

Potential Environmental Impacts of Herbicide-Resistant Crops

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Abstract

Transgenic bromoxynil-, glufosinate-, and glyphosate-resistant crops have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. Bromoxynil-resistant crops have been removed from the market. Few new herbicide-resistant crops (HRCs) are likely to be introduced in the near future. Glyphosate-resistant cotton and soybean have become dominant in those countries where they can be grown. Previous and potential effects of glufosinate and glyphosate on contamination of soil, water, and air are minimal, compared to that caused by the herbicides that they replace when HRCs are adopted. No risks have been found with food or feed safety or nutritional value in products from currently available HRCs. Both glufosinate- and glyphosate-resistant crops promote the adoption of reduced- or no-tillage agriculture. In the U.S.A. and Argentina, the advent of glyphosate-resistant soybeans resulted in a significant shift to reduced- and no-tillage practices, strongly reducing environmental degradation by agriculture. Weed species in HRC fields have shifted to those that can more successfully withstand glyphosate or to those that avoid the time of its application. One species has evolved resistance in glyphosate-resistant crops due to selection pressure from glyphosate. HRCs have a greater potential to become problematical as volunteer crops than do conventional crops. In canola, herbicide resistance transgenes have been found in fields of canola that are supposed to be non-transgenic. Under some circumstances, transgene flow (introgression) to plants that might become problematical in natural ecosystems may be the largest risk of HRCs. The HRC transgene itself is highly unlikely to be a risk in wild populations, but when linked to transgenes that may impart fitness benefits outside of agriculture, natural ecosystems could be affected. The development and use of failsafe introgression barriers in crops with such linked genes is highly encouraged.

Riassunto

Piante transgeniche resistenti ad erbicidi quali il bromoxinil, il glufosinate ed il glifosate sono già da tempo in commercio e sono coltivate in maniera estesa nell'emisfero occidentale e anche, un po' meno, altrove. Le colture resistenti al bromoxinil sono state rimosse dal mercato, mentre sono poche le nuove colture resistenti ad altri erbicidi che stanno per essere introdotte in un prossimo futuro. Cotone e soia resistenti al glifosate sono diventati dominanti in quei paesi dove è stata approvata la loro coltivazione. Gli effetti potenziali del glufosinate e del glifosate sulla contaminazione del suolo, dell'acqua, e dell'aria sono minimi quando si adottano colture resistenti agli erbicidi (CRE), se confrontati con quelli causati dagli erbicidi che vanno a sostituire. Non sono stati riportati rischi per la sicurezza degli alimenti o dei mangimi, o variazioni dei valori nutrizionali nei prodotti derivanti da colture transgeniche attualmente disponibili resistenti agli erbicidi. Sia le piante resistenti al glifosate che al glufosinate favoriscono l'adozione di un'agricoltura basata sulla minima o nulla lavorazione del terreno (no-tillage). Negli Stati Uniti ed in Argentina l'avvento della soia resistente al glifosate ha portato ad uno spostamento significativo verso pratiche di "reduced or no-tillage", con una conseguente riduzione della degradazione dell'ambiente da parte dell'agricoltura. Nei campi coltivati con piante resistenti agli erbicidi la popolazione delle specie infestanti ha subito uno spostamento verso quelle che più facilmente riescono a sopportare il glifosate o che evitano il momento della sua applicazione. Come conseguenza dell'aumentata pressione di selezione si è sviluppata una infestante con resistenza al glifosate. Le CRE hanno un maggiore potenziale per diventare infestanti di quanto non accada per le colture convenzionali. Nel caso della colza, i geni che conferiscono resistenza all'erbicida sono stati trovati anche nei campi dove si suppone sia stata coltivata solo colza non transgenica. In alcune circostanze il flusso di transgeni (introgressione) verso piante che possono diventare un problema nell'ecosistema naturale può rappresentare il rischio maggiore per questo tipo di piante transgeniche. È altamente improbabile che il transgene che conferisce resistenza all'erbicida possa da solo rappresentare un rischio per le popolazioni selvatiche, ma quando legato a transgeni che possono conferire benefici di fitness al di fuori dell'agricoltura, l'ecosistema ne potrebbe essere influenzato. Lo sviluppo e l'adozione di barriere per evitare il problema dell'introgressione in colture con questo tipo di geni legati tra loro è fortemente incoraggiato.

1. INTRODUCTION

Only two types of transgene-conveyed traits for crops have so far had a significant effect on agriculture: herbicide resistance and insect resistance (Gutterson and Zhang, 2004). 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 resistance. HRCs are sometimes called herbicide-tolerant crops. Most of the success and controversy about the safety of HRCs surrounds transgenic HRCs, so this review will concentrate on these products. HRCs have been the sole subject of numerous reviews (e.g., Dekker and Duke, 1995; Duke *et al.*, 1991, 2002; Duke, 1998, 2002, 2005; Dyer *et al.*, 2002; Gressel, 2002b; Hess and Duke, 2000; Silvers *et al.*, 2003; Warwick and Miki, 2004), two books (Duke, 1996; McClean and Evans, 1995), and a special issue of the journal *Pest Management Science* in 2005. Gressel (2002a) covered many aspects of HRCs in his book on the molecular biology of weed control. A recent review covered agronomic and environmental aspects of HRCs (Schütte *et al.*, 2004). Other reviewers have discussed the environmental impacts of all transgenic crops, with coverage of HRCs (e.g., Carpenter *et al.*, 2002; Uzogara, 2000). Lutman *et al.* (2000) and Kuiper *et al.*, (2000) published brief reviews of the environmental consequences of growing HRCs. Meyer and Wolters (1998) reviewed the ecological effects of herbicide use associated with HRCs. None of these publications have focused solely on an in-depth assessment of the potential environmental impacts of all aspects of HRCs.

The present review will not be encyclopedic, as there are thousands of potential references related to this topic. Not all information that we would like to discuss is available in citable sources. We will not discuss the regulatory process for approval of HRCs in the many countries that regulate their approval. We will try to discuss the most important and germane literature, along with selected examples. The chemical, the transgene, and the weed management (e.g., changes in tillage) aspects of HRC environmental impacts will be covered. We will refrain from discussing different formulations of the herbicides, as the actual composition of additives to these products, other than the active herbicide ingredients, are trade secrets and can vary between geographical regions and can change without notice to the user.

The potential environmental impact of a technology is often geographically and/or time dependent. Thus, extrapolation of the results and conclusions of studies to all situations is impossible. The best we can do is make generalizations from reported studies that may not cover every situation.

Analysis of impact cannot be done in a vacuum. Thus, we will at times contrast certain risks of HRCs with the risks that HRCs displace. Lastly, we will de-emphasize bromoxynil-resistant crops, as the last of these products will be removed from the market by 2005. The viewpoints in this analysis are those of the authors and are not meant to reflect those of our employers.

2. AVAILABLE AND FORTHCOMING HERBICIDE-RESISTANT CROPS

Before we can consider potential environmental impacts, we must know what products are being discussed. The first transgenic HRCs, bromoxynil-resistant cotton in the USA and glufosinate-resistant canola in Canada, were first marketed in 1995. Since then, HRCs made resistant to other herbicides, using different genes, have become available in North America (Table 1). We are aware of no HRCs outside of the U.S.A. that are not also available in the USA. In all cases of transgenic HRCs, except for some glyphosate-resistant maize varieties, the transgene conferring herbicide resistance has been of bacterial origin.

2.1. Bromoxynil-resistant crops

Bromoxynil (3,5-dibromo-4-hydroxybenzotrile) inhibits the electron transport of photosystem II (PSII) of photosynthesis (Fedtke and Duke, 2004). It is considered a selective herbicide, in that it kills only certain plant species at recommended application doses, including many crops. It is much more active on dicotyledonous plants than on grasses. Thus, bromoxynil-resistant dicotyledonous crops, such as cotton or canola, would give the farmer of these crops an added tool for weed management. Unlike many other PSII inhibitor herbicides, only one weed species is known to have evolved resistance to bromoxynil (Heap *et al.*, 2004). It is not a low dose rate herbicide (Vencill, 2002).

Crops were made resistant to this herbicide with a transgene from the soil microbe *Klebsiella ozaenae* that encodes a nitrilase that converts the benzonitrile to a non-phytotoxic benzoic acid derivative (3,5-dibromo-4-methoxybenzoic acid) (Stalker *et al.*, 1996). Crops transformed with this gene can resist a more than ten-fold dose of bromoxynil that is normally lethal. Bromoxynil-resistant cotton was grown in the U.S.A. until 2004, and bromoxynil-resistant canola was sold in Canada until 2001 (Table 1). Company mergers resulted in the same company producing both bromoxynil-resistant and glufosinate-resistant crops. Bromoxynil-resistant crops were discontinued for economic reasons.

The adoption rate of these products was never very great, as it only allows

Table 1. Herbicide-resistant crops that have been or are now available to farmers in North America. (adapted from Duke, 2005)

Herbicide or herbicide class	Crop	Year made available
Bromoxynil	cotton ¹	1995
	canola ²	2000
Cyclohexanediones (sethoxydim) ³	maize	1996
Glufosinate	canola	1995
	maize	1997
	cotton	2004
Glyphosate	soybean	1996
	canola	1996
	cotton	1997
	maize	1998
	sugarbeet ⁴	1999
Imidazolinones ³	maize	1993
	canola	1997
	wheat	2002
	rice	2002
	sunflower	2003
Sulfonylureas ³	soybean	1994
Triazines ³	canola	1984

¹ scheduled to be withdrawn after 2004

² withdrawn after 2001

³ not transgenic, not all of these non-transgenic HRCs are still available

⁴ never grown by farmers, withdrawn in 2004

the farmer to add another selective herbicide to others available for these crops. Farmers tended to use bromoxynil with these crops only when there was a particularly bromoxynil-susceptible weed species spectrum.

2.2. Glyphosate-resistant crops

Glyphosate (*N*-[phosphonomethyl]glycine) is a very effective non-selective herbicide. Prior to the 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., 2003a; Franz et al., 1997). It inhibits the shikimate pathway by inhibiting 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). This results in reduced aromatic amino acids and deregulation of the pathway. The latter effect causes a massive flow of carbon into the pathway, with an accumulation of high levels of shikimic acid and its derivatives. Glyphosate is particularly effective because most plants metabolically degrade it 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. Glyphosate is only used as a post emergence herbicide, as it has little or no activity in soil. It is not a low dose rate herbicide. Glyphosate is an anion and is sold as a salt with different cations (e.g., isopropyl amine, trimethylsulfonium, diammonium). Finding a glyphosate-resistant EPSPS possessing sufficient catalytic activity to provide adequate metabolic functioning of the shikimate pathway proved daunting (Padgett et al., 1996a). Eventually, the *CP4* gene of *Agrobacterium* sp. was found to encode a highly efficient, glyphosate-resistant EPSPS. Plants transformed with this gene were highly resistant to glyphosate. Metabolic degradation of glyphosate was also probed as a resistance mechanism. Glyphosate oxidoreductase (GOX), encoded by a gene from the microbe *Ochrobactrum anthropi* (strain LBAA), enhances glyphosate resistance. This enzyme degrades glyphosate to glyoxylate, a ubiquitous and safe natural product, and aminomethylphosphonate (AMPA). A multiple missense mutation in endogenous maize EPSPS produced by site-directed mutagenesis has been utilized to generate commercial glyphosate resistance (Lebrun et al., 1997). This transgenic double variant of maize EPSPS, T102i/P106S is presently sold commercially in some maize hybrids and is known as GA21 (Dill, 2005). To date, glyphosate-resistant soybean, cotton, canola, and maize are available to farmers of North America (Table 1). All varieties use the *CP4* EPSPS gene, except for the GA21 maize varieties. The GOX gene is also found in glyphosate-resistant canola.

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 also contributed to the rapid adoption. Approximately 75% of canola acreage in the U.S.A. was planted in glyphosate-resistant varieties in 2003 (Gianessi, 2005). In Australia, an economic analysis of glyphosate-resistant canola showed significant economic advantages (Monjardino *et al.*, 2005). In Argentina, the adoption of glyphosate-resistant soybeans was even more rapid than in the U.S.A., reaching almost 90% within 4 years of introduction (Penna and Lema, 2003). This level of adoption took more than 25 years for hybrid maize in Argentina. In Argentina, better weed management and reduced cost were about equally important reasons for adoption. The economic advantage is not as clear with glyphosate-resistant maize, with an approximate 18% adoption rate in 2004 (Dill, 2005).

Despite great success with other glyphosate-resistant crops, glyphosate-resistant sugarbeet is not being grown by North American farmers, due to concerns about acceptance of sugar from transgenic plants by the confectionary and other prepared food industries. This HRC was available for several years (Table 1), but not grown. Similar and other concerns resulted in a decision by the company owning glyphosate-resistant wheat technology not to ask for deregulation in 2004 (Dill, 2005).

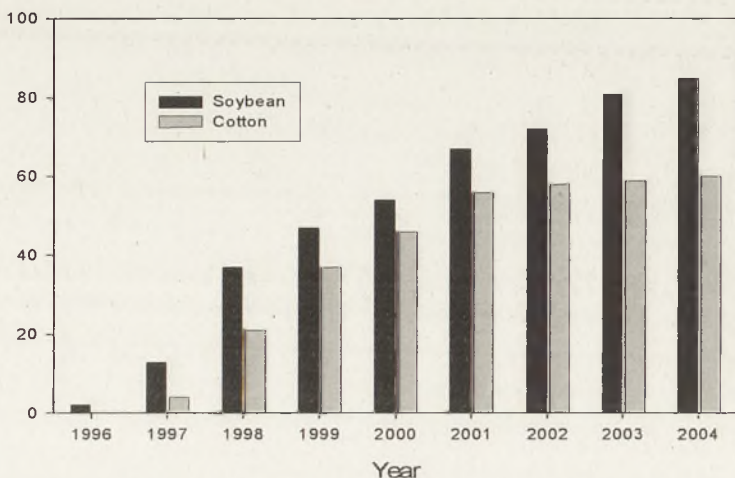


Figure 1. Adoption of glyphosate-resistant soybean and cotton in the U.S.A. by year. (Adapted from Duke, 2005)

2.3 Glufosinate-resistant crops

Glufosinate is the synthetic version of phosphinothricin, a natural compound from *Streptomyces hygroscopicus*. It is a potent inhibitor of glutamine synthetase (GS) of plants. When GS is inhibited, one of its substrates, ammonium ion, accumulates, causing toxic effects. The complete mechanism of action is more complicated than this, involving indirect inhibition of photosynthesis (Lydon and Duke, 1999). GS is present in most living organisms. Glufosinate is a broad-spectrum herbicide that acts faster than glyphosate. It is not a low dose rate herbicide. Glufosinate is sold as the ammonium salt. No weed resistance has evolved to glufosinate. The same organisms that produce phosphinothricin or its precursor, bialaphos, protect themselves from these toxins by metabolically inactivating them with phosphinothricin-*N*-acetyl-transferase (PAT) encoded by the *pat* or *bar* gene. The two genes have a high level of homology and both encode a PAT enzyme. Plants can be made highly resistant to glufosinate with either of these genes. Capitalizing on this, these genes have been used extensively as selectable markers in the transformation of many plant species. Canola, cotton, and maize made resistant to glufosinate with the *bar* gene are commercially available for growing in North America (Table 1).

