

The Current Status and Environmental Impacts of Glyphosate-Resistant Crops: A Review

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ABSTRACT

Glyphosate [N-(phosphonomethyl) glycine]-resistant crops (GRCs), canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. Glyphosate-resistant cotton and soybean have become dominant in those countries where their planting is permitted. Effects of glyphosate on contamination of soil, water, and air are minimal, compared to some of the herbicides that they replace. No risks have been found with food or feed safety or nutritional value in products from currently available GRCs. Glyphosate-resistant crops have promoted the adoption of reduced- or no-tillage agriculture in the USA and Argentina, providing a substantial environmental benefit. Weed species in GRC fields have shifted to those that can more successfully withstand glyphosate and to those that avoid the time of its application. Three weed species have evolved resistance to glyphosate in GRCs. Glyphosate-resistant crops have greater potential to become problems as volunteer crops than do conventional crops. Glyphosate resistance transgenes have been found in fields of canola that are supposed to be non-transgenic. Under some circumstances, the largest risk of GRCs may be transgene flow (introgression) from GRCs to related species that might become problems in natural ecosystems. Glyphosate resistance transgenes themselves are highly unlikely to be a risk in wild plant populations, but when linked to transgenes that may impart fitness benefits outside of agriculture (e.g., insect resistance), natural ecosystems could be affected. The development and use of failsafe introgression barriers in crops with such linked genes is needed.

HERBICIDE AND INSECT RESISTANCE are the two categories of transgene-conferred traits for crops that have had significant effects on agriculture (Gutterson and Zhang, 2004). Herbicide-resistant crops (HRCs) (Duke, 1996), sometimes called herbicide-tolerant crops, are crops made resistant to herbicides either by transgene technology or by selection in cell or tissue culture for mutations that confer resistance. Most of the success and controversy about safety of HRCs surrounds glyphosate-resistant crops (GRCs), the topic of this review. Glyphosate-resistant crops and their environmental impact have never been the sole subject of an in-depth review, although HRCs have been extensively reviewed (Dekker and Duke, 1995; Duke et al., 1991, 2002; Duke, 1998, 2002, 2005; Duke and Cerdeira, 2005a,b; Dyer et al., 1993; Gressel, 2002b; Hess and Duke, 2000; Silvers et al., 2003; Warwick and Miki, 2004) and have been the topic of two edited books (Duke,

1996; McClean and Evans, 1995) and a special issue of the journal *Pest Management Science* in 2005. A recent review covered agronomic and environmental aspects of HRCs (Schuette et al., 2004). Other reviewers have discussed the environmental impacts of all transgenic crops, with coverage of GRCs (Carpenter et al., 2002; Uzogara, 2000). Lutman et al. (2000) and Kuiper et al. (2000) published brief reviews of environmental consequences of growing GRCs. Dill (2005) briefly discussed the current status of GRC products. Meyer and Wolters (1998) reviewed the ecological effects of the herbicide use associated with GRCs. None of these publications have focused solely on an in-depth assessment of the potential toxicological and environmental impacts of all aspects of GRCs.

We will discuss what we consider the most important and germane literature, along with other selected examples. The regulatory process for approval of HRCs in the many countries that regulate their approval will not be discussed. The chemical, transgene, and weed management (e.g., changes in tillage) aspects of GRC environmental impacts will be covered. Unfortunately, we cannot access the potential environmental risks of the compounds with which glyphosate is formulated, due the lack of literature on this topic. This problem is exacerbated by the fact that formulations have varied through time and between geographic areas. This herbicide no longer has patent protection, so many formulations are now sold under several trade names. The potential environmental impact of a technology is often geography and/or time dependent. Thus, extrapolation of the results and conclusions of studies to all situations is impossible. The best we can do is generalize 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 GRCs with the risks that they displace. The viewpoints in this analysis are those of the authors and are not meant to reflect those of our employers.

