Biotechnology and Weed Management

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Abstract
Applications of biotechnology for weed management include research, diagnostics, development of biological herbicides, the use of marker-assisted selection and other techniques to improve breeding for weed competitive crop cultivars, and the development of transgenic crops, including herbicide tolerant cultivars. Herbicide tolerant crops offer significant potential advantages, but are not a magic bullet. Their optimal use will require careful consideration of resistance management and market implications. In the longer term, more significant benefits are likely to come from crop cultivars that are more competitive with weeds. Some of these may be produced with the aid of transgenesis.

Introduction
Biotechnology has been practiced for millennia, including the fermentation of alcoholic beverages, and derivation of medicines from the natural pharmacopoeia. A particular subset of biotechnology is genetic modification (GM), which we have also been doing for centuries with artificial selection of bacteria, plant and animals, including trans-species hybrids. More recently, we have used highly artificial techniques such as radiation and sophisticated "embryo rescue" methods, but as you know, the sort of GM that has people stirred up is "transgenesis", the moving of genes between highly unrelated species. The fact that this seems "unnatural" to many people, plus the involvement of multinational corporations (raising the spectre for some of "globalisation" and the industrialisation of food supply) lends the debate to a high level of rumour and emotion.

However, there is more to the application of biotechnology to pest management than just transgenic organisms. A recent general review of the potential applications of molecular biology in weed management was commissioned by the Weeds CRC and published by Paltridge (2000). Some key current or promising applications include:

Studies of genetics and biochemistry
The multitude of techniques in molecular biology has been widely applied to understanding the basic genetics and ecology of weeds and their natural enemies, including fungi and insects. Modern techniques in genetics and biochemistry have been applied to unravel everything from the effects of introduction practices on the genetic variability of insects introduced from Europe for the biocontrol of weeds to the characterisation of herbicide resistance mechanisms. It is arguable that such research, and the applications to management that have or will result, has been the most important influence of biotechnology on weed management.

Molecular diagnostics for pest detection and identification
These are often through antibody systems, similar to home pregnancy test kits, and some can be used in the field. A commonly used example is the Lepton test kit to distinguish species of cotton bollworms (*Helicoverpa armigera vs H. punctigera*). In other cases, often using more sophisticated PCR and other technologies, the diagnostic services are quite centralised and automated, as at the SARDI soil test centre at the Waite campus in Adelaide. There is no public controversy about such diagnostic tools, and we can expect to see them increase as customer demand develops.

I am unaware of any routine diagnostic methods that have been perfected for use with weeds. However, there have been discussions to do so for cases in which the weeds (or their seeds) are difficult to identify and where there are important biological differences that affect weed management in the field or export requirements. For example, Dr Kathy Evans with the Weeds CRC in Adelaide has developed methods based on microsatellite variants to distinguish between different clones and species of blackberries (*Rubus* spp.), which have differing sensitivities to the rust disease used for their biological control (*Phragmidium violaceum)*. Another problematic case is the Giant Rat's tail grass complex (*Sporobolous* spp.), where some of the species are native and not weedy. Dr Steve Adkins at the University of Queensland has been working on developing diagnostics on this genus through the CRC for Tropical Plant Protection.

On the other hand, molecular diagnostic methods have been developed for identifying and quantifying herbicide resistance in weeds. It is still too early to tell if these will show significant benefits over high throughput bioassay methods that are not limited to particular resistance mechanisms.

Mycoherbicides

The development of fungal pathogens for weed control has been the subject of considerable effort around the world. I don't intend to review it here, except to comment that problems with formulating the fungal spores to provide a sufficient 8-16 hour "dew period" for their germination has been a severely limiting factor in their development for broad area agriculture, and one in which our CRC has put considerable effort. However, there have been limited uses or excellent promise in certain systems (like rice) and "paint on" applications.

Marker-assisted selection and genomics

As I finish this paper, it has just been announced that the entire genome of the plant *Arabidopsis* has been sequenced, a major success which rivals the human genome project in scope and importance. This will no doubt have major ramifications for many breeding efforts, not least of which is the improved breeding of competitive crop cultivars. In contrast to transgenesis, there has been very little controversy about biotechnological approaches that accelerate what some still think passes for "traditional" breeding. It must be obvious that such methods have some marketing advantages over transgenics in the current commercial climate.