2.4 Non-transgenic herbicide-resistant crops

Several HRCs generated by mutation of a crop gene in embryo, tissue, or cell culture are listed in Table 1. These include triazine-, sulfonyleurea-, imidazolinone-, and sethoxydim-resistant crops. Technically, these HRCs were generated by biotechnology, but there is nothing significantly different about these HRCs from crops that are naturally resistant to selective herbicides. No transgenes are used with these crops.

These crops have been previously reviewed: triazine-resistant (Hall *et al.*, 1996); imidazolinone-resistant (Shaner *et al.*, 1996; Tan *et al.*, 2005), sulfonyleurea-resistant (Saari and Mauvais, 1996), and sethoxydim-resistant (Somers, 1996). The most successful of these crops are the imidazolinone-resistant crops with maize, oilseed rape, rice, wheat, and sunflower varieties now available in North America (Tan *et al.*, 2005). The bases for resistance of all of these HRCs are altered molecular target sites.

These crops have less regulatory oversight in the U.S.A. than do transgenic HRCs. This review will not deal with this type of HRC, as there is much less concern about environmental hazards with them than with transgenic HRCs.

2.5 Potential herbicide-resistant crops of the future

Genes exist to make crops resistant to most herbicide classes. Examples of some of these are provided in Table 2. Furthermore, a new gene has been engineered by gene shuffling to make crops resistant to glyphosate (Castle *et al.*, 2004), and an evolved resistant form of EPSPS from *Eleusine indica* has been patented for use in glyphosate-resistant crops (Baerson *et al.*, 2004). Most of these genes are patented, and considerable effort has been put into developing HRCs with some of these transgenes. However, there is currently little effort to commercialize HRCs that are resistant to herbicides other than glufosinate and glyphosate. Bromoxynil-resistant crops have been discontinued. The number of regulatory approvals (deregulations) for new (new crop or new herbicide) commercialized HRCs declined in the U.S.A. after 1999 to a trickle (Duke, 2005). However, this trend is not much different than that for all other transgenic traits combined in the USA (Animal and Plant Health Inspection Service of the U.S.A. Dept. Agriculture website: http://www.aphis.usda.gov/brs/brs_charts.html - accessed November, 2004). The petitioning of the United States Environmental Protection Agency (USEPA) for permits to field test HRCs continues to be 20-30% of permit applications for transgenic plants. Some of the reasons for the reticence of the biotechnology industry to develop and market a new HRC are provided by a recent article (Devine, 2005). Fundamentally, the view expressed in that paper is 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.

3. HERBICIDE AND FOSSIL FUEL USE

3.1 Herbicide use

Since the mid twentieth century, herbicides have been the primary means of weed management in developed countries. In North America, for the past two decades, herbicides have accounted for about 70% of pesticide use in crops (e.g. Anonymous, 1998). Prior to herbicides, extensive tillage and manual weeding were the primary means of weed management. There has been controversy about whether HRCs have increased herbicide use or not. This controversy has been fueled by the assumption by some that an increased amount of chemical use equals increased environmental damage and toxicological risk. This assumption does not take into account the clear fact that the potential environmental damage and toxicological risk can vary by orders of magnitude between different herbicides. Thus, comparing herbicide use rates has relatively little bearing on potential environmental damage or toxicological risk to humans. Very few studies,

Table 2. Some of the transgenes that have been used for making crops resistant to herbicides or classes of herbicides

Herbicide or herbicide class	Gene source and gene product	Reference
2,4-D	microbial degradation enzyme	Llewellyn and Last, 1996 Bisht <i>et al.</i> , 2004
Asulam	resistant microbial dihydropteroate synthase	Surov <i>et al.</i> , 1998
Dalapon	microbial degradation enzyme	Buchanan-Wollaston <i>et al.</i> 1992
Hydroxyphenylpyruvate dioxidase (HPPD) inhibitors	microbial, herbicide-resistant HPPD	Matringe <i>et al.</i> , 2005
Paraquat	chloroplast superoxide dismutase	Sen Gupta <i>et al.</i> , 1993
Phenmedipham	microbial degradation enzyme	Streber <i>et al.</i> , 1994
Phytoene desaturase (PDS) inhibitors	genes from microbes and the aquatic weed <i>Hydrilla</i> encoding resistant PDS	Sandmann <i>et al.</i> , 1996 Arias <i>et al.</i> , 2005
Protoporphyrinogen oxidase (PPO) inhibitors	resistant microbial PPO and resistant <i>Arabidopsis thaliana</i> PPO	Li and Nicholl, 2005

such as that by Nelson and Bullock (2003), have compared toxicological risk, rather than herbicide active ingredient used per unit area.

The two remaining herbicides used with HRCs, glyphosate and glufosinate, are not low use rate herbicides; however, they are considered low risk herbicides in terms of toxicity and environmental effects (see sections 4-7). Nevertheless, we will discuss some of the literature that addresses the question of use rate.

A few studies have claimed that the volume of herbicide use is greater with HRCs (e.g., Benbrook, 2001b, 2003). However, others, such as Heimlich *et al.*, (2000), have concluded that no significant change in the overall amount of herbicide has been observed with the adoption of HRCs in the United States. Heimlich *et al.*, (2000) pointed out that this substitution resulted in the replacement of herbicides that are at least three times more toxic, and that persist nearly twice as long as glyphosate. Gianessi and Carpenter (2000) came to similar conclusions. An analysis by U.S.A. soybean farmers reported by Trewavas and Leaver (2001) showed that 3.27 million kg of other herbicides have been replaced by 2.45 million kg of glyphosate. Carpenter and Gianessi (2002) found that the introduction of glyphosate-resistant soybeans in the U.S.A. resulted in a decrease of the volume of herbicides used. Gianessi (2005) claims that glyphosate-resistant crops generally require less herbicide than that used with non-transgenic crops. Furthermore, he estimates that averaged over all glyphosate-resistant crops, glyphosate-resistance technology has reduced herbicide use by 17 million kg per year in the U.S.A.. In cotton, the amount of herbicide used per unit area in the U.S.A. stayed about the same between 1996 and 2000 (Carpenter and Gianessi, 2003), a period during which adoption of glyphosate-resistant cotton grew from 0 to about 50% (Figure 1). Gianessi's (2005) calculations indicate that if glyphosate-resistant sugarbeets were adopted, the reduction would not be as great, as the herbicides now used in non-transgenic sugarbeets are mostly low use rate compounds in the U.S.A. Coyette *et al.* (2002) estimated that the introduction of glyphosate-resistant sugarbeet to Europe would result in a decrease of herbicide use. Weed control could be achieved with very low use rate herbicides (Benbrook, 2001a, b), reducing the volume of chemicals used for weed management below that used in non-HRCs or with HRCs. If this were more economical and efficacious, farmers would probably adopt such a strategy. But, again, simply reducing the volume of chemical used does not assure that risks are reduced.

Others have pointed out that the introduction of HRCs to underdeveloped countries, where hand weeding is the primary means of weed management, will increase herbicide use in those countries (Shiva, 2001).

At this time, there is no evidence that this has occurred. The economic constraints that prevent these farmers from using selective herbicides will be similar for HRCs. However, should weed management with HRCs become economically viable for poor farmers in underdeveloped areas, herbicide use will increase, displacing tillage and hand labor. Hand labor is rarely used with canola or soybeans, even in developing countries.

As discussed below (Section 8), the amount of any herbicide used with HRCs year after year will probably increase with time, as naturally resistant weed species and biotypes invade fields and resistance evolves. Both increased amounts of the herbicides to which the HRCs are engineered and additional herbicides will be used. Benbrook (2003) claims that this biologically driven increase in herbicide use has already occurred with glyphosate-resistant crops. Owen and Zelaya (2005) also report that this is already happening with glyphosate-resistant crops in some locales.

The worldwide decreasing cost of glyphosate due to loss of patent protection (Woodburn, 2000) also makes higher application rates economical in some cases. The heavy adoption of glyphosate-resistant soybeans in the USA contributed to the dramatic reductions (as much as 80%) in the costs of most other soybean herbicides, due to competition (Nelson and Bullock, 2003) (Table 3). Thus, indirectly, glyphosate-resistant soybeans have, in some cases, helped make it more economical for farmers to use higher rates of other herbicides, sometimes with less desirable toxicological or environmental profiles. Another factor contributing to the reductions in herbicides costs have been patent expirations of many herbicides. Despite the more competitive prices of competing herbicides, adoption of glyphosate-resistant crops increased dramatically during this time period (Figure 1).

In a study, using the environmental impact quotient method of Kovach *et al.* (1992), Kleter and Kuiper (2003) calculated the total environmental impact of herbicides, farm worker exposure impact, consumer impact, and ecology impact associated with the herbicides used with various HRCs versus those used with the same non-transgenic crops. The amount of herbicide used was reduced for all crops. All impacts are reduced in all crops by adoption of HRCs (Table 4). With canola, cotton, and soybean, farm worker and consumer impact are reduced more than ecological impact.

Table 3. Price comparisons of selected herbicides in US dollars in the USA before (1995) and after (1999) the introduction of glyphosate-resistant soybeans. (Adapted from Nelson and Bullock, 2003 with permission)

Formulated Herbicide¹	Unit	1995 price	1999 price	1999/1995 price
Lasso (alachlor)	qt	26.38	5.54	0.21
Assure II (quizalofop-P)	qt	116.61	31.42	0.27
Classic (clorimuron)	oz	17.75	11.02	0.62
Cobra (lactofen)	qt	115.40	33.41	0.29
Command (clomazone)	qt	81.28	15.01	0.18
Fusion (fluazifop + fenoxypop)	qt	148.50	33.56	0.23
Galaxy (bentazon + acifluorfen)	qt	56.58	15.45	0.27
Pursuit (imazethapyr)	oz	19.92	14.09	0.71
Scepter (imazaquin)	oz	6.32	7.1	1.12
Select (clethodim)	oz	190.00	47.3	0.25
Sencor (metribuzin)	lb	26.60	19.21	0.72
Storm (acifluorfen + metribuzin)	qt	68.98	18.97	0.28
Treflan (trifluralin)	qt	32.13	8.16	0.25
Turbo (metolachlor + metribuzin)	lb	83.75	14.24	0.17

¹ Trade name followed by active ingredient

Table 4. Environmental impact (EI) of herbicide use in HRCs based on pesticide use data of Gianessi et al. (2002). (Adapted from Kleter and Kuiper, 2003 with permission)

Crop	Non transgenic	Transgenic	% Difference
Canola			
Herbicide use (lb ai/A) ¹	1.1	0.5	-55
Total impact, EI/A	30.9	16.2	-48
Farm worker impact, EI/A	17	8	-53
Consumer impact, EI/A	9.3	3.5	-62
Ecology impact, EI/A	66.5	37.2	-44
Cotton			
Herbicide use (lb ai/A)	2.3	1.9	-18
Total impact, EI/A	61.0	54.0	-11
Farm worker impact, EI/A	42.2	33.2	-21
Consumer impact, EI/A	18.4	14.6	-20
Ecology impact, EI/A	122.4	114.3	-7
Maize			
Herbicide use (lb ai/A)	3.4	2.4	-30
Total impact, EI/A	102.5	69.9	-32
Farm worker impact, EI/A	52.2	39.9	-24
Consumer impact, EI/A	26.7	19.0	-29
Ecology impact, EI/A	224.3	150.0	-33
Soybean			
Herbicide use (lb ai/A)	1.5	1.0	-38
Total impact, EI/A	40.9	30.8	-25
Farm worker impact, EI/A	29.9	15.2	-49
Consumer impact, EI/A	12.8	6.7	-48
Ecology impact, EI/A	80.1	70.6	-12

¹ pounds of active ingredient per acre

3.2 Fossil fuel use

A major expense and source of pollution in weed management are the fossil fuels used in tillage and herbicide application. This factor is seldom considered in the evaluation of environmental impact of herbicide use. In some countries (e.g., Denmark), mandated herbicide reduction programs have also required fewer applications of herbicides. Certainly, HRCs have greatly reduced tillage (discussed in Section 4.2) and, in some cases, the number of herbicide applications (Gianessi, 2005).

Few studies have carefully evaluated the impact of HRCs on reduced fossil fuel use in weed management, although this is generally recognized as a beneficial aspect of HRCs (e.g., Olofsdotter *et al.*, 2000). In a recent study in Europe using a life-cycle assessment approach, Bennett *et al.* (2004) concluded that the major environmental advantage of growing glyphosate-resistant sugarbeet would be much lower emissions from herbicide manufacturing, transport, and field operations, thus reducing contributions to global warming, smog, ozone depletion, ecotoxicity of water, and acidification and nitrification of soil and water. Some of these effects are illustrated in Figure 2. They qualified their conclusions by stating that the environmental and health impacts of growing HRCs should be assessed on a case-by-case basis, using a holistic approach.

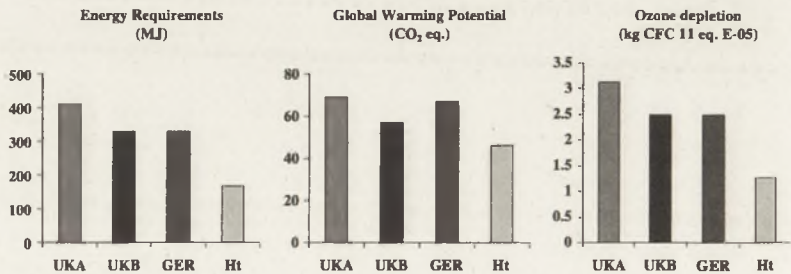


Figure 2. The impacts of typical herbicide regimes for conventional compared with glyphosate-resistant sugarbeet in the UK and Germany in terms of extracted energy use (MJ), global warming potential [kg carbon dioxide (CO₂) equivalent] and ozone depletion [kg chlorofluorocarbon (CFC) 11 equivalent] per functional unit. UKA and UKB are two different herbicide regimes used with non-transgenic sugarbeet in the UK, GER is a typical herbicide regime used with non-transgenic sugarbeet in Germany, and Ht is the use of glyphosate only with glyphosate-resistant sugarbeet. (reprinted from Bennett *et al.*, 2004 with permission)

4. EFFECTS ON SOIL

4.1 Chemical contamination

None of the three herbicides that have been used with transgenic HRCs are thought to be significant soil contaminants when used at recommended doses. All of these herbicides are applied as foliar sprays, so that contamination of soil is from direct interception of spray by the soil surface or from runoff or leaching of the herbicide and/or its breakdown products from vegetation. In the case of glyphosate, the compound can be translocated to roots from foliar tissues and exuded by the roots into the soil (e.g., Coupland and Caseley, 1979).

