TRANSGENIC HERBICIDE-RESISTANT CROPS: CURRENT STATUS AND POTENTIAL FOR THE FUTURE

Stephen O. Duke[1] and Antonio L. Cerdeira[2] bring us up to date on herbicide-resistant crops and discuss the potential future of this technology

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Introduction

The term 'herbicide-resistant crop' (HRC) describes crops made resistant to herbicides by either transgene technology or by selection in cell or tissue culture for mutations that confer herbicide resistance. HRCs are also referred to as herbicide-tolerant crops. Most of the success and controversy about HRCs concerns transgenic HRCs, so this article will focus on these products. Transgenic HRCs are the predominant transgenic crops, and the number of hectares planted in these crops has increased dramatically worldwide since they were introduced in 1994, reaching almost 60 million hectares in 2004 (ISAAA, 2005).

Current and past products

The first transgenic HRCs, bromoxynil (3,5-dibromo-4-hydroxybenzonitrile)-resistant cotton in the USA and glufosinate (4-[hydroxy(methyl)phosphinoyl]-DL-homoalanine)-resistant canola (oilseed rape) in Canada, were initially marketed in 1995 (Table 1). Since then, other transgenic crops made resistant to these two herbicides and transgenic HRCs made resistant to glyphosate (*N*-(phosphonomethyl) glycine) have been introduced into North America and other countries. All HRCs available in the world are found in those approved for use in North America (Table 1). Some of these, especially glyphosate-resistant soybean and cotton are grown extensively outside of North America. All bromoxynil-resistant crops have been removed from the market for economic reasons. In the case of glyphosate-resistant sugarbeet, the product is available, but not grown. In all cases of transgenic HRCs, except for some glyphosate-resistant maize varieties, the transgene conferring herbicide resistance has been of bacterial origin.

Table 1. Herbicide-resistant crops that have been deregulated for commercial production by farmers in North America

Herbicide	Transgene	Crop	Year available
Bromoxynil	bacterial nitrilase	cotton *	1995
	bacterial nitrilase	canola*	1999
Glufosinate	*bar* gene	canola	1995
	bar gene	maize	1997
	bar gene	cotton	2004
Glyphosate	CP4 EPSPS	soybean	1996
	CP4 EPSPS + GOX	canola	1996
	CP4 EPSPS	cotton	1997
	CP4 or GA21 EPSPS	maize	1998
	CP4 EPSPS	sugarbeet**	1999

* no longer available
** never grown commercially

Bromoxynil-resistant crops

Bromoxynil is a selective, post-emergence herbicide, more active on dicotyledonous plants than on grasses. It acts by inhibition of photosystem II of photosynthesis. Thus, bromoxynil-resistant dicotyledonous crops, such as cotton or canola, give the farmer an added tool for weed management. Crops were made resistant to this herbicide with a transgene from the soil microbe *Klebsiella ozaenae* that encodes a bromoxynil-degrading enzyme. Bromoxynil-resistant cotton was grown in the USA until 2004, and bromoxynil-resistant canola was sold in Canada until 2001. Although these crops were useful for weed management under some conditions, adoption rates were never very high, and bromoxynil-resistant crops were eventually discontinued for economic reasons. To our knowledge, no weeds evolved resistance to bromoxynil in bromoxynil-resistant crops.

Glyphosate-resistant crops

Glyphosate is a very effective, non-selective, post-emergence herbicide. Prior to introduction of glyphosate-resistant crops, it was used in non-crop situations, before planting the crop, or with specialized application equipment to avoid contact with the crop (Duke, 1988; Duke *et al.*, 2003). Glyphosate is particularly effective because most plants

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degrade it metabolically very slowly or not at all, and it translocates well to metabolically active tissues such as meristems. Its relatively slow mode of action allows movement of the herbicide throughout the plant before symptoms occur.

To date, glyphosate-resistant soybean, cotton, canola, sugarbeet, and maize are available to farmers of North America (although GM-sugar beet has not been grown). All of the glyphosate-resistant crops have a resistant form of the herbicide target enzyme, 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), a key enzyme in the synthesis of aromatic amino acids. In all except some varieties of maize, a bacterial gene (the CP4 gene of *Agrobacterium* sp.) is used. Site-directed mutagenesis of maize EPSPS has produced the GA21 transgene encoding a resistant form of the enzyme for some maize varieties. Glyphosate-resistant canola also contains a bacterial gene encoding glyphosate oxidase (GOX), an enzyme that degrades glyphosate to aminomethylphosphonate and glyoxylate, compounds that are much less phytotoxic than glyphosate.

