-of-life/gmo/index.html), the enormous effectiveness and benefits of these crops have garnered special attention to make sure that the benefits last indefinitely.

Insect and Herbicide Resistant Transgenic Crops

Transgenic crops that are resistant to herbicides, viruses and insecticides are now commercialised in at least 15 countries, first in China with virus resistant tobacco in 1991. Collectively, countries growing GM crops now include nearly half of the world's population. The largest of these crops by far is Roundup Ready soybeans, accounting for 60% of the world's 70 million hectares of GM crops (www.isaaa.org). Roundup Ready soy is mostly produced in the US, Brazil, and Argentina, but also in Romania, Uruguay, and Bulgaria, and is imported into western Europe.

Pesticide use was reduced by GM crops by 20 million kg in 2001 due to 8 GM crops just in USA alone, in addition to increased profitability and adoption of minimum-till agriculture. GM crops also increased yields by 2 billion kg and saved $1.2 billion in production costs (http://www.ncfap.org/40CaseStudies.htm, see also www.ers.usda.gov/epubs/pdf/aer786/).

The only transgenic field crops commercially grown in Australia are insect resistant (Bt) and glyphosate-resistant (Roundup Ready) cotton, which use genes isolated from common bacteria to produce proteins that protect the crop from insect attack and the herbicide. The insect-resistant cotton uses genes derived from *Bacillus thuringiensis* (Bt), but genetically coded to express well in plants, to produce proteins that attack the midgut of some caterpillars. Bt has been used in sprays for more than 40 years, but due to its poor persistence, still accounts for less than 1% of the total insecticide market. When produced inside the plant, the persistence of Bt is much greater, and even pests that bore into the plant (and might not eat a spray) can be controlled with significant health and economic benefits (Shelton et al. 2002).

In contrast to the limited uses of Bt sprays, about 26% of the area planted to GM crops around the world carries a Bt gene. About 6 million hectares of this is Bt cotton; another 12 million hectares is Bt corn. Countries growing Bt crops include Spain and the Philippines (corn), South Africa and the US (cotton and corn); and China, Mexico, Colombia, and Honduras (cotton) (www.isaaa.org). Perhaps most importantly, India is beginning to adopt Bt cotton in a major way. Although India's current Bt crop is estimated at 100,000 hectares, it is a country with 8 million hectares of low-yielding cotton, with GM targeted for the 44% of the crop grown from hybrid seed.

Cotton production in particular has benefited from Bt varieties. Historically, almost half of the insecticide used in agriculture is applied to cotton, with roughly half of that used against caterpillars. Where grown in the USA, Bt cotton reduces insecticide use by 70-90%. In China, the reductions in insecticide use (and human poisonings) have averaged some 75% (Pray et al, 2000, Huang et al. 2002). Some reports claim effective increase of yields of 80% with Bt cotton in India (Qaim and Zilberman 2003). In Australia, the reductions have been about 50% over the last 3-4 years, but this should further improve with the introduction of "two-gene" cultivars, such as Bollgard II.

Further, there is no risk to the consumer. A key point about many genetically engineered crops is that the foods they produce are not genetically engineered. In the case of cotton (and canola for that matter), the foodstuff is oil, and like most oils and sugars, no detectable protein or DNA remains after processing. That is, sugars and oils produced from insect (or herbicide) resistant crops are the same as from standard crops.

However, the more controversial class of genetically engineered crops are those resistant to herbicides, but it should also be noted that the creation of non-transgenic herbicide tolerant crops such as Clearfield wheat is also an application of biotechnology with most of same potential risks. Herbicide tolerant crops have reduced the use of more persistent herbicides and have increased the adoption of reduced tillage, which reduces erosion, arguably one of the most important of all issues to sustainable agriculture. The Cotton Foundation in the US reported that 78% of growers who have moved to conservation tillage since 1997 credit the change to Roundup Ready technology, with about 59% of US cotton acres now being farmed using some form of conversation tillage (no-till or reduced till). Growers who have adopted conservation tillage indicated in the survey that they believe it saves an average of US$50 per hectare over conventional practices (*Progressive Farmer*, March, 2003). Still, environmental and agronomic concerns about herbicide tolerant crops include increased herbicide use, increased selection for herbicide resistance in weeds, increased weediness of the crops, and transfer of herbicide resistance to weedy relatives by hybridisation.