GLYPHOSATE AND GRCS

Glyphosate is a very effective non-selective herbicide. Before introduction of GRCs, glyphosate 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). Even before the introduction of GRCs, glyphosate was a very successful herbicide. However, glyphosate use increased more than six-fold between 1992 and 2002, to become the most used herbicide in the United

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Abbreviations: AMPA, aminomethylphosphonate; EPSPS, 5-enolpyruvyl-shikimate-3-phosphate synthase; GR, glyphosate-resistant; GRC, glyphosate-resistant crop; HRC, herbicide-resistant crop.

States (Gianessi and Reigner, 2006). Most of this increase in use is due to the adoption of GRCs.

Glyphosate is the only herbicide that acts by blocking the shikimate pathway through inhibition of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). Inhibition of EPSPS results in reduced aromatic amino acids and deregulation of the shikimate pathway (Duke et al., 2003a). The latter effect causes massive flow of carbon into the pathway, with 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, with recommended application doses between 0.21 and 4.2 kg ai ha⁻¹, depending on the use (Vencill, 2002). In most agronomic crops, it is used at rates higher than 1 kg ha⁻¹. Glyphosate is an anion and is sold as a salt with different cations (e.g., isopropyl amine, trimethylsulfonium, diammonium).

At this time, glyphosate-resistant (GR) soybean, cotton, canola, maize, alfalfa, and sugarbeet are available to farmers of North America (Table 1). Other GR crops and turf grasses are under development. Three transgenes are used with these crops. A gene (CP4) encoding a GR form of EPSPS from *Agrobacterium* sp. was found to be very effective at producing GRCs (Padgett et al., 1996a). A gene from the microbe *Ochrobactrum anthropi* that encodes a glyphosate-degrading enzyme (glyphosate oxidase, GOX) is used in GR canola with CP4 EPSPS (Padgett et al., 1996a). GOX degrades glyphosate to glyoxylate, an ubiquitous and safe natural product, and aminomethylphosphonate (AMPA). A form of GR EPSPS from maize was produced by site-directed mutagenesis for use as a transgene in maize (Dill, 2005).

Other genes for glyphosate resistance are under development. A new gene for glyphosate resistance was developed from a gene from *Bacillus licheniformis* (Weigmann) Chester that encodes an enzyme that is a weak acetyltransferase toward glyphosate (Castle et al., 2004; Siehl et al., 2005). N-acetylation of glyphosate inactivates it. Directed evolution of this gene by gene shuffling resulted in a 7000-fold increase in the efficiency of the enzyme. As a transgene, this improved gene confers complete resistance to glyphosate to plants at field rates. A fungal gene-encoding glyphosate decarboxylase

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

Crop	Year made available
Soybean	1996
Canola	1996
Cotton	1997
Maize	1998
Sugarbeet†	1999
Alfalfa	2005

† Never grown by farmers, withdrawn in 2004, but to be reintroduced in 2006.

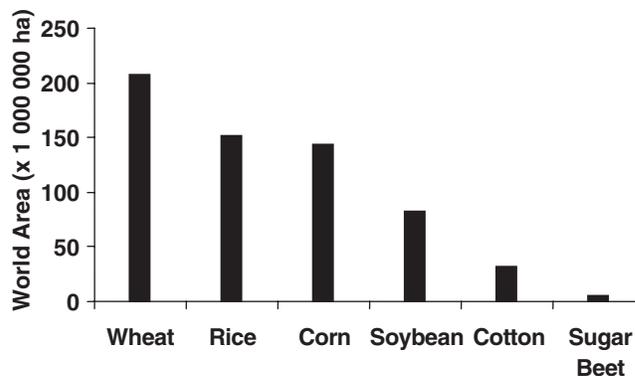


Fig. 1. Worldwide area of major crops. (Food and Agriculture Organization, 2003).

has been patented as a transgene for generating glyphosate-resistant crops (Hammer et al., 2005). An evolved, resistant form of EPSPS from *Eleusine indica* (L.) Gaertn. has been patented for use in GRCs (Baerson et al., 2004).

There is still tremendous potential for growth of GRC market share. The worldwide area of major crops is around 700 million hectares, not counting pasture (Fig. 1). GRCs represent only a small fraction of this crop area. Worldwide, almost all of the approximately 65 million ha of herbicide-resistant crops (ISAAA, 2005) planted in 2004 were GRCs.