The adoption rate of glyphosate-resistant cotton and soybeans in North America has been high (Figure 1). This has been in large part because of the significantly reduced cost of excellent weed control obtained with the glyphosate-resistant crop/glyphosate package (Gianessi, 2005). Simplified and more flexible weed control have also contributed to the rapid adoption of these crops. Approximately 75% of canola acreage in the USA was planted in glyphosate-resistant varieties in 2003 (Gianessi, 2005), and about 50% of the canola grown in Canada is glyphosate-resistant.

Despite great success with other glyphosate-resistant crops, glyphosate-resistant sugarbeet is not being grown by North American sugarbeet farmers, due to concerns about acceptance of sugar from transgenic plants by the confectionery and other prepared food industries. This HRC has been available for several years, but not grown. Similar and other concerns resulted in a decision by the company owning glyphosate-resistant wheat technology not to ask for approval of commercial release in 2004 (Dill, 2005). At the writing of this article, there are petitions for deregulation of glyphosate-resistant bent grass (*Agrostis stolonifera*) and alfalfa (*Medicago sativa*).

Glyphosate-resistant crops have been the dominant transgenic HRC, and glyphosate resistance has been the dominant trait marketed in transgenic crops of all types. The high rates of adoption in soybean, cotton, and canola have been fueled by reduced costs of highly effective weed management. After glyphosate became a generic herbicide in 2000, the price of this herbicide was significantly reduced. Despite concomitant dramatic decreases in prices of competing herbicides (Nelson & Bullock, 2003), the glyphosate/glyphosate-resistant crop combination has been the system of choice for many farmers. Use of only one post-emergence herbicide has also simplified weed management with herbicides. This technology is farm size independent, unlike complicated weed management strategies that are often more difficult and less economical for small farmers.

Where glyphosate-resistant crops are being used frequently, weed resistance is becoming a problem (Nandula *et al.*, 2005). In some cases, naturally resistant weeds have occupied ecological niches of weeds controlled by glyphosate (e.g. *Commelina benghalensis*). Horseweed (*Conyza canadensis*) and common ragweed (*Ambrosia artemisiifolia*) have evolved resistance to glyphosate in glyphosate-resistant crops. Other weed species have evolved resistance to glyphosate in places such as orchards, where there was severe selection pressure by glyphosate use, indicating that there will probably be more glyphosate-resistant weed species evolving or moving into glyphosate-resistant crops. Depending on the level of resistance, farmers have dealt with these problems by increasing the dose rate of glyphosate, mixing other herbicides with glyphosate, or incorporating mechanical weed control methods.

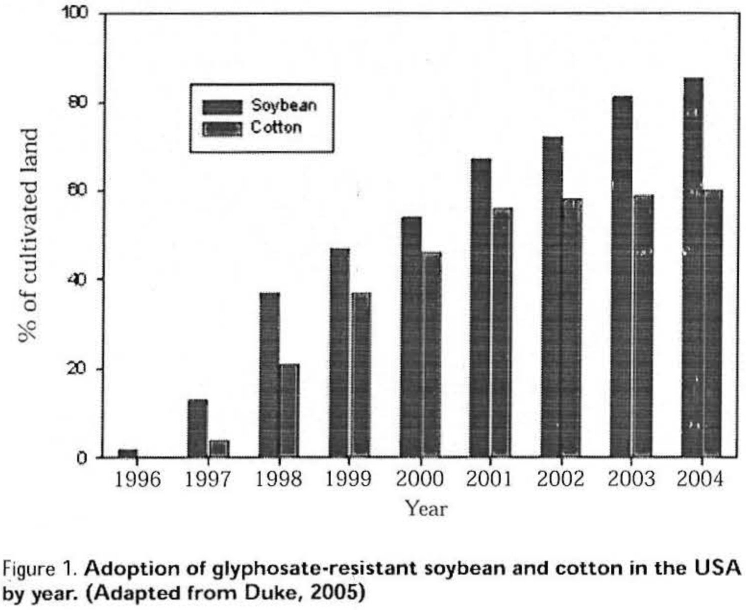

Figure 1. **Adoption of glyphosate-resistant soybean and cotton in the USA by year. (Adapted from Duke, 2005)**