A major concern of environmental activists, that transgenic herbicide tolerant crops will lead to increased herbicide use, is inconsistent with data now available and seems to be due to misconceptions that growers carelessly over apply herbicides. Studies on herbicide use on transgenic crops in the US show clearly that there has been no overall increase. According to a USDA report in 1999, the "technology significantly reduced herbicide treatments for soybeans and, to a lesser extent, for cotton". For a more recent report, see www.ers.usda.gov/epubs/pdf/aer786/.

However, the potential for increased selection for herbicide resistance in weeds is a legitimate concern. Resistance to glyphosate is on the rise in the weed *Conyza* in association with the use of Roundup Ready soybeans in the USA, and is also increasing independent of transgenic crops in annual ryegrass (*Lolium rigidum*) in Australia (personal communication, Chris Preston, University of Adelaide) and California.

Managing Resistance in Bt Transgenic Crops

The concern based on the long history of evolution in pests that resistance can evolve to Bt toxins is borne out by experience with several insect species. After being first demonstrated some 15 years ago (Tabashnik et al. 1990), resistance to Bt sprays is now common in the diamondback moth (*Plutella xylostella*) in several tropical and subtropical areas around the world, to at least two different types of toxins, and these insects survive on Bt transgenic crops in laboratory tests (Perez and Shelton 1997, Imai and Mori 1999, Liu and Tabashnik 1997, Liu et al. 1996, Maruyama et al. 1999, Mohan and Gujar 2000, Sayyed and Wright 2001, Shelton et al. 1993, Tabashnik et al. 1997a, 1997b, Zhao et al. 2003). Resistance has also be selected relatively easily in various bollworms, including in species of *Helicoverpa*, *Heliothis* (Gahan et al. 2001), and *Pectinophora* (pink bollworms, see Tabashnik et al. 2003). Resistance in many of these strains seems to be to single major genes that reduce binding to the midgut and are recessive in inheritance (e.g., Tabashnik et al. 1997a, 1997b; Gahan et al. 2001). In at least some cases, resistance does not seem to come with large fitness costs (Tang et al. 1997). CSIRO Entomology has obtained resistance in the laboratory to both the Cry 1Ac and Cry2Ab toxins used in Bollgard II cotton (personal communication, Ray Akhurst and Rod Mahon).

However, despite intensive use of Bt cotton in some areas of the world, monitoring data show that resistance to Bt has not increased in any cotton (or corn) pest in the field (Tabashnik et al. 2003). There are several possible explanations for this, but the successful application of resistance management strategies, especially of non-transgenic refuges for susceptible insects, probably plays a major role, as documented in laboratory experiments (e.g., Tang et al. 2001). In particular, Bt cotton has been used roughly as intensively as pyrethroid sprays were in the late 1970s and 1980s in the southeastern US, for which resistance was detected to permethrin in 1986 (Luttrell et al. 1987), yet resistance to Bt crops is still unknown.

In the near future, a key to resistance management will be the use of "pyramided" two Bt gene cotton, and avoiding the use of single Bt gene cotton. Two Bt gene cottons, such as Bollgard II, not only provide better control of bollworms within any given season, they can delay resistance much more effectively with smaller refuges (Roush 1998). In essence, individual larvae that are resistant to one Bt toxin can be killed by the other, so long as the expression of each of the toxins is sufficient to kill most (preferably at least 95%) of the individuals susceptible to that toxin.

