Development of crops resistant to herbicides

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Summary

Over the past eight years there has been exciting progress in the application of genetic engineering to the development of crops resistant to herbicides. Two broad scientific strategies have been pursued; herbicide detoxification and alteration of the target site of herbicide action. Notwithstanding the obvious commercial and regulatory issues associated with the introduction of this technology, the main benefits which may accrue are likely to arise from more effective weed control, reduced costs of weed control, overall reduction in herbicide usage and a reduction, or even elimination, of cultivation, thereby providing a long-term soil conservation benefit.

Introduction

Herbicide application is an integral part of most crop, pasture and forestry management practices in developed, and increasingly so in less, developed nations. Ideally, herbicides are used to kill weeds, plants which are in the wrong place at the wrong time; undesirable plants which can contaminate the harvested product and compete for resources, thereby reducing yield.

Direct and immediate weed control can be achieved either by mechanical means, which includes cultivation and hand weeding, and through the application of herbicides. Often both mechanical and chemical-based methods are used in an integrated manner. In a fully integrated and sustainable system prior management can reduce the potential for weed infestation by eliminating or reducing the weed population through exploitation of other land management systems, such as fertilizer application, controlled grazing and alternating the pattern of land use.

Modern herbicides are increasingly selective, phytotoxic at low application rates and pose little to no risk of their residues finding their way into the food chain or general ecosystem. In addition, typically non-selective herbicides have lent themselves to the increasing utilization of reduced tillage practices and the consequential improvement in soil conservation practices.

Herbicides are selective because some plants are naturally able to resist the phytotoxic ingredient of the herbicide formulation. Herbicide tolerance can occur through several mechanisms, including:

- differential uptake or partitioning of the herbicide, so that it is unable to gain access to its target site of action;
- metabolism or detoxification of the herbicide; and
- altered sensitivity to the herbicide at the target site of herbicide action.

It may be possible for plants to utilize more than one of these strategies. Two of these mechanisms, detoxification of the herbicide and altered sensitivity at the target site of herbicide action, have formed the basis of strategies to develop herbicide-resistant plants by genetic engineering.

Development of herbicide-resistant plants

Detoxification of the herbicide

This strategy is attractive in those instances when the biochemical basis of the mode of action of the herbicide is not known, or when the target site of herbicide action is difficult to access by genetic engineering.

Stalker (personal communication) has suggested that detoxification enzymes should satisfy the following criteria:

1. The enzyme should be encoded by a single gene, not require complex co-factors for activity and exhibit high affinity and activity towards the herbicide substrate.
2. Any product(s) of the detoxification reaction should be non-phytotoxic and not be further metabolized to compounds

which are phytotoxic or otherwise deleterious to the plant.

3. The detoxification enzyme should exhibit strict substrate specificity for the herbicide and not be able to react with naturally occurring compounds which may have an essential function within the plant.

There are two well documented examples of transgenic, herbicide-resistant plants developed through application of the detoxification strategy.

Bromoxynil

Bromoxynil (3,5-dibromo 4-hydroxybenzonitrile; Figure 1) is a registered herbicide (Buctril®) for the control of broad-leaved weeds. Cereals are naturally tolerant because they are able to metabolize bromoxynil to a series of products with negligible phytotoxicity (1):

3,5-dibromo 4-hydroxybenzonitrile

↓

3,5-dibromo 4-hydroxybenzamide

↓

3,5-dibromo 4-hydroxybenzoic acid

In soil bromoxynil is rapidly degraded by a nitrilase enzyme to 3,5-dibromo 4-hydroxybenzoic acid (9).

This observation formed the basis of the approach taken by Calgene Inc. to develop bromoxynil-resistant crops. Starting with bromoxynil-treated soil, a nitrilase with strict specificity towards bromoxynil was isolated from *Klebsiella ozaenae* (11). The nitrilase gene *bxn* was cloned (15) and introduced into tobacco and tomato. Transgenic plants expressing the *bxn* gene displayed high levels of resistance to the application of up to 10-fold field rates of bromoxynil application. In subsequent field trials with transgenic tomato and cotton expressing the bromoxynil-specific *bxn* gene there was no effect by the herbicide on growth or yield.