Glufosinate-resistant crops

Glufosinate is the synthetic version of phosphinothricin, a natural compound from *Streptomyces hygroscopicus*. Glufosinate is a broad spectrum herbicide that acts faster than glyphosate through inhibition of the enzyme glutamine synthetase. Canola, cotton, and maize made resistant to glufosinate are commercially available in North America. Glufosinate-resistant crops have been made resistant to glufosinate with the *bar* gene from the same microbe that produces phosphinothricin. This enzyme detoxifies glufosinate by acylation. Since the *bar* gene is commonly used as a selectable marker gene in molecular biology, almost every crop species has been made resistant to this herbicide in the laboratory. However, only a very few glufosinate-resistant crops have been commercialized (Table 1). Glufosinate-resistant cotton was introduced in the USA in 2004 and is being used predominantly in south Texas.

These crops have not captured the market share that glyphosate-resistant crops have.

However, no weeds have evolved resistance to glufosinate in any setting throughout the world. Thus, glufosinate-resistant crops are a good option for rotation with glyphosate-resistant crops. There are no petitions for deregulation (permission for commercialization) of other glufosinate-resistant crops at this time in the USA, although some effort was put into development of glufosinate-resistant sugarbeet, soybean, and bentgrass in the past.

Environmental impacts of herbicide-resistant crops

The environmental impact of transgenic HRCs has been recently reviewed (Duke & Cerdeira, 2005). Glyphosate and glufosinate are not thought to be significant environmental contaminants when used at recommended doses, and both herbicides are considered to have low toxicity to non-target organisms, other than plants. Many of the herbicides for which glyphosate and glufosinate substitute are more environmentally and toxicologically suspect than they are. Both glyphosate and glufosinate are post-emergence herbicides that can substitute for pre-emergence herbicides, allowing the farmer to use them only when and where they are actually needed, with reductions in costs and less pressure to the soil ecology.

The effect of HRCs on total herbicide use has been a matter of debate. An analysis of literature on this topic concluded that, overall, HRCs have had little effect on herbicide use (Duke & Cerdeira, 2005). A more important question is whether adoption of HRCs has reduced the risks associated with weed management. Most studies conclude that the risks have been reduced (Duke & Cerdeira, 2005).

Perhaps the most damaging effect of agriculture on the environment, other than removing land from its natural state, is soil erosion that is exacerbated by tillage. The herbicide/HRC combinations for glyphosate and glufosinate work well with reduced or zero tillage agronomic systems, which contribute to reductions in soil erosion from water and wind. Reduced tillage also contributes to reduced fossil fuel use, less air pollution from dust, improved soil moisture retention, and reduced soil compaction (Holland, 2004). A dramatic increase occurred in the adoption of zero and reduced tillage in soybeans in the USA within five years of the introduction of glyphosate-resistant soybeans (Figure 2). Similar reductions in tillage with glyphosate-resistant soybeans have been documented in Argentina.

The replacement of pre-emergence herbicides with the post-emergence herbicides allowed by these HRCs can also reduce herbicide concentrations in vulnerable watersheds (Wauchope *et al.*, 2002). Glyphosate and glufosinate are not usually found in ground water (U.S. Geological Service, 1998). Many animal feeding studies with glyphosate- and glufosinate-resistant crops have found no nutritional or food safety differences between these crops and conventional crops (summarized by Duke & Cerdeira, 2005).

Potentially, the most long-lasting environmental damage of a transgenic crop is for an ecologically important transgene to escape to other plant species (gene flow). Herbicide resistance genes have no ecological significance in places where the corresponding herbicide is not used.