Bromoxynil is not photostable (Kochany *et al.*, 1990), and it photolyzes on the plant or soil surface. It is adsorbed moderately to soil at neutral and alkaline pHs. Bromoxynil is not considered to be very persistent in soils, and the half-life is about ten days to two weeks at 25 °C. Most literature reports rapid degradation in most soil types in most climates (e.g., Collins, 1973; Ingram and Pullin, 1974; Smith, 1971, 1984). Much of the compound is degraded completely to CO₂ within a few weeks (Collins, 1973). Soil was not contaminated with bromoxynil in a long-term use site in Alberta, Canada (Miller *et al.*, 1995). It is degraded by numerous soil microbes, such as *Agrobacterium radiobacter*, being broken down by a denitrification process (Kidd and James, 1991; Muller and Gabriel, 1999).

Glyphosate strongly adsorbs to soil particles and is rapidly degraded by soil microbes (reviewed by Duke, 1988; Duke *et al.*, 2003a). It has little or no herbicidal activity after it reaches the soil. It is degraded by many microbes to glyoxylate and AMPA (e.g., Araujo *et al.*, 2003). Other microbes can convert glyphosate to inorganic phosphate and sarcosine, and some can use glyphosate as both a carbon and phosphorus source. After long-term use of glyphosate in Canadian soils, no detectable residues could be detected (Miller *et al.*, 1995). Glyphosate is rapidly degraded by soil microbes, even at high application rates, without adversely affecting microbial activity (Haney *et al.*, 2000). Haney *et al.* (2000) found a cumulative soil carbon mineralization with increasing glyphosate rate. The CO₂ flush 2 d after application suggested that glyphosate was either readily and directly utilized by soil microbes or made other resources available (Figure 3).

Glyphosate has a moderate half-life in soils with an average value of approximately 47 days, but reaching 174 days in some soils under some conditions (Vencill, 2002; Wauchope *et al.*, 1992). Studies with lysimeters have shown less leaching and higher concentrations of glyphosate and AMPA in soil where low-tillage agriculture had been practiced (Fomsgaard *et al.*, 2003).

Like glyphosate, glufosinate has little or no herbicidal activity once it enters the soil. Glufosinate, also like glyphosate, has a relatively short half-life in soil under field conditions. For example, Langelueddeke *et al.* (1982) reported it to be completely decomposed within a few weeks of application. Under laboratory conditions, the half-life in Canadian prairie soils was 3-7 days at 20 °C and 8-11 days at 10 °C (Smith, 1988). Gallina and Stephenson (1992) found glufosinate to have a half life of 3 to 7 days in Ontario soils, degrading to metabolic intermediates (3-(methylphosphinyl) propionic acid (MPPA) and 2-(methylphosphinyl)acetic acid), until eventually being degraded to CO₂.

Escherichia coli strains were able to degrade glufosinate to its corresponding 2-oxoacid {2-oxo-4-[(hydroxy)(methyl)phosphinoyl] butyric acid} by transamination, and *Rhodococcus* sp., was able to utilize glufosinate as a sole source of N, forming 2-oxo-[(hydroxy)(methyl) phosphinoyl]butyric acid by oxidative deamination (Bartsch and Tebbe, 1989). In a field study, Smith and Belyk (1989) found no leaching of glufosinate nor MPPA to soil depths below 10 cm.

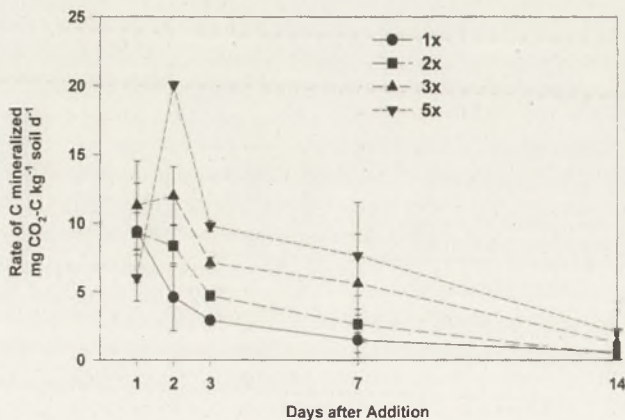


Figure 3. Effect of glyphosate addition rate on soil carbon mineralization. Carbon mineralized from basal microbial respiration in control samples has been subtracted. The 1, 2, 3, and 5X represent glyphosate addition rates of 47, 94, 140, and 234 $\mu\text{g ai g}^{-1}$ soil, respectively. Error bars indicate ± 1 standard deviation (reprinted from Haney *et al.*, 2000 with permission)

4.2 Soil loss and compaction

Another benefit from the use of HRCs is that they facilitate reduced or zero tillage agronomic systems, which contribute to reductions in soil erosion from water and wind, fossil fuel use, air pollution from dust, loss of soil moisture, and soil compaction (Holland, 2004). Reduced tillage also improves soil structure, leading to reduced risk of runoff and pollution of surface waters with sediment, nutrients, and pesticides.

Considering the relatively high level of potential environmental improvement that can be gained by reducing tillage, there is a remarkable paucity of refereed publications on the influence of HRCs on tillage practices and associated environmental effects. Loss of topsoil due to tillage is perhaps the most environmentally destructive effect of agriculture. Even taking land out of its natural state for agriculture is more rapidly reversible than the loss of top soil, which, once lost, can take centuries or eons to replace.

A survey by the American Soybean Association (2001) found that 53% of U.S.A. soybean farmers made an average of 1.8 fewer tillage passes per year through their soybean fields since glyphosate-resistant soybeans were introduced. This translates to a savings of \$385 million per year in reduced tillage costs. In a five-year period in the U.S.A., during which the planting of glyphosate-resistant soybeans increased from only a few per cent to about 70% (Figure 1), there was a dramatic increase in the adoption of no-tillage and reduced tillage management (Figure 4). Most of this change was associated with the growing of glyphosate-resistant soybeans (Figure 5). Whether this trend has continued is unknown; however, weed changes (Section 8) in glyphosate-resistant crops have caused some farmers to return occasionally to tillage as a weed management tool.

Similarly, there has been a rise in no-tillage agriculture in soybeans in Argentina with the adoption of glyphosate-resistant soybeans, where there is a loss of 10 tons of topsoil per hectare in soybeans produced with conventional tillage (Penna and Lema, 2003). Dramatic reductions in soil erosion were documented where no-tillage, glyphosate-resistant soybeans were grown.

A lesser-studied effect is that of HRCs on soil compaction. Use of glyphosate-resistant crops has generally resulted in few herbicide applications, meaning fewer trips across the field with a tractor. This should result in less soil compaction; however, to our knowledge, the shift to no-tillage agriculture that many farmers using HRCs have made has not been studied in the context of soil compaction.

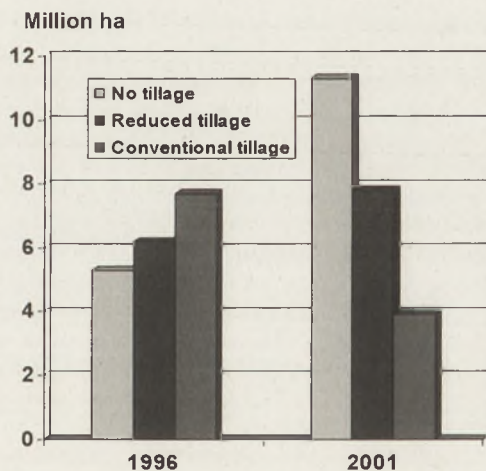


Figure 4. Soybean tillage methods by hectares farmed in the U.S.A. in 1996 and 2001. In 1996 and 2001, there were 19.2 and 23 million ha, respectively, of soybeans grown. (Drawn from American Soybean Association, 2001 data)

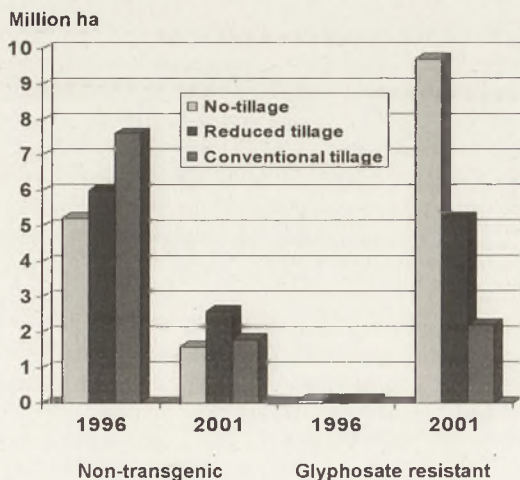


Figure 5. Tillage practices and glyphosate-resistant soybean use by hectares in the USA in 1996 and 2001. (Drawn from American Soybean Association, 2001 data)

5. EFFECTS ON WATER AND AIR

One study has focused on impacts of HRCs on both water and air. Bennett *et al.* (2004), using a life-cycle assessment model comparing the environmental and human health impacts of conventional sugarbeet growing regimes in the United Kingdom and Germany with those that might be expected if glyphosate-resistant sugarbeet were grown, suggested that growing this HRC would be less harmful to the environment and human health than growing the conventional crop, largely due to lower emissions from herbicide manufacture, transport and field operations. Most of this analysis dealt with air and water pollution. Emissions contributing to negative environmental impacts, such as global warming, ozone depletion, ecotoxicity of water and acidification and nitrification of soil and water, were much lower for the HRC than for the conventional crop. Emissions contributing to summer smog, toxic particulate matter and carcinogenicity, which have negative human health impacts, were also substantially lower for the herbicide-tolerant crop (Figure 2).

5.1 Water

Bromoxynil photolyzes in surface layers of water (Millet *et al.*, 1988) and is biodegraded in natural water systems. In test ponds, bromoxynil did not persist in sediment beyond 15 days after treatment. It does not bioconcentrate in aquatic organisms. Studies conducted in Canada did not detect bromoxynil in water and ponds after spraying (Waite *et al.*, 1992). In a study with five different soils of Alberta, Canada, bromoxynil was found to have a moderate level of leaching from soil, compared to strongly leaching herbicides (e.g., dicamba) and herbicides that do not leach at all (trifluralin) (Hill *et al.*, 2000). A three-year study was conducted to determine the seasonal effects, such as herbicide application and rainfall/irrigation, on herbicide levels in shallow groundwater (Hill *et al.*, 1996). Bromoxynil (0.03-8.4 ppb) and three other herbicides were detected in 17-61% of the wells sampled. Other long term studies conducted in Canada with the herbicides glyphosate, dicamba, 2,4-D, bromoxynil, methylchlorophenoxyacetic acid (MCPA), diclofop, and triallate showed no residues of glyphosate in groundwater (Miller *et al.*, 1995). Of the HRC herbicides used, only bromoxynil was found at concentrations exceeding levels set by the Canadian drinking water quality guidelines. In a study conducted by McNaughton and Crowe (1995), bromoxynil was also found in shallow groundwater in Canada, along with the herbicides 2,4-D, diclofop-Me, dicamba, MCPA, and triallate, but below the limits set by the

Canadian drinking water quality guidelines.

Both glyphosate and glufosinate are strongly adsorbed to soils particles, and, even though both are highly water soluble, they do not leach to ground water. Various studies have shown that glyphosate contaminates surface water less than several alternative herbicides (summarized by Carpenter *et al.*, 2002). Once in surface water, it dissipates more rapidly than most other herbicides.

In the intensely farmed maize-growing regions of the mid-western USA, surface waters have often been contaminated by herbicides, principally as a result of rainfall runoff occurring shortly after application of these to maize and other crops (Wauchope *et al.*, 2002). A model was used to predict maize herbicide concentrations in the reservoirs as a function of herbicide properties comparing broadcast surface pre-plant atrazine and alachlor applications with glyphosate or glufosinate post-emergent herbicides incorporating both glyphosate-resistant and glufosinate-resistant maize (Wauchope *et al.*, 2002). Because of their lower post-emergent application rates and greater soil sorptivity, glyphosate and glufosinate loads in runoff were generally one-fifth to one-tenth those of atrazine and alachlor, indicating that the replacement of pre-emergent maize herbicides with these post-emergent herbicides allowed by genetic modification of crops would dramatically reduce herbicide concentrations in vulnerable watersheds. Similarly, Estes *et al.* (2001) found in a higher tier modeling examination, that various herbicide use regimes employed in the U.S.A. in maize caused more ground and surface water contamination than did glyphosate when used with glyphosate-resistant maize, thereby reducing the risk to drinking water and related ecosystems.

By modeling, Peterson and Hulting (2004) predicted less risk to groundwater and aquatic plants by a glyphosate-resistant wheat/herbicide system than from a non-transgenic wheat/conventional herbicide system. In a comprehensive survey of the U.S. Geological Service (1998), more than 95% of all samples collected from streams and rivers contained at least one pesticide, compared to about 50% for ground water. None of the pesticides were among those used on HRCs. Although this study was done before the widespread adoption of HRCs, glyphosate was widely used as both a preplant and postharvest herbicide, as well as a harvest aid before the advent of HRCs.

Other studies also found no HRC herbicides in ground water in the United States where glyphosate is applied on no-tillage cropping systems (Kolpin *et al.*, 1988) and in Brazil in various cropping systems (Bonato *et al.*, 1999; Cerdeira *et al.*, 2000, 2002, 2003; Lanchote, *et al.*, 2000; Paraíba *et al.*, 2003). Similar results were found for surface waters (Clark *et al.*, 1999).