Phosphinothricin

Phosphinothricin is a non-selective herbicide (glufosinate; Basta®) which is phytotoxic due to its inhibition of glutamine synthetase, the enzyme responsible for ammonia assimilation in plants. When glutamine synthetase is inhibited, ammonium ions accumulate and these are phytotoxic (16). Phosphinothricin, an analogue of glutamic acid, was originally discovered as the phytotoxic constituent of bialaphos, a tripeptide antibiotic comprising two alanine residues and phosphinothricin (Figure 1). Bialaphos is produced by *Streptomyces hygroscopicus* and has little to no inhibitory activity. However, intracellular peptidases can remove the L-alanyl-L-alanine dipeptide and, as a consequence, release the phosphinothricin residue which is autotoxic to the *S. hygroscopicus*. In order to cope with this unwelcome event, *S. hygroscopicus* has evolved an effective mechanism to detoxify the phosphinothricin through acetylation of the free NH_2 of phosphinothricin by the enzyme phosphinothricin acetyltransferase (17). The gene encoding the phosphinothricin acetyltransferase *(bar)* has been isolated and characterized (12).

Transgenic tobacco, potato and tomato plants resistant to phosphinothricin have been developed by transfer and constitutive expression of the *bar* gene (4). Field trials with transgenic tobacco and potato confirmed the herbicide resistance (10).

Altered sensitivity at herbicide target site

In simple terms, resistance could be achieved by over-production, or by modification, of the herbicide target so that it is no longer sensitive to the herbicide. In order to exploit this approach for the development of herbicide-resistant plants, it is necessary to identify the biochemical target for herbicidal activity. There are two well documented examples of herbicide resistance developed with this approach.

Glyphosate

N-phosphonomethylglycine (Figure 1) is a highly phytotoxic, non-selective herbicide which inhibits the enzyme enolpyruvyl shikimate 3-phosphate synthase (EPSP synthase), an enzyme essential to the synthesis of aromatic amino acids. EPSP synthase is encoded by the *aroA* gene.

Comai *et al.* (3) were the first to report the isolation of mutant *aroA* genes from *Salmonella typhimurium* mutants able to grow in the presence of glyphosate. Resistance to the herbicide was due to a single base mutation; cytosine was exchanged for thymine and, as a consequence, serine was substituted for proline at position 101 of the 349 amino acid EPSP synthase polypeptide (14).

Sulfonylureas
The sulfonylurea family of herbicides (Figure 1) inhibit the enzyme acetolactate synthase (ALS), which is involved in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (2). ALS is also the target enzyme for two other unrelated classes of herbicides, the imidazolinones and the triazolopyrimides (6). Although several important crops are naturally tolerant to various sulfonylurea herbicides by detoxifying the herbicide (13), the development of sulfonylurea-resistant crops has been based on the transfer of a gene encoding a form of the ALS enzyme which is unaffected by this class of compounds (5,7).

Impact of herbicide-resistant crops on weed management

In general terms, herbicides are used so extensively in agriculture, horticulture and forestry in developed nations that it is unlikely that the widespread use of plants genetically engineered for herbicide resistance will increase the overall proportion of plants sprayed with herbicides. What seems more likely, notwithstanding the cost of the technology, is that the total quantity of herbicides applied to a herbicide-resistant crop may actually decline in comparison to non-genetically engineered plants. This may result in fewer applications as the farmer is able to monitor the extent of weed infestation and make a decision based on that assessment. This may reduce the use of pre-emergence herbicides where application is obligatory because there may be no selective post-emergence herbicide or where application is considered as an insurance against an anticipated problem.

Herbicide usage may decline because of the high phytotoxicity of new herbicides and their low application rates. However, use of the particular herbicide for which the crop is resistant may increase. Repeated use of such a herbicide may increase the likelihood of the target species becoming tolerant, not by gene transfer from the crop to the weed, but by the natural processes of selection that occur when weeds are exposed to repeated applications of the herbicide (8). Sensible herbicide application practices can minimize this issue, and herbicide-resistance technology may also contribute if different herbicides and their respective resistance genes are rotated between crops.