Work in our CRC has already shown that more competitive crops accomplished through increased seeding rates can reduce herbicide use. The Centre for International Economics in Canberra has estimated that our work in accelerating the adoption of competitive crops has the potential to deliver $124 million of benefits in present value terms over the next 30 years compared with outlays of $4.5 million. The internal rate of return on this project is estimated to be 43 per cent. However, differences between cultivars show that still more can also be done with the genetics of crop varieties. With the increased onset of resistance to herbicides in weeds, particularly in southern cropping systems, and with the very limited offerings of new herbicides, it may well be that crop competitiveness will form a cornerstone of weed management in a few decades, which will increase its significance in crop breeding programs.

There are a number of potential ways to improve crop competitiveness, including allelopathy, improved access to water, nutrients and light, and improved exploitation of microorganisms, especially in the soil (1). However, one of the most important outcomes from plant molecular biology would be to improve the efficiency of photosynthesis. RuBisCO is the world's most abundant protein and the principal catalyst for photosynthesis. It has also been described as the "nearly the world's worst, most incompetent enzyme" (2). Following discoveries in the late 1990's of red algae with RuBisCO some three-fold more efficient than that in higher plants, genetic engineers have been aiming for a more efficient RuBisCO in crops. In addition to the obvious ramifications for yields, such crops might simply outgrow weeds because of their energetic superiority. It may be possible to achieve some or most of these approaches to increased competitiveness without moving genes between plant species, but some interventions may be feasible in the next few decades only by transgenic approaches.

Transgenic crops

Transgenic crops that are resistant to herbicides, viruses and insects are now commercialised in several countries, but first in China. The only transgenic agronomic crops commercially grown in Australia are insect resistant (INGARD) and glyphosate-resistance (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 isolated from *Bacillus thuringiensis* (Bt), 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.

The benefits from Bt cotton are enormous. 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 Australia, the reductions have been about 50% over the last 3 years, but this should further improve with the introduction of "two-gene" cultivars, perhaps in 2003. In short, Bt cotton alone has the potential to wipe out 10- 25% of the world's agricultural insecticide use, and probably an even greater proportion of its risks. Preliminary estimates from China suggest that Bt cotton is already saving at least 60 lives per year from reductions in accidental insecticide poisonings. I have worked on cotton insect pest management since 1975, and have never seen a more promising approach for long term reductions in pesticide use.

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, the foodstuff is cottonseed 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.

Environmental and agronomic concerns include increased resistance in insects and effects on non-target species. Resistance management strategies for insect pests are well advanced (3,4). Impacts on non-target species are under investigation, but in spite of the publicity generated by a small laboratory study on Monarch butterflies (and the lack of publicity on field studies refuting the claims), the effects of Bt crops on non-target species are clearly much less than in conventional agriculture.

Herbicide Tolerant Crops

However, the more controversial class of genetically engineered crops and more relevant to my topic are those crops resistant to herbicides, but it should also be noted that the creation of non-transgenic herbicide tolerant crops is also an application of biotechnology with most of same potential risks. Environmental and agronomic concerns 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.

One herbicide tolerant crop, triazine tolerant canola, has been planted on a more than a million hectares of southern Australia in a single year, but has attracted little public attention. Why not? The resistance was transferred to canola (from a weedy relative) through classical breeding and not by molecular means. Nonetheless, the use of this canola has produced a major shift in cropping in southern Australia, essentially because the triazine tolerance allows superior weed control, so good in fact that it overcomes a 10-20% yield penalty inherent in the resistance.

The use of herbicides is sufficiently vexing in its own right, with much of the public believing that there are alternatives that can be used in broad scale agriculture. A less impassioned view is that our job as public servants is to try to understand how to balance and reduce the overall impacts of agriculture on the environment and risks to human health. The environmental costs of one alternative to herbicides, cultivation, are clearly much higher than herbicides. Not only does reduction in tillage reduce soil loss, it increases soil organic matter and reduces loss of soil carbon to the air as CO_2, thereby potentially reducing atmospheric warming.