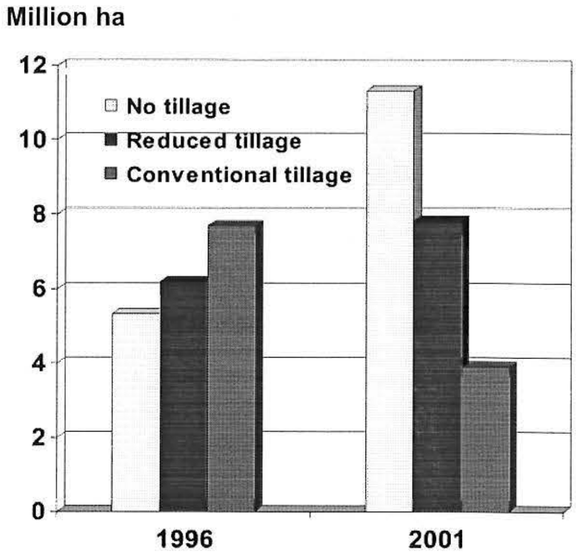

Figure 2. **Soybean tillage methods by hectares farmed in the USA in 1996 and 2001. In 1996 and 2001, there were 19.2 and 23 million ha, respectively, of soybeans grown. (Drawn from American Soybean Association, 2001 data).**

However, when paired with a gene that might have an effect in a natural ecosystem (e.g., a *Bt* gene for insect resistance), there is a potential problem with gene flow. Repeated application of the herbicide (especially a non-selective herbicide) would select for and protect crosses and backcrosses, increasing the probability of successful gene flow to wild, related species. Gene flow from transgenic canola to weedy relatives is probable and has been documented in commercial fields, with gene flow from glyphosate-resistant canola to *Brassica rapa* (Warwick *et al.*, 2003). Fortunately, *Bt* genes and herbicide resistance have not been "stacked" in canola.

So far, the environmental benefits of the current HRCs appear to outweigh any environmental harm, when compared with the herbicides and agronomic practices that they displace. However, this may not be true for every situation with every crop and every HRC.

Farmer problems with herbicide-resistant crops

Some varieties of glyphosate-resistant maize, cotton and soybean have been sufficiently susceptible to glyphosate under some conditions to exhibit phytotoxicity symptoms with recommended application rates, although yield losses have been reported only in cotton (Pline-Srnic, 2005). These problems have been minor, as evidenced by the increased adoption of these crops.

Because HRCs and conventional cultivars of crops cannot be visually distinguished from each other, herbicide drift from HRCs and unintentional spraying of conventional crops has been a bigger problem than when two different crop species are grown in the same area. A more significant problem is gene flow from transgenic to non-transgenic cultivars of the same crop. Preserving non-transgenic canola identity has been a problem in some places in Canada, due to gene flow from HRC canola to non-transgenic canola. The accumulation of a seed-bank of canola seed with single and multiple herbicide resistance traits is a potential long-term problem for producers.

Potential transgenic herbicide-resistant crops

Transgenes exist to make crops resistant to most herbicide classes. Examples of some of these herbicides are 2,4-D, acetolactase synthase inhibitors, asulam, dalapon, paraquat, phenmedipham, phytoene desaturase inhibitors, and protoporphyrinogen oxidase inhibitors. Most of these genes are patented, and considerable effort has been put into developing crops with some of these transgenes. For example, maize that is highly resistant to protoporphyrinogen oxidase-inhibiting herbicides was developed by transformation with a gene encoding a resistant form of the enzyme (Li & Nicholl, 2005). This product proceeded to the trade name stage, but there appear to be no plans to commercialize it in the near future. A new transgene conferring resistance to glyphosate was generated from a microbial acyltransferase via directed evolution (Castle *et al.*, 2004). Currently, few resources are being allocated to commercialization of HRCs that are resistant to herbicides other than glufosinate and glyphosate or to those that are not major crops.

Why are there not more transgenic herbicide-resistant crops?

Since the year 2000, only one new transgenic herbicide-resistant crop has been introduced, two products have been withdrawn, and one that is available has not been grown (Table 1). Current HRCs are resistant to only two herbicides, glyphosate and glufosinate, both broad spectrum products for post-emergence use. Only four transgenes are used in all of these crops. Glyphosate and glufosinate are both broad spectrum herbicides for which there is only one product available in their respective chemical families. Almost all other potential HRCs that have been patented each confer resistance to several selective herbicides of the same chemical class. Thus, the herbicide will not kill as many different weed species as glyphosate or glufosinate, and there will be several herbicides to which the HRC is cross resistant that could be used with the HRC. In these cases, tailoring the HRC to be resistant to only one herbicide may be impossible, reducing the chances of linking profits from the HRC to profits from a single herbicide.