Adoption of GR soybeans has been rapid in the USA, Argentina, and Brazil, with almost 90% of the acreage in the USA (ca. 30 million ha) planted with such varieties in 2004 (Fig. 2). In Argentina, the adoption of GR soybeans was even more rapid than in the U.S.A, reaching almost 90% (ca. 14 million ha) within 4 yr of introduction (Penna and Lema, 2003). This level of adoption took more than 25 yr for hybrid maize in Argentina. In Argentina, better weed management and reduced cost were about equally important reasons for adoption. In the USA, rapid adoption of GR soybeans has been due to increased yields, the reduced cost of excellent weed control, and simplified and more flexible weed control (Reddy, 2001; Gianessi, 2005; USDA-ERS, 2005b).

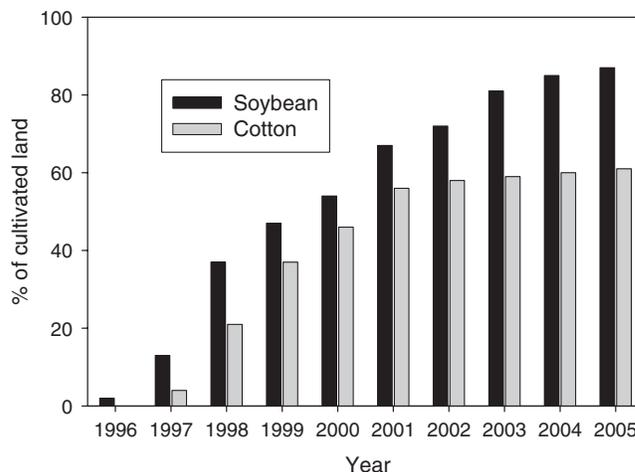


Fig. 2. Adoption of glyphosate-resistant soybean and cotton in the U.S.A. by year. (Duke, 2005; USDA-ERS, 2005a).

Adoption of GR soybean is increasing rapidly in Brazil, where it was only recently approved. Some GR soybeans are also grown in Uruguay and Mexico.

The potential area in the world for GR maize is even bigger than for soybeans (Fig. 1). However, the economic advantage is not as clear with GR maize, with an approximate 18% adoption rate in the USA in 2004 (Dill, 2005). Johnson et al. (2000) concluded that input costs for GR maize are slightly higher than for non-transgenic hybrids, but net economic returns are similar, and the use of glyphosate allows greater flexibility in postemergence weed management decisions.

Approximately 75% of canola acreage in the U.S.A. was planted in GR varieties in 2003 (Gianessi, 2005). In Australia, an economic analysis of GR canola showed significant economic advantages (Monjardino et al., 2005).

Despite great success with other GRCs, GR sugarbeet (*Beta vulgaris* L.) was not grown by North American sugarbeet farmers after its first approval for use, due to concerns about acceptance of sugar from transgenic plants by the confectionary and other prepared food industries. Similar and other concerns resulted in a decision by the company developing GR wheat (*Triticum aestivum* L.) technology not to ask for deregulation in 2004 (Dill, 2005). GR sugarbeet was deregulated again in 2005, for planting in 2006. Savings for farmers in Europe if GR sugarbeet were adopted are estimated to be 220 € ha⁻¹ yr⁻¹ (Pidgeon et al., 2004).

Glyphosate-resistant alfalfa (*Medicago sativa* L.) was deregulated in 2005, and there is a petition for deregulation of GR creeping bentgrass (*Agrostis stolonifera* L.). Glyphosate-resistant onions (*Allium cepa* L.), Kentucky bluegrass (*Poa pratensis* L.), and peas (*Pisum sativum* L.) have approval for field testing. However, as was the case with wheat, this does not ensure that these GRCs will be commercialized. There is already concern about gene flow from creeping bentgrass to weedy relatives (Watrud et al., 2004).