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. First, 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 http://www.ers.usda.gov/epubs/pdf/aer786/

Second, we all know that growers even now don't use the full labelled rates of many herbicides just to save costs, so the mere fact that a crop can tolerate a high level of herbicide use has provided no incentive to growers to actually use that much herbicide. Finally, even if there was an increase in use, the key issue should be what is the environmental impact of the use. Even if glyphosate, for example, did replace sulfonylureas or imidazolinones used at a lower rate, there is lower effective persistence with glyphosate. What's more important, a simplistic calculation of kilograms applied, or an assessment of the impacts?

Herbicide resistant crops may provide many advantages to Australian agriculture, as triazine tolerant canola has already done. However, the potential for increased selection for resistance in weeds and management of herbicide resistant volunteers, in particular, must be addressed if herbicide resistant crops are to provide their maximum sustained value to growers. In the short term (at least the next 5 years), these crops will in essence provide new uses for old herbicides and old herbicide groups. However, in the future, herbicide tolerant crops may facilitate the introduction of new broad spectrum herbicides.

Herbicide tolerant varieties of a number of crops are under development, but the most important initially outside cotton will be the various herbicide tolerant canola and wheat varieties, and they are useful to illustrate many of the issues involved. In addition to the triazine tolerant canola, imidazolinone ("imi") tolerant canola, which was developed via mutation and selection, was introduced last year, with imi-tolerant wheat to follow this year. The next few years may see the introduction of other herbicide resistant canola varieties including the genetically-engineered glufosinate resistant ("Liberty Link") and glyphosate resistant ("Round-up Ready") canolas.

Is classically bred resistance safer or more environmentally friendly than genetically engineered? Just for comparison, atrazine is used on triazine tolerant canola at 1-2 kilograms active ingredient per hectare. Atrazine has a history of ground and surface water contamination, especially in North America. In contrast, glyphosate would probably be used at about 1 kg/ha on the "Round-up Ready" canola. Rarely cited as an environmental contaminant, the US National Academy of Sciences in 1987 (5) rated glyphosate as among the least risky pesticides to human health. Both canolas offer similar threats from outcrossing to weeds and weedy volunteers.

As with canola, the use of some kinds of herbicide tolerant crops could encourage the replacement of persistent and potentially risky herbicides with less persistent and relatively safe ones like glyphosate. By allowing new uses of herbicides, these crops open new options for weed management, particularly for weeds that are increasingly resistant to currently used and often more selective herbicides. However, this may be a case of simply shifting selection pressure.

The increased use of herbicides which are already extensively used could increase the incidence of herbicide resistant weeds. There is already evidence that atrazine use on triazine tolerant canola has increased triazine (eg., simazine) resistance in annual ryegrass in WA. In addition, the detection of glyphosate resistance in a few populations of annual ryegrass has heightened concerns that glyphosate-resistant canola will increase selection for glyphosate resistance in annual ryegrass and other weeds.

The best way to avoid increased problems with resistance in weeds from the use of transgenic crops will be to avoid over-reliance on the relevant herbicides, and especially to avoid the use of the same herbicide group on the same paddock two years in a row. Glyphosate is a special case, since it is often used annually as a pre-plant application with only moderate selection pressure. One possible method to maintain moderate selection pressure even with Round-up Ready canola would be to avoid pre-plant use of glyphosate in the year following canola, which would allow susceptible seeds from the seed bank to escape glyphosate selection after germination and dilute resistance among offspring of glyphosate survivors.

However, there is also concern about imi-tolerant ("Clearfield") canola and wheat. Resistance to the ALS-inhibiting group B herbicides (eg., sulfonylureas such as Ally and Glean, and imidazolinones such as Spinnaker) is very common in annual ryegrass and increasingly so in Western Australia and South Australia. The introduction of both Clearfield canola and Clearfield wheat onto the same paddocks in a crop rotation will surely increase selection pressure on group B herbicides, and hasten their ineffectiveness. Growers and their advisors need to carefully consider how to use their group B herbicides to maximise their long term returns.