Degradation of pesticides in aquifers has been evaluated, and glyphosate was found to be degraded under both anaerobic and aerobic conditions, as opposed to some other herbicides such as MCPA and mecoprop (Albrechtsen *et al.*, 2001). Certain pesticides were not degraded in water under aerobic or anaerobic conditions (dichlobenil, bentazon, isoproturon, and metsulfuron-methyl). This could be important when using glyphosate on transgenic crops, if the herbicide leached sufficiently to reach ground water, which is a more anaerobic environment. Half-lives of glyphosate vary from 60 h for ground water samples exposed to sunlight to 770 h for those stored under dark conditions (Mallat and Barcelo, 1998). Glyphosate was also evaluated for ecological risk assessment, and it was found not to bioaccumulate, biomagnify, or persist in an available form in the environment (Solomon and Thompson, 2003). This study also showed that the risk to aquatic organisms is negligible or small at application rates <4 kg/ha and only slightly greater at application rates of 8 kg/ha.

Based on results from the literature, one can conclude that there is a low risk of significant water contamination with bromoxynil, but the risk is negligible for glyphosate and glufosinate.

5.2 Air

Herbicides can pollute the air by drift or volatility. The three herbicides that have been used with HRCs are essentially not volatile at 25 °C (Vencill, 2002) and have not been reported as atmospheric contaminants (e.g., Van Dijk and Guicherit, 1999).

Most herbicides are applied by spraying, resulting in movement to non-target sites and organisms through the air. Air movement of sprayed herbicides to unintended crops and other vegetation (termed 'herbicide drift' in the U.S.A.) has been a problem since the use of potent, synthetic herbicides began. After glyphosate-resistant soybeans were introduced, Owen (1998) reported that complaints of herbicide drift problems increased in Iowa. Growing a HRC next to a non-HRC of the same species may exacerbate such problems, as there is no visual difference between the two crops to the herbicide applicator. Furthermore, with glyphosate-resistant crops, the herbicide can be used during later crop development by aerial application, further increasing the risk of drift.

6. EFFECTS OF THE HERBICIDES AND HERBICIDE-RESISTANT CROPS ON NON-TARGET ORGANISMS

6.1 Other plants

The original goal of using herbicides was to kill all vegetation in a crop with as little damage as possible to the crop. Even a small infestation of weeds can reduce crop yield by competition and/or allelopathy. In most cases, the farmer also wants to kill or reduce most vegetation within a meter or two of the crop, to prevent the spread of weed seeds and/or other propagules to cropland. So, for the farmer in most parts of the world, non-target plants are usually those more than a couple of meters from the field.

Drift of herbicides to non-target plants has been a problem since synthetic herbicides were introduced. As mentioned above (Section 5.2), drift to non-transgenic crops of the same species is a new problem with HRCs. Although effects of herbicides on non-target plants are not a new problem, there are numerous studies on such effects of herbicides used with HRCs. For example, De Snoo et al. (2001) found in studies simulating drift of glufosinate that phytotoxicity (reduced growth) to vegetation could be detected at 2% of the field dose. At doses that would not be expected more than a meter or two outside the field (32 and 64% of field dose), the vegetation biomass and number of species within the sprayed area was reduced. There was nothing surprising in this study, as glufosinate is a broad-spectrum herbicide.

Ellis and Griffin (2002) evaluated the response of non-transgenic soybean and cotton to simulated drift of glyphosate and glufosinate. Soybean and cotton injury and height reductions occurred in most cases. Soybean height was reduced by no more than 11%, regardless of herbicide rate or timing. There was no substantial difference in the sensitivity of soybean to glyphosate and glufosinate. When herbicides were applied late, soybean was more sensitive to glufosinate in the first year (Table 5). Cotton was more sensitive to glufosinate than to glyphosate 7 days after application in both years, regardless of timing, but by 28 days differences between the herbicides were less apparent, as there were little or no residual effects of either herbicide at this time. A similar study was done with rice and maize with similar results (Ellis et al., 2003). Injury to both crops was seen, particularly when applied early at the highest rate (12.5% of the recommended rate for weed management).

Table 5. Injury of non-transgenic soybean after simulated drift rates of glyphosate and glufosinate at two application timings (adapted from Ellis and Griffin, 2002 with permission)

Soybean injury (%)			
7 DAT ¹			
Herbicide	Rate g ai/ha ³	Early timing	Late timing
Glyphosate	140	29(21) ²	25(17)
	70	18(8)	3(5)
	35	3(4)	0(0)
	18	0(0)	3(0)
	9	0(0)	0(0)
Glufosinate	53	14(19)	40(17)
	26	9(6)	16(5)
	13	0(1)	0(0)
	7	0(0)	0(0)
	4	0(0)	0(0)
14 DAT			
Glyphosate	140	35(5)	3(0)
	70	9(1)	0(0)
	35	1(0)	0(0)
	18	0(0)	0(0)
	9	0(0)	0(0)
Glufosinate	53	4(6)	14(0)
	26	0(0)	6(0)
	13	0(3)	0(0)
	7	0(1)	3(0)
	4	0(0)	0(0)
28 DAT			
Glyphosate	140	8	0
	70	0	0
	35	0	0
	18	1	0
	9	0	0
Glufosinate	53	1	0
	26	1	0
	13	0	0
	7	0	0
	4	0	0

¹ 1 Days After Treatment

² Application timings correspond to 2 to 3 trifoliolate (early timing) and first flower (late timing). Data averaged across years. Data are for 1988 and 1999. The 1999 data are in parentheses

³ Rates correspond to 12.5, 6.3, 3.2, and 0.8% of the labeled rates of 1.12 g ai/ha glyphosate and 420 g ai/ha glufosinate

Glyphosate, sprayed during seed maturation, can dramatically affect seed quality (Cerqueira *et al.*, 1985). Blackburn and Boutin (2003) determined whether glyphosate would have an effect on the germination and growth of the F1 generation of seeds produced by plants sprayed with the herbicide. Of the 11 species tested, using treatments of up to 890 g a.i./ha sprayed near seed maturity, seven showed a significant effect of the glyphosate treatment on germination and/or growth characteristics. The authors concluded that results of this experiment, together with several previous studies reviewed in this paper, suggest that there are significant effects to keep in mind when using herbicides such as glyphosate, as significant ecological changes could occur.

The objective of using herbicides in agroecosystems is to cause severe changes in plant species composition and density within the agroecosystem. The desired effect dissipates over a relatively short distance from the field, if the herbicide is used properly. As stated above, these effects are no different than those caused by herbicides in non-HRCs.

In Europe there has been some controversy about the effects on biodiversity by HRCs in farm-scale evaluation studies. In Europe, much more than in North America, there is a desire to incorporate the maintenance of some weed species within crops to maintain ecological diversity. Studies have linked the presence of weeds to biodiversity of invertebrates, wildlife, and birds, since weeds provide a food supply for these animals. Marshall (2001) speculated that the currently available HRCs seem unlikely to provide the required flexibility of management for leaving sufficient weeds for these purposes. Perry *et al.* (2004) found larger weed abundance in fodder maize with HRCs than with conventional weed management. Dewar *et al.* (2003) devised a strategy to use band spraying in HRCs to increase biodiversity within the crop and providing a habitat for birds and wildlife. Their method of leaving weeds between crop rows could, in some cases, be used without compromising crop yield. Some have suggested that biodiversity would be increased with HRCs that use broad-spectrum, foliar-applied herbicides like glyphosate and glufosinate, since the farmer can wait to spray weeds after there has been some weed growth, providing habitats and/or food for birds, arthropods, other herbivores, etc. However, Freckleton *et al.* (2004), using weed phenologies and a population model, found that such effects would probably be transient. They suggested that if herbicide application could be ceased earlier, a viable population of late-emerging weeds could be maintained. Most North American farmers strive to rid their fields of weeds and weed seeds. In a multi-year study in Argentina in glyphosate-resistant soybean in which glyphosate was used continuously, weed species diversity decreased

or remained stable early in the growing season and increased by harvest time as a result of this weed management choice (Vitta *et al.*, 2004).

Perhaps an even greater effect on non-target plant life than that caused by the herbicide associated with the HRC is the effect of tillage on vegetation. No-tillage agriculture results in weed species shifts (see section 8.2) and results in more vegetation on the field before and after the period of crop production, resulting in improved habitat for other organisms.

HRC turf grass may present another method of affecting non-target plants. Grass clippings from glyphosate-resistant creeping bentgrass that had been sprayed with glyphosate for weed control possessed enough residual glyphosate for three days after treatment to cause injury to other species when used as a mulch (Goss *et al.*, 2004).

As we mentioned earlier, glyphosate and glufosinate are less likely to pollute ground and surface waters than many of the herbicides that they replace. A life-cycle assessment technique used to compare conventional sugarbeet agricultural practices with risks that might be expected if glyphosate-resistant sugarbeet were grown suggested that growing this HRC would be less harmful to the ecology of water for the herbicide-tolerant crop than for the conventional crop (Bennett *et al.*, 2004, Figure 6). These results suggest less impact of glyphosate-resistant crops on aquatic vegetation than conventionally grown crops.

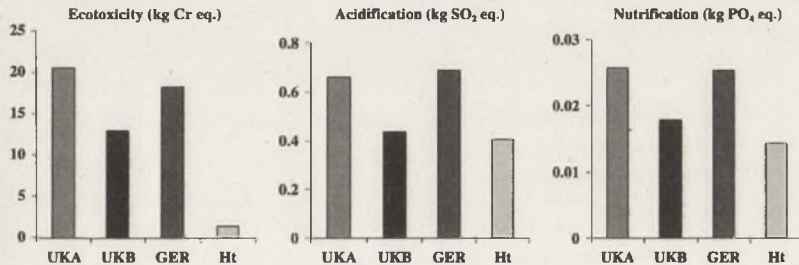


Figure 6. The impacts of typical herbicide regimes for conventional compared with genetically modified (GM) glyphosate-resistant sugarbeet in the UK and Germany in terms of ecotoxicity [kg chromium (Cr) equivalent], acidification [kg sulphur dioxide (SO₂) equivalent] and nutrification [kg phosphate (PO₄) equivalent] per functional unit. UKA and UKB are two different herbicide regimes used with non-transgenic sugarbeet in the UK, GER is a typical herbicide regime used with non-transgenic sugarbeet in Germany, and Ht is the use of glyphosate only with glyphosate-resistant sugarbeet (reprinted from Bennett *et al.*, 2004 with permission)

The indirect effect of HRC herbicides on plants through their influence on plant pathogens is discussed in Section 6.3. Subtle effects of sublethal concentrations of these herbicides on non-target vegetation through this mechanism have not been studied in the field.

6.2 Soil organisms

The potential direct effects of HRCs and their management include changes in soil microbial activity due to direct effects of the herbicides used with the HRC, differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as changes in other herbicide applications and tillage (reviewed in part by Dunfield and Germida, 2004). Most of the available literature addresses the first effect. Bromoxynil is more toxic to soil microbes than are glufosinate and glyphosate. Debona and Audus (1970) found bromoxynil to be in the midrange of herbicides inhibiting soil nitrification processes. The toxicity of fourteen herbicides was assessed by their effects on pure cultures of *Nitrosomonas europaea* and *Nitrobacter winogradskii* and soil nitrification processes (Ratnayake and Audus, 1978). Bromoxynil was one of the least toxic herbicides in this study. Kristufek and Blumauerova (1983) found lower amounts and numbers of actinomycetes in three horizons of forest soil treated with bromoxynil than in untreated soil. Studies on the interaction of bromoxynil with *Azospirillum* species and the growth of maize have shown that neither inoculation nor herbicide application with or without inoculation had any significant effect on the major groups of soil microflora (bacteria, actinomycetes, and fungi) (Fayez, et al., 1983). Incorporation in soil of bromoxynil at the recommended field dose had no effect on the nitrogenase or dehydrogenase activities. Bromoxynil increased dry weight of roots and shoots of crops after 45 days, and this effect was more pronounced when applied with *Azospirillum* spp..

Gyamfi et al. (2002) evaluated possible shifts in eubacterial and *Pseudomonas* spp. rhizosphere community structures due to the release of glufosinate-resistant oilseed rape and its associated herbicide use. Treatments included cultivation of the transgenic plant as well as of the wild-type cultivar in combination with mechanized removal of weeds and the application of the herbicides glufosinate and metazachlor. Rhizosphere soil was sampled from early and late flowering plants, as well as from senescent plants. A culture-independent approach was chosen to characterize microbial communities based on analysis of 16S rRNA gene fragments amplified from rhizosphere DNA. Dominant pseudomonads in

the rhizosphere were analyzed by sequence analysis. Whole community and *Pseudomonas* fingerprints revealed slightly altered microbial communities in the rhizosphere of transgenic plants; however, the effects were minor as compared to the plant developmental stage-dependent shifts. Both herbicides (glufosinate and metazachlor) caused transient changes in the eubacterial and *Pseudomonas* spp. population structure, whereas differences due to the genetic modification were still detected at the senescent growth stage. The observed differences between transgenic and wild-type lines may have been due to unintentionally modified plant characteristics such as altered root exudation.

In general, there is little or no effect of glyphosate on soil microflora. For example, Gomez and Sagardoy (1985) found no effect of glyphosate on microflora of soils in Argentina at twice the recommended rates of the herbicide. Studies on the effect of glyphosate on microbial activity of typical Hapludult and Hapludox Brazilian soils measured by soil respiration (evolution of CO₂) and fluorescein diacetate (FDA) hydrolysis revealed an increase of 10-15% in the CO₂ evolved and a 9-19% increase in FDA hydrolyses in the presence of glyphosate (Araujo *et al.*, 2003). Soil which had been exposed to glyphosate for several years had a strong response in microbial activity. After 32 days incubation with glyphosate, the number of actinomycetes and fungi had increased, while the number of bacteria was slightly reduced. After the incubation period, HPLC detected the glyphosate metabolite AMPA, indicating glyphosate degradation by soil microorganisms. Other studies (Haney *et al.*, 2000, 2002) have generated data strongly suggesting that glyphosate causes enhanced microbial activity directly. An increase in the carbon mineralization rate occurred the first day following glyphosate addition and continued for 14 d (Figure 3). Glyphosate appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, even at high application rates, without adversely affecting microbial activity. In relation to leaching of glyphosate and/or its metabolite AMPA, it was studied in four lysimeters, from a low-tillage field and from a normal tillage field. A significant difference between the soil residual concentrations of the herbicide has been seen under different management regimes. The higher concentration was found in the lysimeters where low-tillage had been practiced (Fomsgaard *et al.*, 2003).

Siciliano and Germida (1999) found differences in rhizosphere-associated microbes between a glyphosate-resistant and two non-transgenic canola cultivars. Endophytic bacterial populations also varied between cultivars. In a later study, Dunfield and Germida (2001) concluded that there were differences in bacterial communities in the rhizosphere of HRC canola

varieties compared to non-transgenic varieties. However, the changes were temporary, and did not persist until the next field season (Dunfield and Germida, 2003). The microbial communities associated with glufosinate-resistant maize did not differ in their single strand conformation polymorphism patterns from those associated with non-transgenic maize (Schmalenberger and Tebbe, 2002).