EFFECTS ON HERBICIDE AND FOSSIL FUEL USE

Effects on 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 (Agrow World Crop Protection News, 1998). Before herbicides, extensive tillage and manual weeding were the primary means of weed management. There has been controversy about whether GRCs have increased herbicide use or not. Glyphosate is normally often used at rates of a kg or more per ha, whereas some of the newer herbicides such as carfentrazone-ethyl are effective at only about 10–50 g per ha (Vencill, 2002). The controversy over increased volume of herbicide use has been fueled by others' assumption that an increased amount of chemical use equals increased environmental damage and toxicological risk. This assumption does not take into account the facts that the potential environmental damage and toxicological risk can vary consid-

erably between different herbicides as determined by many factors, including use rate and toxicity. Thus, comparing herbicide use rates has relatively little bearing on potential environmental damage or toxicological risk to humans. A very few studies, such as those by Nelson and Bullock (2003), have compared toxicological risk, rather than herbicide active ingredient used per unit area.

Even though glyphosate is not a low use rate herbicide, it is considered to be a low risk herbicide in terms of toxicity and environmental effects. 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 GRCs (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 GRCs in the U.S.A. Heimlich et al. (2000) noted that substitution of glyphosate for other herbicides 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 Trewavas and Leaver (2001) showed that 3.27 million kg of other herbicides have been replaced by 2.45 million kg of glyphosate in U.S. soybean fields. Carpenter and Gianessi (2003) concluded that introduction of GR soybeans in the U.S.A. resulted in a decrease in the total volume of herbicides used. Gianessi (2005) claims that GRCs generally require less herbicide than non-transgenic crops. Furthermore, he estimates that averaged over all GRCs, glyphosate-resistance technology has reduced herbicide use by 17 million kg per yr 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 GR cotton grew from 0 to about 50% (Fig. 2). Among the herbicides replaced in cotton were arsenic-containing compounds. Gianessi's (2005) calculations indicate that if GR sugarbeets were adopted, herbicide use reduction would not be as great as for combined GRCs, 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 introduction of GR 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 to levels below that currently used in non-glyphosate HRCs or with GRCs. 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. For example, many of the low use rate herbicides have long persistence in soil compared to glyphosate.

Shiva (2001) claims that introduction of GRCs to underdeveloped countries, where hand weeding is the primary means of weed management, will increase herbicide use (Shiva, 2001). At this time, there is no evidence that this has occurred. The economic constraints that prevent these farmers from using selective herbi-

cides will be similar for GRCs. However, should weed management with GRCs 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 in this review, the amount of glyphosate used with a GRC year after year may increase with time, as naturally-resistant weed species and biotypes invade fields and resistance to glyphosate evolves. Both increased amounts of glyphosate and other herbicides will be used in these cases. Benbrook (2003) claims that this biologically-driven increase in herbicide use has already occurred with GRCs. Owen and Zelaya (2005) also report that this is already happening with GRCs in some locales.

The worldwide decrease in cost of glyphosate due to loss of patent protection (Woodburn, 2000) also makes higher application rates economical in some cases. The heavy adoption of GR soybeans in the USA contributed to the dramatic (as much as 80%) reductions in the costs of most other soybean herbicides due to competition (Nelson and Bullock, 2003). Thus, indirectly, GR 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 than glyphosate. Another factor contributing to the reductions in herbicide costs have been patent expirations of other herbicides. Despite the more competitive prices of competing herbicides, adoption of GRCs increased dramatically during this period (Fig. 2).

In a study using the environmental impact quotient method of Kovach et al. (1992), Kleter and Kuiper (2003) calculated total environmental impact of the herbicides, farm worker exposure impact, consumer impact, and ecology impact associated with the herbicides used with various GRCs versus those used with the same non-transgenic crops. Their calculations were based on herbicide use data of Gianessi et al. (2002). The amount of herbicide used was reduced for all crops. All impacts are

reduced in all crops by adoption of HRCs (primarily GRCs). With canola, cotton, and soybean, farm worker and consumer impact are reduced more than ecological impact in their study. Brimmer et al. (2005), using the same method, found the environmental impact of herbicide-resistant canola in Canada markedly lower than that of conventionally grown canola.

Effects on 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 evaluations of environmental impacts of herbicide use. In some countries (e.g., Denmark), mandated herbicide reduction programs have also required fewer applications of herbicides. Certainly, GRCs have greatly reduced tillage (discussed below) and, in some cases, the number of herbicide applications (Gianessi, 2005). Energy use with tillage is much higher than that with herbicide spraying.