Outcrossing Between Herbicide Resistant Crops and Weeds: Potential for Gene Transfer

Another concern is the potential for herbicide resistance genes to be transferred from crops to weeds. Fortunately, few crops grown in Australia have any wild relatives (much less weedy relatives!) with which they can interbreed. However, there are concerns about canola, which can potentially hybridise with wild radish and Buchan weed (*Hirschfeldia incana*). Drs Mary Rieger, Chris Preston and Steve Powles of the CRC for Weed Management Systems have been studying the potential for hybridisation between canola and wild radish in large scale field experiments that use herbicide resistance to identify potential hybrids. These experiments, which have screened more than 52 million seeds, have found two plants that are hybrids on the basis of their herbicide resistance and chromosome numbers.

These experiments already imply that hybridisation is no more common than the naturally occurring genes for resistance to imidazolinones (at about 10^{-5}) and triazines in weeds. However, there may still be concerns about the potential for the transfer of glyphosate resistance to wild radish, even though most growers seem not to rely on glyphosate for control of wild radish anyway.

Herbicide tolerant volunteers

Genetically engineered crops will also require generally more detailed record keeping and accountability (particularly those crops that must be grown under license) and greater crop hygiene to avoid problems with resistant volunteers (eg., of resistant canola). The have already been reports of hybridisation of different herbicide resistance types from neighbouring cultivars in Canada, with irritating effects on the flexibility of growers in controlling the volunteers in other crops. Thus, it may prove useful to plant cultivars with different herbicide tolerances at least a few hundred metres from one another to avoid natural crosses that produce volunteers with multiple herbicide tolerances, and to avoid the intentional stacking of multiple herbicide resistance genes in the same cultivar. I understand that European regulations forbid the development of plants resistant to several herbicides (New Scientist, 21 October 2000, page 6).

Good Agricultural Practice Guidelines

In an effort to address the problems discussed above (and more), a committee for the Standing Committee on Agriculture and Resource Management (SCARM) developed a report entitled "Good Agricultural Practice Guidelines for the Use of Genetically Modified Plants". 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 successive years, to avoid the development of herbicide-resistant weeds.

Market Access for Transgenic Crops

A further issue of which all will be aware is that some potential markets have refused access for genetically engineered crops, especially in Europe. Thus, it will be very important for the Grains Council of Australia and other such organisations to consider these trends and take appropriate steps to advise growers and perhaps government officials to be certain that Australian grain is not disadvantaged on the world market.

The CRC for Weed Management Systems, the GRDC and other organisations are actively investigating the advantages and potential problems of herbicide tolerant crops. Ultimately, it will be grain growers who will have to weigh up the risks and benefits of herbicide tolerant crops, and decide which to grow and under what management systems. For the best future of the industry as a whole, however, grain growers should make this decision collectively through their organisations rather than individually. Herbicide resistance in weeds and international marketing are issues that clearly transcend individual growers.

Conclusion

I often hear that consumers never asked for genetically engineered crops and stand to gain little from them. However, consumers have consistently demanded a reduction in the health and environmental risks of agriculture. At least some transgenic crops answer that demand. More importantly, the risks of pesticide exposure are far greater to farm workers that to the general public. We need to think carefully about both the risks and benefits of transgenic crops.

There are potential risks of genetically modified crops, but many of those raised in the popular press have achieved the status of urban myths. For example, there are no tomatoes or strawberries with fish genes to resist cold. The much publicised hazards of insect resistant Bt corn to Monarch butterflies were based on a biased laboratory study and have since been debunked by extensive field trials that supported the original risk assessment by the US EPA. Genetically modified foods are much more extensively scrutinised than many potentially risky conventional foods that are on the market and not receiving attention. Still, thorough regulation is needed, and not all proposed genetic modifications should be allowed.

The public has every right to reject genetic modification of plants, but it also has the right to be well informed about its choices. Otherwise, the choices may not be wise either for the general public or the people who produce our crops.

However, with the continuing evolution of resistance in weeds, and the relative lack of herbicides to replace them, it seems unlikely that herbicide resistant crops can be relied upon for much of the long-term solution to weed problems. Other applications of biotechnology will also be important, especially the more sustainable use of crop cultivars that are highly competitive with weeds.

References

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