The soybean nitrogen-fixing symbiont *Bradyrhizobium japonicum* possesses a glyphosate-sensitive EPSPS, and upon exposure to glyphosate accumulates high concentrations of shikimate and certain benzoic acids that can be plant growth inhibitors (Moorman et al., 1992). These effects are accompanied by growth inhibition and/or death of the microbe, depending on the glyphosate concentration. This paper suggested that there could be unexpected effects of glyphosate on nitrogen metabolism in glyphosate-resistant soybeans. Furthermore, glyphosate is translocated to nodules of glyphosate-resistant soybeans (Reddy and Zablotowicz, 2003). Subsequent research (King et al., 2001; Reddy et al., 2000) summarized by Zablotowicz and Reddy (2004) has indicated that such effects as the reduction in nodulation, nodule size, and leghemoglobin content of nodules can be caused in glyphosate-resistant soybeans sprayed with glyphosate. However, the effect of glyphosate on nitrogenase activity in nodules from glyphosate-resistant soybeans in field studies was inconsistent. Greenhouse studies indicated that the effects should be maximal under moisture stress. Sensitivity of *B. japonicum* strains varied. In the field, the effects are transient, and there is no evidence that crop yield is affected.

Motavalli et al. (2004) concluded in a review that there is so far no conclusive evidence that those HRCs and other transgenic crops which have been deregulated and used in many cropping situations in many climates and soil types over the past 10 years have had any significant effect on nutrient transformations by microbes. However, they point out that this topic needs further study, as not every situation has been adequately researched. In another recent review, Dunfield and Germida (2004) stressed that the effects shown are field site and season dependent and that the method of analysis can affect the results. They point out that the changes in microbial communities associated with HRCs are more variable and transient compared to those caused by other agricultural practices such as crop rotation, tillage, use of certain other herbicides, and irrigation. Nevertheless, they stated that minor alterations in the total diversity of the soil microbial community, such as the removal or appearance of certain microbes, for example rhizobacteria or plant pathogens could affect soil health and ecosystem functioning. So far, there

appear to be no significant harmful effects of the three HRC herbicides (glyphosate, glufosinate, and bromoxynil) and their use with HRCs on soil microorganisms. Kowalchuk *et al.* (2003), in a review of the effects of all transgenic crops on soil microbes, states that observed effects have generally been minor and that they are very small in comparison with other sources of variation. They propose case-by-case approaches that target both potentially vulnerable microbes, as well as community parameters in evaluating the impact of transgenic crops on soil microorganisms.

6.3 Plant pathogens

The effects of pesticides on plant pathogens that affect crops have been an understudied and controversial topic (Altman, 1993). Since HRC crops made resistant to bromoxynil and glufosinate use rapid metabolic inactivation of the herbicide by a transgene-encoded enzyme as a resistance mechanism, it seems unlikely that these herbicides, when used with HRCs, will affect the crop's response to a plant pathogen. However, this is not the case with glyphosate. Furthermore, glyphosate is toxic to many microorganisms, including plant pathogens (e.g., Toubia-Rahme *et al.*, 1995; Wyss and Muller-Scharer, 2001) and even some animal pathogens, such as apicomplexan parasites (e.g., *Plasmodium* spp.) containing the apicoplast (Roberts *et al.*, 1998). Not all fungi are susceptible to pure glyphosate. For example, Morjan *et al.* (2002) found that glyphosate alone was not fungicidal to the entomopathogenic fungi *Beauveria bassiana*, *Metarrhizium anisopliae*, *Nomuraea rileyi*, and *Neozygites floridana*. However, when formulated, *N. floridana* and *M. anisopliae* were susceptible to all glyphosate formulations. The four fungi were susceptible to various glyphosate formulations when exposed to field concentrations.

Kremer *et al.* (2001) compared the effects of glyphosate, a conventional herbicide mix (pendimethalin plus imazaquin), and glyphosate plus the conventional herbicide mix on soil microbes in four glyphosate-resistant soybean varieties at eight sites. The frequency of *Fusarium* spp. on roots increased 0.5 - 5X at 2 or 4 weeks after the application of glyphosate or glyphosate plus conventional herbicides compared with the conventional herbicide alone. In another study, *Fusarium* spp. populations increased after glyphosate treatment of weeds in the field, but crops subsequently grown in these fields were not affected by *Fusarium* spp. (Levesque *et al.*, 1987).

Glyphosate inhibits the growth of the plant pathogen that causes red crown rot (*Calonectria crotalariae*) on soybean (Berner *et al.*, 1991). Field trials showed a reduction in red crown rot incidence with preplant applications of low rates of glyphosate. In a laboratory study, growth of *Pythium ultimum* and *Fusarium solani* could be stimulated or inhibited,

depending on glyphosate concentration (Kawate et al., 1992). Dead or dying weeds can provide a good microenvironment for plant pathogens. *Pythium ultimum* and *Fusarium solani* populations increased in soils containing glyphosate-treated weeds (Kawate et al., 1997). Smiley et al. (1992) found that the incidence of *Rhizoctonia* root rot was more severe and yields lower when intervals between glyphosate treatment and crop planting were short, which they attributed to greater availability of nutrients from dying weeds for pathogen populations.

Glyphosate can also affect how a plant responds to a pathogen. In non-glyphosate-resistant plants, it is well documented that glyphosate can make the plant more susceptible to plant pathogens (e.g., Johal and Rahe, 1988; Liu et al., 1997), largely or at least partly by inhibiting the production of defense-related compounds derived from the shikimate pathway such as some phytoalexins and lignin. Low doses of glyphosate can sometimes make resistant cultivars susceptible to plant disease (Brammall and Higgins, 1988). Glyphosate was even patented as a synergist for a plant pathogen that controls weeds (Christy et al., 1993). Theoretically, reduced resistance to plant pathogens caused by glyphosate through these processes should not occur in glyphosate-resistant crops.

Nevertheless, there have been reports of increased susceptibility of glyphosate-resistant crops to plant pathogens. Farmers in Michigan have reported an increased susceptibility of glyphosate-resistant soybean to *Sclerotinia sclerotiorum* (Lee et al., 2003). Neither glyphosate, nor its formulation components, nor the glyphosate resistance transgene were implicated in the increased susceptibility (Lee et al., 2000). In this case, there was no effect of glyphosate or the shading from the narrower rows that farmers use with this crop on the plant's defense. In a wider study, Harikishnan and Yang (2002) concluded that glyphosate-resistant and -susceptible soybeans reacted similarly to most herbicide treatments with respect to root rot and damping off diseases cause by *Rhizoctonia solani*. Similarly, the response of glyphosate-resistant soybeans to *Fusarium solani*-caused sudden death syndrome (SDS) was not different than that of conventional soybeans and was not affected by the application of glyphosate (Sanogo et al., 2001). Nijiti et al. (2003) had similar results with *F. solani*-caused SDS in soybeans, as influenced by glyphosate and the glyphosate resistance trait. Nelson et al. (2002) had mixed results with different glyphosate-resistant soybean cultivars and the application of different herbicides to these cultivars with respect to susceptibility to *Sclerotinia sclerotiorum*-caused stem rot. Thifensulfuron treatment resulted in lower disease severity in isogenic glyphosate susceptible cultivars than with glyphosate-resistant cultivars. Sulfonylurea herbicides,

such as thifensulfuron, have been reported to stimulate the production of products of the shikimate pathway (e.g., Suttle *et al.*, 1983), from which some phytoalexins are derived.

Evaluating pest management implications of glyphosate-resistance in wheat, Lyon *et al.* (2002), considered that a lack of an equally effective and affordable herbicide to control glyphosate-resistant volunteer wheat, could increase wheat diseases such as wheat streak mosaic and *Rhizoctonia* root rot.

Field observations in Ohio suggested a possible interaction between soybean cyst nematode (SCN) and glyphosate in a transgenic glyphosate-resistant variety that also expresses SCN resistance derived from the 'PI88788' soybean line (Yang *et al.*, 2002). To investigate this possible interaction under controlled conditions, greenhouse experiments were conducted. Inoculation with SCN reduced shoot fresh weight of glyphosate-resistant soybean 8 to 29% across all experiments, but there was no interaction of glyphosate and SCN in glyphosate-resistant soybean. Glufosinate is toxic to various microbes (e.g., Tubajika and Damann, 2002) and could act as a fungicide when sprayed on glufosinate-resistant crops. This has been demonstrated with *Pseudomonas syringae* Pathovar *Glycinea* in glufosinate-resistant soybeans (Pline *et al.*, 2001). Colony-forming units were reduced 45 and 60 % in inoculated soybeans with 0.5 and 1.0 kg/ha glufosinate, respectively. In glufosinate-resistant rice, glufosinate application decreased symptoms caused by the pathogen *Magnaporthe grisea* (Tada *et al.*, 1996). Sheath blight caused by *Rhizoctonia solani* was prevented by the application of glufosinate or bialaphos (a peptide that is converted to glufosinate by the plant) to glufosinate-resistant rice (Uchimiya *et al.*, 1993) and creeping bentgrass (Liu *et al.*, 1998). *Rhizoctonia solani* and *Sclerotinia homeocarpa* were controlled by glufosinate in two glufosinate-resistant *Agrostis* (bentgrass) species (Wang *et al.*, 2003), indicating that this herbicide could be used to control both weeds and fungal plant pathogens in these transgenic turf grasses.

One unexpected aspect of the interactions between HRCs and pathogens is that if the expression of the herbicide resistance transgene is promoted by cauliflower mosaic virus 35S promoter, the expression of the resistance gene can be reduced if the crop is infected with cauliflower mosaic virus, leaving the crop susceptible to the herbicide (Al-Kaff *et al.*, 2000). This has not yet been reported in the field.

In summary, there are cases in which the herbicide or the HRC itself may influence plant pathogens either negatively or positively. However, most of the data suggest that the herbicide/HRC combination for glufosinate and glyphosate may be providing some disease reduction. Much more work needs to be done to study these effects to better use HRCs in integrated

pest management.

6.4. Arthropods

Bromoxynil and glyphosate have not been reported to have insecticidal or other activities against arthropods. However, any herbicide can indirectly affect arthropod populations and species compositions in an area by its effects on vegetation. Furthermore, changes in cropping systems (e.g., changing from tillage to no-tillage) can drastically influence arthropod populations.

Virtually all studies on the direct effects of glyphosate on arthropods show no significant effects. For example, Haughton *et al.* (2001), in a study of the effects of glyphosate on spiders, stated that "their results support other limited data which suggest that glyphosate is harmless to non-target arthropods." Gomez and Sagardoy (1985) found no effects of glyphosate on microarthropods in soil at double the recommended application rates. An indirect effect of the herbicide through effects on weed species compositions and densities is more likely. For example, Jackson and Pitre (2004) found that populations of adult *Cerotoma trifurcata*, adult *Spissistilus festinus*, larvae of *Plathypena scabra*, and the caterpillar of *Anticarsia gemmatalis* were unaffected by glyphosate-resistant soybeans or by recommended or delayed doses of glyphosate. But, adult *Geocoris punctipes* populations were decreased by the herbicide. The authors concluded that this effect was due to reduced weed densities after glyphosate treatment.

Host plant suitability to green cloverworm (*Hypena scabra*) was evaluated on two conventional soybean varieties and two glyphosate-resistant varieties, with and without exposure to glyphosate (Morjan and Pedigo, 2002). No differences among treatments were detected on developmental time and survivorship. No sex bias or morphological effect was detected among treatments. Soybean genetic differences (between conventional varieties and analogous transgenic varieties) or plant stress (induced by glyphosate) did not affect the plant suitability to *H. scabra*.

Weed management systems that allowed more weeds generally had higher insect population densities (Buckelew, *et al.*, 2000). But, some species did not fit this generalization, as systems with fewer weeds appeared to be preferred by potato leafhoppers (*Empoasca fabae*). Bean leaf beetles (*Cerotoma trifurcate*) and potato leafhoppers preferred certain soybean varieties, but these effects were attributed to soybean plant height. Their findings (Table 6) indicate that although the glyphosate-resistant soybean varieties did not strongly affect insect populations, weed management systems can affect insect populations in soybean.

Table 6. Mean number of insects per 20 sweeps collected from transgenic and non-transgenic soybean varieties in two weed management systems (Adapted from Buckelew *et al.*, 2000 with permission)

Soybean variety/ Management system	Bean leaf beetle	Potato leafhopper	Early timing	Early timing	Late timing	Late timing
Glyphosate-resistant	3.96a	5.94c	0.49a	1.13a	1.11a	1.17a
Glyphosate-resistant ^a	3.87ab	7.29b	0.44a	1.14a	0.92a	1.05ab
Glufosinate-resistant	3.53b	6.02bc	0.57a	1.28a	1.65a	0.89b
STS ^b	3.48b	6.14bc	0.53a	1.10a	0.82a	1.10ab
Jack ^a	4.52a	4.95c	0.29a	0.97a	1.13a	0.91b
Kenwood 94	3.55ab	11.00a	0.53a	1.13a	1.14a	1.14a
Conventional weed management	3.69a	6.70b	0.64a	1.25a	1.66a	1.16a
Control/ Hand-weeded	3.95a	7.6a	0.32b	1.00b	0.59b	0.92b

Means followed by the same letter are not significantly different ($p > 0.05$).

^aDenotes a variety that is also resistant to soybean cyst nematode.

^bDenotes a non-transgenic variety that is resistant to sulfonylurea herbicides.

Glufosinate can be toxic to insects. Kutlesa and Caveney (2001) found glufosinate applied to leaf surfaces to be toxic to the first instar caterpillars of the butterfly *Calpododes ethlius*. The ingested levels that cause toxicity were comparable to those that might realistically be consumed from feeding on glufosinate-treated crops. The toxicity was apparently due to inhibition of the insect's glutamine synthetase, the same target site that it acts on in plants.

In studies of the effects of glufosinate or glufosinate-resistant sugarbeet, compared to non-transgenic sugarbeet with other herbicides, on epigeous predators of insects (Araneae, Carabidae, Staphylinidae), no long term effects could be predicted (Volkmar *et al.*, 2003). There was no evidence of any negative effects during the 3-year study. Furthermore, numerous endangered spiders and carabids were found on both the transgenic and the conventional sugar beet plots. Acute toxicity of glufosinate to springtails (*Paronychiurus kimi*) was negligible in a laboratory study (Kang *et al.*, 2001).