Apparently, in the short term, relatively few new HRCs will be introduced. Devine (2005) concluded that the high cost, lengthy development time, and high economic risk have been the primary reasons for the slow development and introduction of new HRCs. Another major consideration in committing resources to introduction of an HRC is the fear of consumer rejection of HRC-based products. Thus, most of the growth in HRC use during the next few years appears to be with existing products. Any predictions beyond this would be highly speculative.

References and further reading

American Soybean Association. (2001) Conservation tillage study, St.Louis, MO http://www.soygrowers.com/ctstudy.

Castle, L. A., D. L. Siehl, R. Gorton, P. A. Pattern, Y. H. Chen, B. Bertain, H.-J. Cho, N. Duck, J. Wong, D. Liu, and M. Lassner (2004) Discovery and directed evolution of a glyphosate resistance gene. *Science* **304**: 1151–4.

Devine, M. D. (2005) Why are there not more herbicide-resistant crops? *Pest Manag. Sci.* **61(3)**: 312–7.

Dill, G. M. (2005) Glyphosate-resistant crops: History, status and future. *Pest Manag. Sci.* **61(3)**: 219–24.

Duke, S.O. (1988) Glyphosate. In: P. C. Kearney and D. D. Kaufman. (Eds.) *Herbicides-Chemistry, Degradation and Mode of Action Vol. III.*, pp. 1–70. Marcel Dekker, Inc., New York, NY, USA.

Duke, S.O. (Ed.) 1996 *Herbicide-Resistant Crops*. CRC Press, Boca Raton, FL.

Duke, S. O., S. R. Baerson, and A. M. Rimando (2003) Herbicides: Glyphosate. In: *Encyclopedia of Agrochemicals.* (Plimmer, J.R., Gammon, D. W., Ragsdale, N. N., Eds.) John Wiley & Sons, New York, NY, USA, http://www.mrw.interscience.wiley.com/eoa/articles/agr119/frame.html.

Duke, S. O. and N. N. Ragsdale (Guest Eds.) (2005) *Herbicide-Resistant Crops from Biotechnology*. Special Issue of *Pest Manag. Sci.* **61:(3)**.

Duke, S.O. (2005) Taking stock of herbicide-resistant crops ten years after introduction. *Pest Manag. Sci.* **61(3)**: 211–8.

Duke, S. O. and A. L. Cerdeira (2005) Potential Environmental Impacts of Herbicide-Resistant Crops. In *Collection of Biosafety Reviews, Vol. II*, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, (In Press).

Gianessi, L. P. (2005) Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Manag. Sci.* **61(3)**: 241–5.

Holland, J.M. (2004). The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. *Agricultural. Ecosystems. Environment* **103**: 1–25.

ISAAA (2005) http://www.isaaa.org/kc/Bin/cbtupdate/index.htm.

Li, X., and D. Nicholl. (2005) Development of PPO inhibitor-resistant cultures and crops. *Pest Manag. Sci.* **61(3)**: 277–85.

Nandula, V. K., K. N. Reddy, S. O. Duke, and D. H. Poston (2005) Glyphosate-resistant weeds: Current status and future outlook. *Outlooks Pest Manag.* (in press).

Nelson, D.S., and G.C. Bullock. (2003) Simulating a relative environmental effect of glyphosate-resistant soybeans. *Ecol. Econom.* **45**: 189–202.

Pline-Srnic, W. (2005) Technical performance of some commercial glyphosate-resistant crops. *Pest Manag. Sci.* **61(3)**: 225–34.

U.S. Geological Service. (1998) Pesticides in Surface and Ground Water of the United States: Summary of Results of the National Water Quality Assessment Program (NAWQA), http://ca.water.usgs.gov/pnsp/allsum.

Warwick, S. I., M.-J. Simard, A. Légère, H. J. Beckie, L. Braun, B. Zhu, P. Mason, G. Séguin-Swartz, and C. N. Stewart (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. *Theor. Appl. Genet.* **107**: 528–39.

Wauchope, R.D., T.L. Estes, R. Allen, J.L. Baker, A.G. Hornsby, R.L. Jones, R.P. Richards, and D.I. Gustafson (2002) Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Manag. Sci.* **58(2)**:146–60.

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