Few studies have carefully evaluated the impact of GRCs on reduced fossil fuel use in weed management, although this is generally recognized as a beneficial aspect of GRCs (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 GR sugarbeet would be much lower emissions from herbicide manufacturing, transport, and field operations (overall, approximately 50% less energy required), 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 Fig. 3. They qualified their conclusions by stating that the environmental and health impacts of growing GRCs should be assessed on a case-by-case basis, using a holistic approach.

EFFECTS ON SOIL

Glyphosate is rapidly adsorbed and tightly complexed by most soils (Goldsborough and Brown, 1993). The pH

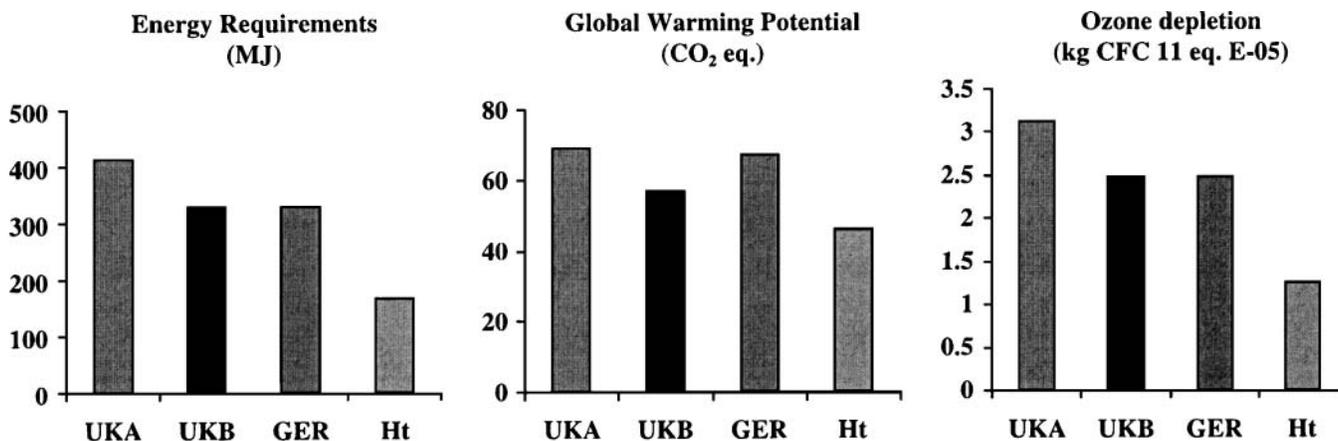


Fig. 3. Comparison of impacts of typical herbicide regimes for conventional, compared with glyphosate-resistant sugarbeet in the UK and Germany in terms of energy requirements (MJ), global warming potential [kg carbon dioxide (CO₂) equivalent], and ozone depletion [kg chlorofluorocarbon (CFC) 11 equivalent] 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 with use of glyphosate only with glyphosate-resistant sugarbeet (Bennett et al., 2004).

and content of organic matter have little effect on binding of the herbicide. Mobility is increased a little at high pH and with high levels of inorganic phosphate. Inactivation of glyphosate through adsorption is of critical importance. In most soils, leaching is very limited compared to most other herbicides, and glyphosate is not volatile (Franz et al., 1997; Getenga and Kengara, 2004).

Soil Contamination

Glyphosate is not considered to be a significant soil contaminant when used in recommended doses. It is 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. Glyphosate can be translocated to roots from foliar tissues and exuded by the roots into the soil (Coupland and Caseley, 1979). Glyphosate strongly adsorbs to soil particles and is rapidly degraded by soil microbes (Duke, 1988; Duke et al., 2003a).

After long-term use of glyphosate in Canadian soils, no residues were detected (Miller et al., 1995). 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 (Fig. 4). Glyphosate has a moderate half-life in soils with an avg value of approximately 47 d, but reaching 174 d in some soils under some conditions (Vencill, 2002; Wauchope et al., 1992). Gimsing et al. (2004) indicated up to 40–50% of glyphosate is mineralized in 90 d, with AMPA accumulating as the major metabolite. They hypothesized that the soil pseudomonad population is responsible for most of the degradation. Abiotic oxidative degradation of glyphosate and AMPA by manganese oxide in the form of birnessite, a common component of soils, was recently reported (Barrett and McBride, 2005). Manganese oxide caused breakage of both the C-P and C-N bonds.