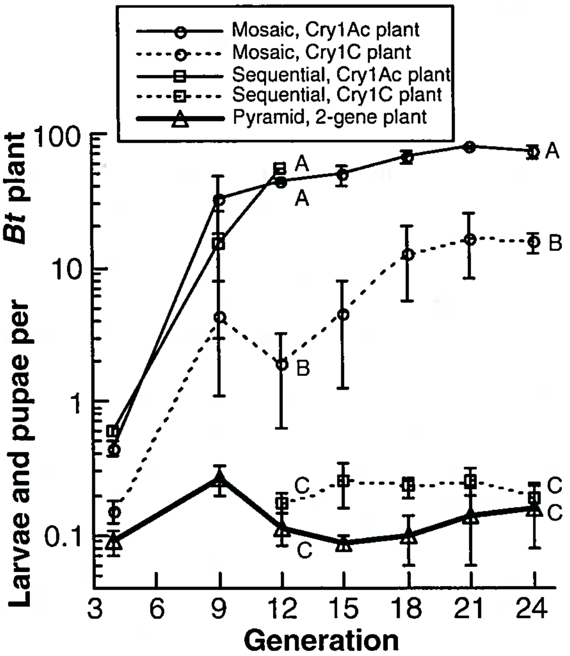
The relatively greater effectiveness of pyramided two gene plants can be illustrated by experiments that have been conducted with the diamondback moth, using resistance genes collected from populations in which have evolved resistance to Cry1Ac and Cry1C toxins, which like Cry 1Ac and Cry 2Ab for bollworms, show no evidence of cross-resistance. In cage experiments using broccoli plants that express either one of the toxins or both, and including a 20% refuge of non-transgenic plants, resistance evolved more quickly to single gene plants whether used in a mosaic (with half of the Bt plants expressing Cry1Ac or Cry1C), or to the use of Cry1Ac plants alone, than to pyramided plants, despite an initial frequency of the resistance alleles that is much higher than expected in the field (Zhao et al., 2003). After resistance to the toxins evolved to high frequencies, the densities of larvae increased dramatically, whereas as the densities stayed low in the cages with pyramided plants (Fig. 1). Additional experiments have shown that a mosaic of plants with one gene or two also leads to more rapid resistance (Zhao et al. submitted). Thus, an important part of resistance management with the introduction of Bollgard II will be the removal of INGARD varieties to reduce selection pressure where the Cry1Ac gene is unprotected by Cry 2Ab.

Further, where single Bt gene cotton is used, a much larger refuge is required for an equivalent delay of resistance compared to two-gene cottons. Thus, the Australian strategy will continue to require much larger refuges for single gene than two gene cottons.

Even with pyramided plants, some non-host plants are needed as a refuge to delay resistance. Otherwise, it remains possible that some doubly resistant insect families will occur, and without a refuge to promote outcrossing and a separation of the resistance genes, these resistant insects are likely to generate another generation.

The Australian resistance management strategy to date has focused on protecting Cry 1Ac, to make sure that any resistance genes to Cry1Ac would remain at a low frequency. In this way, Cry1Ac would remain effective in killing the vast majority of larvae on pyramided plants. Bt cotton with just one Bt protein was never going to be an effective long-term product for Australia. Because *Helicoverpa* species are much less susceptible to Cry 1Ac than *Heliothis virescens* and pink bollworm, which have been the most important pest species in the US with respect to resistance, pyramids are more important here than in the US. INGARD was only ever seen to be a stepping-stone to a much more sustainable two-Bt gene product, which was originally expected to be available within 4 years of INGARD being released.

Figure 1. Population densities of Cry1Ac/Cry1C-resistant diamondback moth in cages with different *Bt* broccoli plants: (Mosaic) 40% Cry1Ac plants + 40% Cry1C plants +20%refuge; (Sequential) 80% Cry1Ac plants + 20% refuge until control failures occurred, when the Bt plants were replaced by Cry1C plants; and (Pyramid) 80% plants with pyramided expression of Cry1Ac and Cry1C + 20% refuge of non-*Bt* broccoli. Within the same generation, means (± SEM) followed by the same letter are not significantly different ($P > 0.05$, HSD).



Of particular concern in Australia is that although the frequency of the resistance genes for Cry1Ac in *Helicoverpa armigera* is something less than 0.001 (Ahmad and Roush 1999), the frequency of resistance to Cry2Ab unexpectedly seems more common, in the range of 0.002 (personal communication, Rod Mahon, CSIRO Entomology), which is higher than estimates for the same resistance in *Helicoverpa zea* in the US. This will require continued vigilance, and adaptation of the resistance management strategy as new information becomes available over time.

By managing the major pests of Australian cotton with Bt transgenics, the focus of pest management may change to dealing with what had been the minor or secondary pests. Bt cotton probably reduces the risks of resistance in pests not directly suppressed by Bt toxin, for two reasons. First, with fewer sprays for bollworms, there is less exposure of aphids and mites (as examples) to insecticides. Second, studies from around the world consistently show that populations of predators and parasitoids, the natural enemies of secondary pests, are higher in Bt crops than non-Bt crops managed conventionally. This in turn reduces the need for pesticide applications for pests such as aphids and mites. By further reducing the need for sprays, two gene cottons like Bollgard II can be expected to further reduce insecticidal selection pressure on aphids, mites, and plant bugs, and further encourage the reliance on non-chemical tactics for their control.