In studies on the toxicity of glufosinate-ammonium to predatory insects and predatory mite species at a concentration of 540 ppm (an application rate for weed control in apple orchards), glufosinate-ammonium was only weakly toxic to eggs of *Amblyseius womersleyi*, *Phytoseiulus persimilis*, and *Tetranychus urticae* but was highly toxic to nymphs and adults of these three mite species, indicating that a common mode of action between predatory and phytophagous mites might be involved (Ahn *et al.*, 2001). Little or no harm to larvae or pupae of the predatory insect *Chrysopa pallens* was seen, but the herbicide significantly affected *Orius strigicollis* (Table 7). The larvae and nymphs of predators died within 12 h after treatment, suggesting that the larvicidal and nymphicidal action may be attributable to a direct effect. The authors suggested that glufosinate-ammonium merits further study as a key component of integrated pest management.

In summary, there is little evidence of any direct effect of the herbicides used with HRCs on arthropods in the field or in natural environments. Effects of HRCs and associated cultural practices can affect arthropods indirectly.

6.5. Birds and wildlife

Using the LD₅₀ dose of glyphosate and other herbicides for rats, the environmental effect of the use of glyphosate-resistant soybeans compared to the use of non-transgenic soybeans for over 1400 US Midwest farms was estimated by Nelson and Bullock (2003). Unlike most previous studies, this one considered the relative toxicity of herbicide choices

Table 7. Toxicity of glufosinate-ammonium to each developmental stage of *O. strigicollis* using direct contact application (from Ahn *et al.*, 2001 with permission)

Concentration (ppm, a.i.)	Mortality (%)		
	Egg	Nymph ^a	Adult
2,160	ND	ND	95a
1,080	90a	90a	72b
540	71a	65b	58b
270	64bc	55c	40c
135	61c	47c	34c
67	43d	35d	33c

a.i., active ingredient. *ND.*, Not determined. Means within a column followed by the same letter are not significantly different ($p=0.05$). ^aThird instars

available to farmers. The simulation model results suggested that glyphosate-resistant soybean technology is more environmentally friendly, especially with regard to mammalian toxicity, than other technologies for all farms in the midwest of the U.S.A. (Figure 7). The effect was generally more pronounced in the southern part of the midwest, where a longer growing season makes overall weed pressure more serious, resulting in more herbicide use.

Peterson and Hulting (2004) compared the ecological risks of glyphosate used in glyphosate-resistant wheat with those associated with 16 other herbicides used in spring wheat in the northern Great Plains of the USA. A Tier 1 quantitative risk assessment method was used. They evaluated acute dietary risk to birds and wild mammals and acute risk to aquatic vertebrates, aquatic invertebrates, and aquatic plants, and effects on seedling emergence and vegetative vigor to non-target terrestrial plants. They also estimated groundwater exposure. They found less risk with glyphosate than with most other herbicides to aquatic plants, groundwater, and non-target plant seedling emergence (Table 8).

HRCs can affect birds and wildlife indirectly by altering habitat and food sources by effectively reducing weed biomass and/or changing weed species composition within the agricultural field. We mentioned above the studies by Dewar *et al.* (2003) and Perry *et al.* (2004) that have shown or predicted indirect effects of HRCs on wildlife through effects on habitat. The herbicide can be applied later in the growing season with HRCs that are resistant to glyphosate or glufosinate, because these herbicides are generally effective against most weed species at later growth stages. If desired, this allows the farmer to design a weed management scheme that would not reduce yield, but would benefit wildlife. Strandberg (2004) studied such possibilities in HRC maize, canola, and sugarbeet and found some improvements in both flora and fauna during early summer, due to a longer pesticide-free window during the spring. However, he points out that glyphosate and glufosinate use reduces weed seed production, with possible eventual negative consequences on wildlife. He concluded that long term investigations of cropping systems with each HRC should be conducted to understand the full effects (both positive and negative) on farm land wildlife.

Vegetation changes due to the adoption of no-tillage agriculture will almost certainly have effects on wildlife. We are not aware of studies on this indirect effect of HRCs.

Regarding the safety of the crop to wildlife, this topic is covered in detail in Section 7.

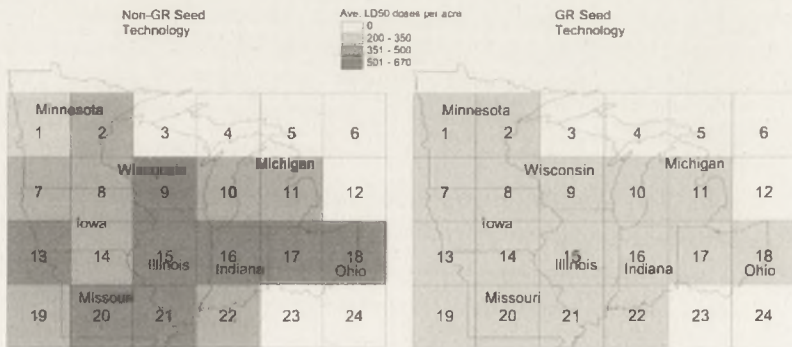


Figure 7. Average LD_{50} doses of herbicides in 3 by 3° grids of the upper Midwest of the U.S.A. with and without glyphosate-resistant (GR) soybean. (reprinted from Nelson and Bullock, 2003 with permission)

Table 8. Predicted relative ecological risks of herbicide active ingredients based on modeling (adapted from Peterson and Hulting, 2004 with permission)¹

Active Ingredient	Application rate (g ai/ha)	Groundwater value (ppb)	RR ²	Aerobic soil half-life (days)
Glyphosate	840	0.0005	1	2
2,4-D	560	0.005	10	5.5
Bromoxynil	1,100	0.0004	0.8	2
Clodinafop	67	0.00003	0.06	1
Clopyralid	146	0.06	120	26
Dicamba	280	0.1	220	18
Fenoxaprop	90	0.000006	0.01	1
Flucarbazone	34	0.2	400	NA
MCPA	1,457	0.26	520	25
Metsulfuron	9	0.004	8	28
Thifensulfuron	22	0.0001	0.2	6
Tralkoxydim	280	0.001	2	5
Triallate	1,100	0.04	80	54
Triasulfuron	34	0.05	100	114
Tribenuron	16	0.00003	0.06	2
Trifluralin	1,100	0.009	18	169

¹ Abbreviations: RR, relative risk; NA, not available²RR: Relative Risk compared with glyphosate, value in bold indicates greater risk relative to glyphosate

7. FOOD AND FEED SAFETY

There are two components of food safety associated with HRCs. The herbicides used with the HRC can alter food safety if they or their metabolic products are found in the edible portions of the crop. The transgene itself could alter food safety, either directly or indirectly. For regulatory approval, transgenic crops are scrutinized to a far greater level than conventional crops, using analytical, nutritional, and toxicological methods (Atherton, 2002; König *et al.*, 2004; Malarkey, 2003), although some have proposed that even more extensive tests be done by metabolomic, proteomic, and transcriptomic analysis to detect potential unintended effects of the transgene and its insertion on food safety and quality (Cellini *et al.*, 2004).

7.1 Herbicide residues

Surprisingly little has been published on herbicide residues in HRC foods. Most of what we know is from studies with non-HRC crops. However, herbicide residue data must be supplied for regulatory approval of HRCs. In the U.S.A., bromoxynil is a restricted use pesticide (RUP), and is not registered for homeowner use. RUPs may be purchased and used only by certified pesticide applicators. Its acute oral LD₅₀ is 440 mg/kg (Vencill, 2002). Developmental toxicity to rats fed daily doses of bromoxynil has been reported (Rogers *et al.*, 1991). It is categorized as toxicity class II - moderately toxic. Animals do not contain the herbicide molecular target site of bromoxynil (D1 protein of photosystem II). We have found no published studies of bromoxynil residues in edible parts of cotton (oil and cotton seed meal) or canola (oil). Since bromoxynil-resistant crops are no longer available, such studies are unlikely to be done in the future.

Glyphosate acid and its salts are moderately toxic compounds in EPA toxicity class II. Glyphosate (either the anion or the isopropylamine salt) is practically nontoxic by ingestion, with a reported acute oral LD₅₀ of >5000 mg/kg in rat (Vencill, 2002). The trimethylsulfonium salt of glyphosate is more toxic, with an oral LD₅₀ of about 705 mg/kg. It is not a RUP and is a best-selling weed killer for home use. Animals do not contain the herbicide molecular target site (EPSPS) of glyphosate.

Perhaps, because of its widespread use by the general public, glyphosate is among the pesticides most frequently reported to the California EPA Pesticide Illness Surveillance Program (Goldstein *et al.*, 2002). It analyzed glyphosate-related calls to the Pesticide Illness Surveillance Program in order to assess the number of reports involving symptoms and to better understand the nature and severity of reported cases. Data on glyphosate

and other pesticides available for the years 1982-1997 including target organ(s) affected (skin/eye/respiratory/systemic) is available. Of the 187 systemic cases, only 22 had symptoms recorded as probably or definitely related to glyphosate exposure alone. The reported symptoms were not severe, expected to be limited in duration, and frequently inconsistent with the route of exposure and/or previous experience with glyphosate. Occasional reports of severe effects of ingestion of formulated glyphosate occur (e.g., Sorensen and Gregersen, 1999; Stella and Ryan, 2004), however, the glyphosate-molecule itself is considered one of the most toxicologically benign herbicides available. Williams *et al.* (2000) extensively reviewed the toxicology literature on glyphosate and its metabolites and concluded that under present and expected conditions of use, glyphosate does not pose a significant health risk to humans.

In a testing program to detect whether glyphosate-resistant soybeans had been sprayed with glyphosate or not, Lorenzatti *et al.* (2004) found glyphosate and AMPA in green, immature seeds. Recent work of ours (Duke *et al.*, 2003b) found both glyphosate and its degradation product, AMPA, in harvested seeds of different glyphosate-resistant soybean varieties grown in widely separated geographical regions. Even though the glyphosate applications were at legal, but at relatively high rates and late timing, the residues were within established tolerance levels. We were surprised to find higher AMPA than glyphosate levels, since plants are thought to degrade glyphosate very little, if at all (Duke, 1988; Duke *et al.*, 2003a). This work also indicated that residues can be found in seeds of non-transgenic soybean grown in proximity to glyphosate-resistant soybeans, apparently due to herbicide drift. These findings led to a study that indicated the occasional phytotoxicity caused by glyphosate in glyphosate-resistant soybeans is due to AMPA accumulation (Reddy *et al.*, 2004), a compound known to be phytotoxic (Hoagland, 1980). We have found no publications on glyphosate residues in glyphosate-resistant crops other than soybean.

Glufosinate is not a RUP and is sold for home weed control in the USA. Its acute oral toxicity in rats is an LD₅₀ of ca. 2.2 g/kg. The herbicide target site, glutamine synthetase is also found in animals. Glufosinate chemically resembles glutamine, a molecule used to transmit nerve impulses in the brain.

Ebert *et al.* (1990) concluded in an extensive review that glufosinate is safe under conditions of recommended use. Similarly, Hack *et al.* (1994) also concluded from their studies that glufosinate is unlikely to cause health effects of either users or consumers when used as directed.

Neurotoxic symptoms observed in laboratory animals following ingestion

of high levels of glufosinate include convulsions, diarrhea, aggressiveness, and disequilibrium. Glufosinate can cause neurotoxicity, although the mechanism is unclear (Watanabe and Sato, 1998). This herbicide apparently does not poison mammals by its inhibition of glutamine synthetase, the herbicide target, but causes epileptic activity via nitric oxide production through activation of the N-methyl-D-aspartate receptor (Lapouble *et al.*, 2002).

In a study to determine if glufosinate applied to glufosinate-resistant maize and canola could lead to an increase in herbicide residues or to the formation of new metabolites, Ruhland *et al.* (2004) found that L-glufosinate was in the form of known metabolites and the parent compound in both maize and canola. The highest content was in leaves, and the lowest in grain. No levels were found above the established tolerance levels.

7.2 Changes in feed and food safety and quality due to the transgene

A transgene might pose a food safety risk for two basic reasons. First, the transgene itself could be toxic, due to direct toxicity, antinutritive effects, or allergenic effects. Second, the gene could cause a change in the metabolic pathways of the crop changing the levels of already existing metabolites or introducing a new metabolite. The latter risk can be due either to a direct effect of the transgene product or to insertion into the genome at a place that alters expression of other genes. In the case of HRCs, where no metabolic pathway is purposefully altered, there is less probability that new compounds other than herbicide residues will end up in the edible parts of the crop. New profiling methods can be used to detect changes in metabolic pathways (Cellini *et al.*, 2004; Kuiper *et al.*, 2002).

Harrison *et al.* (1996) provide the details of the safety evaluation for the CP4 EPSPS enzyme introduced into soybean to provide glyphosate resistance. The protein was found to be 1) non-toxic to mice when consumed at doses thousands of times higher than potential human exposure, 2) readily degraded by digestive fluids, and 3) not structurally or functionally related to any known protein allergens or toxins, based on amino acid sequence homology searches.

Health Canada's review of the information presented in support of the food use of refined oil from glufosinate resistant canola line HCN92 concluded that such refined oil does not raise concerns related to safety. Health Canada is of the opinion that refined oil from canola line HCN92 is as safe and nutritious as refined oil from current commercial varieties (http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_nf7web00.html). The nutritional properties of glufosinate-resistant

sugarbeets and maize grains were found to be essentially equivalent to non-transgenic cultivars in feeding studies with swine and ruminants (Bohme *et al.*, 2001).

Studies with glyphosate-resistant maize line GA21 evaluated the compositional and nutritional safety of maize line GA21 compared to that of conventional maize (Sidhu *et al.*, 2000). Compositional analyses were conducted to measure proximate, fiber, amino acid, fatty acid, and mineral contents of grain and proximate, fiber, and mineral contents of forage collected from sixteen field sites over two growing seasons. Similarly, Tutel'ian *et al.* (2001) found no compositional differences between conventional maize and maize line GA 21. The nutritional safety of maize line GA21 was also evaluated by Sidhu *et al.* (2000) in a poultry feeding study. Results from the poultry feeding study showed that there were no differences in growth, feed efficiency, adjusted feed efficiency, and fat pad weights between chickens fed with GA21 grain or with parental control grain. These data taken together demonstrate that glyphosate-resistant GA21 maize is as safe and nutritious as conventional maize for food and feed use. Several other studies have found no substantial difference in the nutrient content of glyphosate-resistant and non-transgenic crops. These studies include maize (Ridley *et al.*, 2002; Autran *et al.*, 2003), soybean (Padgette *et al.*, 1996b), wheat (Obert *et al.*, 2004), and cotton (Nida *et al.*, 1996). In the Autran *et al.* (2003) study, the characteristics of glyphosate- and glufosinate-resistant maize in different foods (e.g., beer, hominy, oil, grits) were compared and found to be not substantially different than the respective, non-transgenic parental lines.