Impact of herbicide resistance on soil conservation

Traditionally, cultivation was employed to control weeds and to establish a seed-bed for germination and establishment of the crop. In principle, selective herbicides can address the issue of weed control, but in practice, the broad applicability of such herbicides is limited. As a consequence, cultivation continues to be important to weed control management.

Herbicide-resistant crops developed systematically by genetic engineering will increase the likelihood that herbicide application will substitute for cultivation as an effective means of weed control. In addition, the overall environmental impact of herbicide application will be reduced because current programs for the development of herbicide resistant crops are focused on herbicides which are:

- highly phytotoxic
- non-selective
- relatively non toxic, or of low toxicity, to non-plants
- rapidly degraded in soil and,
- not likely to move into the groundwater.

Adoption of herbicide-resistance technology

For most production inputs such as seed, fertilizers and herbicides, the individual market niches are mature in the developed nations and hence, increasing market-share can only be achieved at the expense of competitors. For this reason the difference between competing products must be large and obvious.

The factors determining adoption of herbicide-resistance technology are complex because a "package" of two technologies must be offered to the farmer:

Transgenic seed containing the specific herbicide-resistance gene

Seed companies would be attracted to the development of such plants in order to;

- increase profit margins through increasing the seed price,
- increase market-share, or
- maintain market-share in the face of their

competitors adopting the technology within their own product lines.

Herbicide

Usually the emphasis is on non-selective herbicides, such as glyphosate, because the "package" has the potential to open up new markets for the herbicide manufacturer. There is less incentive to develop resistance genes for herbicides which are already partly selective, because of the likelihood that other selective herbicides may be introduced to address this specific market opportunity.

The "package" will have to compete with analogous "packages" from competitors. Also, the cost-effectiveness of the "package" must compete with other, or newly developed, herbicides able to achieve the same degree of selectivity, and with non-genetically engineered seed which has the same agronomic performance, but is likely to be cheaper.

Add to this equation the intangible variable of farmers being reluctant to rely on one source for two of their most important crop inputs. Thus, the pathway to widespread adoption of herbicide-resistance technology is complex and challenging.

Finally, there is the important issue of registering the herbicide for its new use and the attendant costs of registration. The perceived market opportunity will obviously dictate the economic feasibility of undertaking this task and determine what, if any, increase in price is tenable. For example, horticultural crops are often characterized by high value per hectare, but often have very high weed control costs due to the lack of registered, selective herbicides appropriate to the crop. This has meant that weed control costs can be relatively high due to a higher proportion of cultivation and hand weeding. This may provide a suitable margin for the development, registration and marketing of an appropriate "package" of seed and herbicide.

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bromoxynil by *Klebsiella pneumoniae* subsp. *ozaenae*. *Applied Environmental Microbiology* 52, 325.

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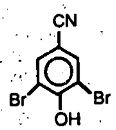
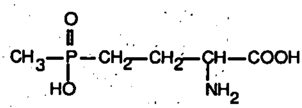
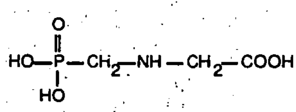
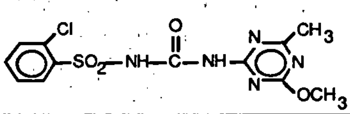
Table 1 *Examples of herbicides used in the development of herbicide-resistant plants by genetic engineering*

Compound/herbicide	Chemical formula	Inhibited pathway	Target protein
Bromoxynil		Photosynthesis	Q_b protein
Phosphinothricin (Basta)		Nitrogen assimilation	Glutamine synthase
Glyphosate (Roundup)		Aromatic amino acids	5'-Enolpyruvylshikimate 3'-phosphate synthase
Chlorsulfuron		Branched chain amino acids	Acetolactate synthase