Managing Herbicide Resistant Transgenic Crops

Finally, resistance management for herbicides, especially glyphosate given its widespread use even independent of Roundup Ready cotton, must not be overlooked. Resistance has not been an issue yet in weeds of cotton, perhaps because of the extent of chipping that removed resistant weeds. However, the use of chipping may diminish, especially with the use of ever more efficient herbicide tolerant cotton varieties.

Although glyphosate resistant weeds does not appear to be an eminent threat, it is not one that should be taken lightly, especially given that it may be fairly easily avoided. From research undertaken thus far in Australian on annual ryegrass, it appears that simply avoiding the use of glyphosate at key times of the season in at least one year in three in any given paddock will be a key to extending the durability of this herbicide. Thus, as Australian resistance expert Professor Steve Powles of the University of Western Australia has often said for herbicide resistance management, "When on a good thing, don't stick to it!"

In an effort to address the problems discussed above (and more), a committee for the Standing Committee on Agriculture and Resource Management (SCARM) developed report in 1998 entitled "**Good Agricultural Practice Guidelines for the Use of Genetically Modified Plants**", which are rather self-explanatory but still useful to review. On the subject of herbicide tolerant crops, the "GAP report" recommended that:

- Deployment of the technology should ensure the sustainable use of herbicides and/or should lead to use of more benign herbicides.
- If the herbicide to which resistance has been introduced is currently used to control volunteers of the crop, or other weedy outbreaks of the crop, management plans will be required for control of these weeds by other means.
- Ideally, pyramiding of genes for resistance to more than one herbicide in a given cultivar should be avoided, unless experimentally demonstrated to be useful/effective in a particular farming system.
- Ideally, the same herbicide resistance trait should not be introduced into different crops used in a rotational system in a given region. However, if this does occur, management plans should be devised to limit the use of the same herbicides on the same paddocks in the successive years, to avoid the development of herbicide-resistant weeds.

Conclusions

Biotechnology has and is contributing to agriculture in a variety of ways that reduce the environmental impacts of agriculture and lower or even eliminate some risks to farm workers.

Cotton has been a particularly important beneficiary of agricultural biotechnology, with reductions in insecticide use of as much as 90% in some cases from Bt cotton, increases in yields, and reductions in tillage from herbicide tolerant cotton.

Unfortunately, the long-term benefits of both insecticidal Bt cotton and herbicide-resistant cotton are at some threat from the evolution of resistance in insects and weeds. Resistance to Bt sprays is now common in the diamondback moth (*Plutella xylostella*) in several tropical and subtropical areas around the world, and these insects survive on Bt plants in laboratory tests. Resistance has also be selected relatively easily in various bollworms, including in species of *Helicoverpa*, *Heliothis*, and *Pectinophora* (pink bollworms). Herbicide resistance is also on the rise to glyphosate in association with the use of Roundup Ready soybeans in the USA, and is also increasing independent of transgenic crops in annual ryegrass (*Lolium rigidum*) in Australia and California.

Despite intensive used of Bt cotton in some areas of the world, monitoring data show that resistance to Bt has not increased in any cotton pest in the field. There are several possible explanations for this, but the successful application of resistance management strategies, especially of non-transgenic refuges for susceptible insects, probably plays a major role. It is nonetheless far too early to become complacent about resistance.

A key to the future will be the use of two Bt gene cotton, and avoiding the use of single Bt gene cotton. Where single Bt gene cotton must be used, a larger refuge will be required for an equivalent delay of resistance compared to two-gene cottons. In all cases, an appropriate refuge of some non-Bt cotton or non-cotton alternative food plants ("hosts") will be needed to slow or prevent resistance.

Bt cotton probably actually reduces the risks of resistance in pests not directly suppressed by Bt toxin, for two reasons. First, with fewer sprays for bollworms, there is less exposure of aphids and mites (as examples) to insecticides. Second, studies from around the world consistently show that populations of predators and parasitoids, the natural enemies of secondary pests, are higher in Bt crops than non-Bt crops managed conventionally. This in turn reduces the need for pesticide applications for pests such as aphids and mites.

Finally, resistance management for herbicides, especially glyphosate given its widespread use even independent of Roundup Ready cotton, must not be overlooked. From research undertaken thus far in Australian on annual ryegrass, it appears that simply avoiding the use of glyphosate at key times of the season in at least one year in three will be a key to extending the durability of this herbicide. "When on a good thing, don't stick to it!"

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