Glyphosate targets the shikimate pathway (Duke *et al.*, 2003a), and the estrogenic isoflavones of soybeans are products of this pathway. Glyphosate resistance from the CP4 EPSPS gene is not always complete (Pline *et al.*, 2002), and glyphosate preferentially translocates to metabolic sinks such as seeds (Duke, 1988). Therefore, we reasoned that at relatively high and late applications of glyphosate to glyphosate-resistant soybeans, a reduction of the content of these compounds could occur. In a well-replicated field study at two sites, hundred of kilometers apart, we found no significant effects of glyphosate on isoflavones (Duke *et al.*, 2003b). Earlier, Lappe *et al.* (1999) reported reductions of isoflavone levels on glyphosate-resistant soybean varieties in the absence of glyphosate (*i.e.*, a pleiotropic effect of the CP4 gene). However, this study was not done by comparing isogenic lines. Padgette *et al.* (1996b) found no effects of the transgene on isoflavone content of soybean.

Table 9 summarizes most of the published results of animal feeding studies with glyphosate-resistant crops. All studies support the view that food from

Table 9. Results of animal feeding studies with glyphosate resistant crops

Crop	Animal	Result	Reference
maize	rat	no effect	Hammond <i>et al.</i> , 2004
maize	swine	no effect	Hyun <i>et al.</i> , 2004
maize	cattle	no effect	Erickson <i>et al.</i> , 2003
maize	dairy cattle	no effect no effect no effect	Donkin <i>et al.</i> , 2003 Ipharraguerre <i>et al.</i> , 2003 Grant <i>et al.</i> , 2003
maize	poultry	no effect	Sidhu <i>et al.</i> , 2000
soybean	rat	no effect no effect	Zhu <i>et al.</i> , 2004 Hammond <i>et al.</i> , 1996
soybean	mice	no effect	Brake and Evenson, 2004
soybean	swine	no effect	Cromwell <i>et al.</i> , 2002
soybean	dairy cattle	no effect	Hammond <i>et al.</i> , 1996
soybean	catfish	no effect	Hammond <i>et al.</i> , 1996
soybean	poultry	no effect	Hammond <i>et al.</i> , 1996
canola	rainbow trout	no effect	Brown <i>et al.</i> , 2003
canola	poultry	no effect	Taylor <i>et al.</i> , 2004

glyphosate-resistant crops is substantially equivalent to non-transgenic crops. In addition to these studies, no evidence of the *CP4* gene or its protein product could be detected in pork from swine fed glyphosate-resistant soybean meal (Jennings *et al.*, 2003). No effects on glyphosate-resistant soybeans could be found on the immune system of mice (Teshima *et al.*, 2000).

The potential allergenic properties of the protein products of transgenes must be determined before approval. These data are provided to regulatory agencies, but publications on this topic are scarce. However,

there are a few published studies showing no allergenic properties of transgene products associated with HRCs. Sten et al. (2004), in a study with soybean-sensitized patients, found that the allergenicity of ten glyphosate-resistant and eight non-transgenic soybean cultivars were not different. Chang et al. (2003) found no significant allergenicity to rats of the CP4 EPSPS gene product conferring glyphosate resistance.

A last, but understudied, aspect of food quality and HRCs is their influence on contamination of food with poisonous weed seeds. Weed seeds can be sources of toxic compounds (e.g., Powell et al., 1990). HRCs are generally more weed-free than conventional crops, resulting in less foreign matter, including weed seeds, in the harvested product (Shaw and Bray, 2003; Canola Council of Canada, 2001). Therefore, there is less likelihood of significant contamination of harvested food with toxic weed seeds in HRCs than with conventional crops.

8. HERBICIDE-RESISTANT WEEDS

This section will deal with weed problems for farmers that may or will occur because of the use of HRCs. Although glyphosate and glufosinate are non-selective, broad-spectrum herbicides, they cannot control all plant species or biotypes at recommended dose rates. Thus, weed species or biotypes with high levels of natural resistance can fill the ecological niches vacated in the agroecosystem in HRC cropping systems (Section 8.1). Weeds can evolve resistance to herbicides, especially when they are used year after year (Section 8.2). HRCs can become feral in some situations, creating a weed that cannot be controlled with the herbicide to which it has been engineered to be resistant (Section 8.3). The gene(s) conferring herbicide resistance to the crop can move to weedy relatives by outcrossing, causing a more problematic weed (Section 8.4). Lastly, the HRC gene can move to non-transgenic crops of the same species, creating weed and economic problems (Section 8.5). All of these scenarios are occurring already in glyphosate-resistant crops. Thus, to combat the weed problems associated with these developments, farmers are beginning to apply other herbicides with these 'non-selective' herbicides (e.g., Grichar et al., 2004). In a modeling study that analyzed 1356 potential interactions between biological, chemical, and physical factors for potential hazard associated with herbicide-resistant canola, Hayes et al. (2004) found the incidence of herbicide-resistant weeds (both wild weed and crop volunteers) to be the most probable risk. Almost all of the changes in vegetation resulting from HRC use at this time are problems for farmers, but not for the general public.

8.1 Weed shifts

Although glyphosate and glufosinate are broad-spectrum, non-selective herbicides, different species and different biotypes within species have different levels of natural resistance to these herbicides. Levels of natural resistance also can vary dramatically with growth stage. For example, the following weeds, with their Brazilian common name, are not weeds that have evolved resistance, but are difficult to control with glyphosate due to their natural resistance: *Chamaesyce hirta* (erva-de-Santa-Luzia), *Commelina benghalensis* (Trapoeiraba), *Spermacoce latifolia* (erva-quente), *Euphorbia heterophylla* (amendoim-bravo), *Richardia brasiliensis* (poaia-branca), and *Ipomoea* ssp. (corda-de-viola) (Brighenti, 2004). Genetic variation in glyphosate resistance exists in weedy morningglory species (Baucom and Mauricio, 2004). The more glyphosate-resistant biotypes were found to produce fewer seeds, so this trait may be a fitness disadvantage in the absence of glyphosate. The populations of naturally-resistant species were expected to increase in glyphosate-resistant crops if the crops are grown continuously (Shaner, 2000), leading to higher glyphosate application rates or the necessity of using other herbicides. Furthermore, with glyphosate and the slow evolution of resistance to it, weed species or biotype shifts should occur more rapidly than evolution of resistance. The predictions of Shaner (2000) have been borne out.

Waterhemp (*Amaranthus rudis* and *A. tuberculatus*) biotypes possess extremely variable levels of susceptibility to glyphosate (Patzoldt et al., 2002). Common waterhemp (*Amaranthus rudis*) and velvetleaf (*Abutilon theophrasti*), which are not effectively controlled by glyphosate, became a greater problem in glyphosate-resistant soybean in Iowa after a short period (Owen 1997). A Kansas study showed that ivyleaf morningglory (*Ipomoea hederacea*) and large crabgrass (*Digitaria sanguinalis*) that were not controlled well by glyphosate dominated the weed community in a corn-soybean rotation using glyphosate applications for weed control (Marshall et al., 2000). In a similar study, Coble and Warren (1997) reported that morningglory species increased in abundance with the continuous 3-year use of glyphosate compared with other herbicide programs. Other weed species that possess some level of natural resistance to glyphosate include nutsedge species (*Cyperus* spp.), marestail (*Conyza canadensis*), and hemp sesbania (*Sesbania exaltata*) (Shaner, 2000). The relatively high level of natural resistance of marestail to glyphosate could have aided in its evolution of a higher level of resistance (see Section 8.2).

A field study that was conducted from 1999 through 2001 in Mississippi, to determine the effects of bromoxynil-resistant and glyphosate-resistant cotton rotation systems on weed control and cotton yield, indicated a shift

in the spectrum of weeds toward more naturally herbicide-resistant species (Reddy, 2004). Reddy concluded that a yield decline in continuous bromoxynil-resistant cotton due to species shifts can be prevented by rotating bromoxynil-resistant with glyphosate-resistant cotton. The farmer no longer has this option due to the withdrawal of bromoxynil-resistant cotton from the market.

Not all cases of weed shifts are due to natural resistance to the herbicide. Hilgenfield et al. (2004) pointed out that weed shifts could also be due to the avoidance of glyphosate by development at times that are unlikely to be under glyphosate selection pressure. In a study with a range of weed species, varying in time of seedling emergence and sensitivity to glyphosate, they found ivyleaf morningglory to both better survive the herbicide after emergence and to avoid it by late emergence. Shattercane (*Sorghum bicolor*) avoided glyphosate applications by late emergence.

In the U.S.A., there have already been increasing populations of naturally resistant species and biotypes in glyphosate-resistant crops, requiring other herbicides to be used frequently with HRC herbicides in HRCs (e.g., Grichar et al., 2004; Johnson et al., 2002). Evaluating pest management implications of glyphosate-resistance in wheat, Lyon et al. (2002), considered that a lack of an equally effective and affordable herbicide as glyphosate would increase the possibility of over reliance on glyphosate, leading to species shifts, with unknown consequences for weed management in wheat.

The reduction and elimination of tillage that has been encouraged by the adoption of HRCs (Figure 3 & 4) also causes shifts in weed species (e.g., Swanton et al., 1999).

8.2 Evolved resistance

An excellent web site exists that catalogues all verified cases of evolved resistance to herbicides (Heap et al., 2004). After a long lag phase, the cases of evolved herbicide resistance to all herbicides have grown linearly since the late 1970s, reaching more than 250 resistant biotypes by 2004. Only one of these cases has been associated with a HRC. Relatively little evolved resistance is associated with the three herbicides that have been used with HRCs.

Evolved bromoxynil resistance was detected in 1995 in common groundsel (*Senecio vulgaris*) growing in a non-transgenic cotton field in Oregon (Mallory-Smith, 1998). The mechanism of resistance has not been reported. No other cases of evolved resistance to bromoxynil have been reported with any crops, including bromoxynil-resistant crops (Heap et al., 2004). Apparently, no resistance has evolved to glufosinate (Heap et al., 2004).

This herbicide has not been used as extensively as glyphosate or most of the other herbicides to which resistance has evolved.

Considering the complex manipulations that were required to produce an effective transgene for imparting glyphosate resistance, Monsanto Company considered it unlikely that a similar type of resistance would evolve in weeds (Bradshaw *et al.*, 1997). However, since this publication, several weed species have evolved resistance to glyphosate (Heap *et al.*, 2004) (Table 10), apparently through several different mechanisms.

We do not have a good understanding of the mechanism of resistance in *Lolium* spp., although it is apparently not due to an altered EPSPS (Baerson *et al.*, 2002a). When the susceptible and resistant biotypes of the California *L. rigidum* are treated with glyphosate, the susceptible biotype accumulates ten-fold more shikimic acid than the resistant biotype (Simarmata *et al.*, 2003). This result, coupled with the evidence of no enhanced degradation of shikimate in the resistant biotype, indicates differential effects of glyphosate on *in vivo* EPSPS activity. This conclusion is consistent with the conclusion of Lorraine-Colwill *et al.* (2003) that resistance is based on differences in cellular transport and translocation, indicating that glyphosate is not reaching the molecular target site in much of the plant tissues. Evolved resistance in *Eleusine indica* is due to a resistant form of EPSP (Baerson *et al.*, 2002b). This gene has been patented for use in producing glyphosate-resistant crops (Baerson *et al.*, 2004). All of these cases of evolved glyphosate resistance took place in non-transgenic crop situations except that of *Conyza* spp. In the U.S.A., this species evolved resistance to glyphosate largely or entirely in glyphosate-resistant soybeans (e.g., VanGessel, 2001). Its mechanism of resistance is apparently reduced translocation of the herbicide (Feng *et al.*, 2004). With the continued extensive use of glyphosate-resistant crops, more cases of evolved glyphosate-resistant weeds are expected to emerge.

8.3 Feral crops

Feral versions of crop species exist, and gene flow to these biotypes is of great concern. This topic is dealt with in Section 8.4. However, HRC technology may contribute to current HRCs themselves becoming a greater problem as volunteer or feral crops. Most domestic crops do not persist in a natural ecosystem, but they can be problems in agricultural fields when rotating crops, especially if they are resistant to the herbicide(s) used with the subsequent crop. This problem can be exacerbated with HRCs if two crops used in rotation are made resistant to the same herbicide. For example, York *et al.* (2004) found glyphosate-resistant cotton to be a problem in subsequent crops of glyphosate-resistant soybean.

Table 10. Weed species that have evolved resistance to glyphosate (data from Heap *et al.*, 2004)

Species	Year first reported	Countries
<i>Conyza bonariensis</i>	2003	South Africa
	2004	Spain
<i>Conyza canadensis</i>	2000	USA
<i>Eleusine indica</i>	1997	Malaysia
<i>Lolium multiflorum</i>	2001	Chile
	2003	Brazil
<i>Lolium rigidum</i>	1996	Australia
	2001	South Africa
	1998	USA

Feral cotton in soybeans can be a problem in harboring undetected cotton boll weevils. Volunteer HRC canola and wheat could pose problems in weed control in conservation tillage systems in the Pacific Northwest of the U.S.A. (Rainbolt *et al.*, 2004). These systems currently rely on glyphosate for weed control during fallow and before planting. Thus, continued use of a single herbicide with a HRC will make this problem worse.

8.4 Introgression of herbicide resistance transgenes to weeds

Introgression is the movement of a gene or genes from donor plants to sexually compatible recipient plants of a different genotype (e.g., different species, variety, or biotype) by sexual crossing, followed by backcrossing of the hybrid with the recipient population until the gene is stabilized in the population. This process is sometimes called gene flow. Gressel (2002a) points out that there are more confirmed cases of gene flow from weeds to crops than vice versa. Gene flow between plants may occur if the source and recipient plants are grown close enough to each other. However, pollen can be carried for long distances by wind, water, insects, and

animals. So, increased distance only reduces, but does not eliminate the probability of gene flow. Also, to occur, the potential gene source and recipient populations must flower at the same time and must be open-pollinated. For full movement of the gene or genes into another population, several backcrosses are required. Introgression of herbicide resistance transgenes into weedy species has the potential to exacerbate the problems with existing weed species in HRCs or to create a new weed problem with species that are normally not a problem. Introgression is more likely for HRC transgenes than for other transgenes, in that the herbicide used with the HRC selects for crosses between species, eliminating competition from plants without the transgene. Hybrids between species or between crops and weedy variants of the crop are often unfit (e.g., Lefol et al., 1996; Scheffler and Dale, 1994). The herbicide may enhance the survival of unfit crosses that might not survive under normal competitive situations, allowing the survivors to backcross with the non-crop parent, resulting in eventual introgression of the HRC transgene into the highly fit, wild population.

All crops are naturally resistant to most of the selective herbicides that are used with them. This natural resistance has a genetic basis. Although there are hundreds of cases of evolved resistance of weeds in fields of non-transgenic crops (Heap et al., 2004), there are no proven cases of introgression of herbicide resistance gene(s) from a naturally-resistant crop to an associated weed. This may, in part, be due to the fact that closely related weeds are often also naturally resistant to the same selective herbicides. This is not the case with HRCs.

Gressel (2002a), Gressel (2002b) and Kwon and Kim (2001) have reviewed the risks for introgression of transgenes from various crops into weedy relatives.

Among the transgenic HRCs grown in the U.S.A., only canola has weedy relatives with which it could interbreed. In North America *Brassica rapa* is the only species with which it readily interbreeds (Légère, 2005). However, there is no evidence yet of introgression of herbicide resistance genes into wild plants where HRC canola is grown. Scheffler and Dale (1994) reviewed outcrossing of canola with weedy relatives in Europe and concluded that poor vigor and high sterility in the hybrids will generally mean that hybrids and their progeny will not survive in either an agricultural or a natural habitat. In a study to measure the fate of a herbicide resistance transgene escaping from canola to *B. rapa* in the absence of the herbicide, Lu et al. (2003) found the gene frequency was reduced from 50% in the first backcross to 0.1% in backcross generation four if the gene was on an A chromosome. Transmission was less if the transgene was on a C

chromosome. Under the selection pressure of spraying the herbicide to which the transgene conferred resistance, the frequency of the transgene reached a stable value of about 5.5% within six generations of successive backcrossing. They suggested that the transgenic cultivars should be developed by integrating the herbicide resistant gene on a C chromosome or a cytoplasm genome and cultivated rotationally by year(s) with other non-transgenic varieties in order to reduce the transfer of the transgene to wild *B. rapa* species. With some weedy relatives of *B. napus*, such as *Sinapis arvensis*, gene transfer from *B. napus* in the field is very rare or non-existent (Moyes *et al.*, 2002).

Maize genes could theoretically introgress into teosinte (*Euchlaena mexicana*), the species from which maize originated, since the two species can interbreed (Doebly and Stec, 1993). Teosinte is found only in Mexico and Central America, and has not yet been reported to be contaminated with transgenes, although this possibility and the consequences of it are discussed in detail in a recent report (North American Commission for Environmental Cooperation, 2004).

Rice and wheat are two crops that have been made resistant to herbicides through non-transgenic methods. Both have weedy relatives with which they can interbreed in the USA. Tracking gene flow to these species should give us an accurate indication of how rapidly transgenes would move to these crop relatives under the selection pressure of the herbicide. Non-transgenic, imidazolinone-resistant rice is now available (Table 1), and transgenic HRC rice may reach the market in the near future. Rice readily interbreeds with a feral form of rice called red rice in North America (Messeguer *et al.*, 2004; Zhang *et al.*, 2003) and in Asia it interbreeds with feral forms of *Oryza sativa* and a weedy related species, *O. rufipogon* (Chen *et al.*, 2004). Oard *et al.* (2000) found under field conditions, that the progeny from crosses of red rice and transgenic rice with the *bar* gene conferring glufosinate resistance were apparently fit.

In the case of wheat, crosses of non-transgenic, imidazolinone-resistant wheat and *Aegilops cylindrica* were discovered after only one year of introduction of the crop (Seefeldt *et al.*, 1998). Crosses between these species are apparently more likely with some wheat varieties than others (Stone and Peeper, 2004). Putting the herbicide resistance transgene on the A or B genome of wheat will apparently prevent the movement to *Aegilops cylindrica* (Wang *et al.*, 2001). However, genes of the D genome of wheat can readily introgress into *Aegilops cylindrica* (Kroiss *et al.*, 2004). Glyphosate-resistant creeping bentgrass (*Agrostis stolonifera* L.), a wind-pollinated perennial, is being tested as a herbicide-resistant plant for use as a turf grass. Recent studies have shown that the CP4 EPSPS gene is

readily transmitted to non-transgenic bentgrass (Watrud *et al.*, 2004). This was not a surprising result. Bentgrass is not a troublesome weed, but if it were glyphosate-resistant it might become a problem in glyphosate-resistant crops.

There is good potential for introgression of transgenes from sunflower (Linder *et al.*, 1998) and sugarbeet (Desplanque *et al.*, 1999), as well as many other crops, to wild relatives.

The transfer of transgenes from soybean to weedy relatives is not considered a risk in the Western Hemisphere (which accounts for about 83% of the total soybean acreage worldwide), because there are no sexually compatible relatives of soybean growing wild in the Americas. Similarly, there are no weedy relatives of cotton in North America. However, it is difficult to completely restrict a cultivar to a particular area, as evidenced by the illegal growing of glyphosate-resistant soybean by some farmers in Brazil (Flaskerud, 2003) and the gene from transgenic maize moving into non-transgenic maize land races in Mexico, where the transgenic crop is not legal (North American Commission for Environmental Cooperation, 2004).

8.5. Gene flow to non-transgenic crops

Gene flow to non-transgenic crops of the same species is much more likely than outcrossing with other species. The transgene's presence in an unintended cultivar can result in weed problems with volunteer plants in the subsequent year when the farmer grows another crop that has been made resistant to the herbicide for which the gene confers resistance. Non-transgenic crops that are contaminated with transgenes may not be accepted by some markets, depending on the degree of contamination and the market. For some crops, such as soybean, outcrossing is not considered a significant problem, but for rice, maize, and canola, considerable outcrossing can occur.

In Canada, gene flow between fields of bromoxynil-, glufosinate-, and glyphosate-resistant canola and non-transgenic canola has resulted in herbicide resistance transgenes in several combinations being present in fields that were supposed to contain only non-transgenic canola (Hall *et al.*, 2000). Gene flow between transgenic and non-transgenic canola can be substantial (Rieger *et al.*, 2002). Models have been used to predict the effects of cropping systems on gene flow from herbicide-resistant canola to non-transgenic canola (Colbach *et al.*, 2001a, b). Methods have been developed to control multiple herbicide-resistant volunteer canola caused by gene flow (Beckie *et al.*, 2004).

Maize genes could theoretically introgress into land races of maize, and at

least one paper has claimed that this has already happened (Quist and Chapela, 2001). There was considerable controversy over this paper, and the journal concluded that although "the evidence is not sufficient to justify the publication of the original paper", it was best "to allow readers to judge the science for themselves" (Anon, 2002). However, a recent report has again stated that there has been gene flow to maize landraces in Mexico (North American Commission for Environmental Cooperation, 2004).

9. INTROGRESSION OF TRANSGENES AND THE NATURAL ENVIRONMENT

We know relatively little about introgression of genes from crops into wild relatives and the potential impact of this on natural ecosystems. Many of the traits necessary for survival in a natural environment have been bred out of crop species, as demonstrated by the very few crops that have feral representatives in natural habitats. Few crops can survive in the wild for more than a year. Most herbicide resistance transgenes would provide no survival benefit in a natural environment. This topic has been previously discussed (Stewart *et al.*, 2003). However, there are transgene-imparted mechanisms of herbicide resistance, such as non-specific metabolic degradation (Didierjean *et al.*, 2002) or non-specific metabolic pumps for xenobiotics (Windsor *et al.*, 2003), that could provide cross resistance to pathogens that have phytotoxins as part of their virulence mechanism. The biotechnology industry has thus far chosen not to commercialize crops with such transgenes.

A persuasive argument can be made that a herbicide resistance gene should have no fitness advantage in a natural habitat. In a few cases, herbicide resistance appears to result in lowered fitness; however, the HRC genes used in commercial crop varieties at this time seem to be fitness neutral. Thus, we would expect no effect of these genes on natural ecosystems if introgressed into wild plants. When the herbicide resistance gene is coupled with another transgene that would provide a natural ecosystem fitness advantage (e.g., disease, insect, drought, or temperature extreme resistance), there is a potential problem with the herbicide resistance transgene. When both genes are used, the use of the herbicide in the presence of the hybrid will favor backcrossing until the gene conferring the fitness advantage is introgressed into a wild population. At this time, insect and herbicide resistance transgenes are coupled in maize and cotton. The North American Commission for Environmental Cooperation (2004) report claims that both transgenes have entered the maize land race populations of Mexico.

Movement of fitness-enhancing transgenes into wild populations is the only non-reversible risk of transgenic crops. Thus, controlling or mitigating movement of transgenes to wild populations is highly desirable. This topic has been reviewed by Gressel (2002a). Some of the approaches for the mitigation or elimination of introgression of transgenes proposed by Gressel (2002a) and others are listed below:

- 1) Do not couple herbicide resistance genes with transgenes imparting fitness in natural habitats. If herbicide resistance genes are used with transgenes imparting fitness in natural ecosystems, there will be less chance of introgression being enhanced by the herbicide if the two genes are put on different chromosomes.
- 2) Putting the resistance gene into the plastid genome (the plastome) would prevent or greatly reduce gene flow through pollen flow (e.g., Daniell *et al.* 1998). Advances are being made in plastid transformation (reviewed by Zhang *et al.*, 2003 and Maliga, 2003). There is very little information on how "failsafe" such a strategy might be, since there is evidence of movement of plastome genes through pollen in several angiosperms (e.g., Wang *et al.*, 2004; Zhang and Sodmergen, 2003).
- 3) Use sterile varieties. Some varieties or cultivars of plants propagate vegetatively, producing no pollen. Webster *et al.* (2003) recommended triploid, non-pollen-producing or -receiving cultivars of bermudagrass for the introduction of herbicide resistant turf. Some cultivars are male sterile. Luo *et al.* (2004) designed and synthesized chimeric gene constructs that produced complete male sterility in creeping bentgrass.
- 4) The transgene can be linked to one that would be deleterious to survival in the wild (e.g., genes that prevent dormancy or seed shattering) (Al-Ahmad *et al.*, 2004; Gressel and Al-Ahmad, 2004).
- 5) Use regulatory elements that will not function in weedy relatives (Gressel and Al-Ahmad, 2004).
- 6) Transgenes can be located in parts of the genome that are normally not subject to introgression (Stewart *et al.*, 2003). For example, in wheat, the A and B genomes of this triploid crop will not introgress into jointed goatgrass (Anderson *et al.*, 2004).
- 7) Hybrid technology can also be used. Placing a dominant transgene for herbicide resistance in the male sterile line, with close linkage with the male sterility gene will prevent introgression (Gressel, 2002a).
- 8) The controversial "terminator" technology, that prevents seed viability from crosses with the transgenic crop, would stop introgression (Oliver *et al.*, 1998). To our knowledge, this technology has not been tested in the field.

We view development and use of "failsafe" methods for eliminating

introgression as the most important thing that could be done to reduce the potential environmental impact of transgenic crops.

10. POTENTIAL EFFECTS ON INVASIVE PLANT SPECIES

The introduction of exotic, invasive plant species to habitats where they are not native has caused incalculable environmental harm that is rarely reversible and extremely costly to mitigate (Carruthers, 2003). Part of the spread of these species is due to contamination of crops seeds with those of weeds. In Canada, harvested HRCs are more weedfree than conventional canola, resulting in less contamination of harvested seed with weed seed (Canola Council of Canada, 2001). Foreign matter in representative glyphosate-resistant and conventional soybean systems was determined by evaluating elevator receipts collected from soybean producers in the southern and midwestern United States (Shaw and Bray, 2003). A total of 16,535 ha were represented, of which 13,903 were from glyphosate-resistant soybean and 2,632 were from conventional soybean. The average foreign matter content from the glyphosate-resistant soybean was 1.9%, compared with 2.5% from the conventional soybean. The authors conclude that the glyphosate-resistant program reduced foreign matter, an indication of reduced weed seed. We assume that similar results would be found for most other HRCs. Thus, the reduced potential for contamination of harvested seeds of HRCs with weed seed could result in a small but valuable reduction to the risk of the spread of exotic, invasive weed species.

11. SUMMARY AND CONCLUSIONS

We have provided an abbreviated survey of the potential impacts (risks and benefits) of HRCs and the herbicides that are used with them. Clearly we, and many of the authors that have written on this topic, emphasize that risks and benefits are very geography and time dependent. In the context of the replaced herbicides and agronomic practices, the health and environmental benefits of the herbicide/HRC combinations that have been used are significant.

The only herbicides currently being used with HRCs, glyphosate and glufosinate, are more environmentally and toxicologically benign than many of the herbicides that they replace. Their effects on soil, air, and water contamination and on non-target organisms are relatively small.

Soil erosion causes longterm environmental damage. Being broad spectrum, foliarly applied herbicides, with little or no activity in soil,

glyphosate and glufosinate are highly compatible with reduced- or no-tillage agriculture and have contributed to the adoption of these practices in the Western Hemisphere. This contribution to environmental quality by HRCs is perhaps the most significant one.

Transgenic foods are tested and evaluated for safety to a much greater extent than traditional foods that are usually derived by conventional breeding methods involving the transfer of many genes. New crops and new crop varieties of conventional crops are usually introduced without any testing for safety or nutritional problems related to genetics. In contrast, a rigorous safety testing paradigm has been developed for transgenic crops which utilises a systematic, stepwise and holistic approach (reviewed by Cogburn, 2002). Regarding food quality, we agree with Cogburn (2002), who concluded in an exhaustive review of the approval processes for transgenic food, that foods and feeds derived from genetically modified crops are as safe and nutritious as those derived from traditional crops. The lack of any adverse effects resulting from the consumption of transgenic crops grown on hundreds of millions of cumulative hectares over the last 10 years supports his conclusion.

All of the potential environmental risks that we discuss above are reversible (even that of soil erosion) and are in most cases not exclusive to transgenic crops, except for those associated with flow of transgenes to other plants (the same species or other species). Little or no impact or risk is expected from the HRC transgene if it introgresses into wild populations, as it is fitness neutral. However, when the HRC transgene is linked with genes that could provide a fitness advantage in a natural habitat, introgression could be aided by elimination of competing plants of the hybrid by the herbicide. Over the long term, this could be the greatest risk of HRCs. Several methods could be used to prevent introgression, but more research should be done to discover and/or develop technology to prevent it.

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