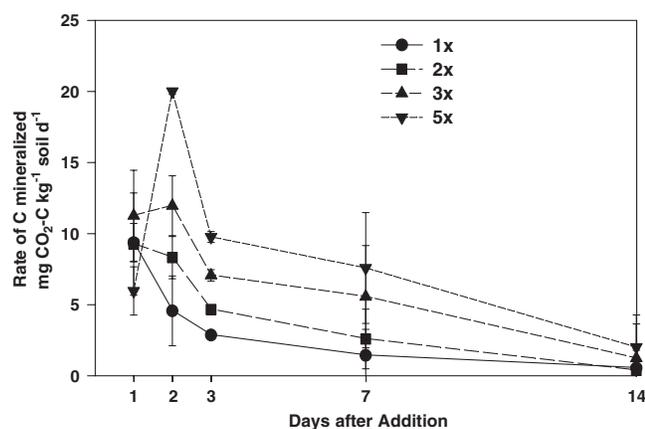


Fig. 4. Effect of glyphosate addition dose on soil carbon mineralization. Carbon mineralized from basal microbial respiration in control samples has been subtracted. The 1, 2, 3, and 5X represent glyphosate addition doses of 47, 94, 140, and 234 mg ai kg⁻¹ soil, respectively. Error bars indicate ± 1 standard deviation (Haney et al., 2000).

Other studies with glyphosate have shown soil half-life values of less than 30 d (Smith and Aubin 1993) and between 11 and 17 d (Grunewald et al., 2001). Mamy et al. (2005) found the half-life of glyphosate to be shorter than those of the herbicides trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine], sulcotrione {2-[2-chloro-4-(methylsulfonyl)benzoyl]-1,3-cyclohexanedione}, metazachlor [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide], and metamitron [4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] for which glyphosate would substitute in GRCs in three different soils, except for that of sulcotrione in one of the soil types. Glyphosate and sulcotrione dissipated primarily by microbial degradation, whereas trifluralin loss was mainly due to volatilization, and metazachlor and metamitron were lost due to non-extractable residue formation. However, they found AMPA to have a much longer half-life than glyphosate and pointed out that this soil contaminant could accumulate with the extensive use of glyphosate. The half-life of glyphosate in water ranged from 1.5 to 3.5 d (Goldsborough and Beck, 1989). Leaching of glyphosate and/or its metabolite AMPA was studied in four lysimeters from a low-tillage field and from a normal tillage field (Fomsgaard et al., 2003). Soil residual concentrations of AMPA were highest where low-tillage had been practiced in soils to which glyphosate had been applied several times in the years before sampling of the lysimeter soil.

Effects on Soil Biota

The potential direct effects of GRCs and their management on soil biota include changes in soil microbial activity due to direct effects of glyphosate, differences in the amount and composition of root exudates of GRCs vs. non-GRCs, 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 (Dunfield and Germida, 2004). Most of the available literature addresses the first effect.

In general, there is little 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 a transient increase of 10–15% in the CO₂ evolved and a 9–19% increase in FDA hydrolysis 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 d 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 AMPA, indicating glyphosate degradation by soil microorganisms. Haney et al. (2000, 2002) generated data

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 (Fig. 4). 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. Liphadzi et al. (2005) found no differences in the effects of glyphosate and conventional herbicides in GR maize and soybean on nematode densities, soil microbial biomass, or substrate-induced respiration in soil, suggesting that soil health was similar under the two herbicide regimes.

Siciliano and Germida (1999) found differences in rhizosphere-associated microbes between a GR 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 GR 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 soybean nitrogen-fixing symbiont *Bradyrhizobium japonicum* Kirchner possesses a glyphosate-sensitive EPSPS, and on exposure to glyphosate accumulates high concentrations of shikimate and certain benzoic acids that could 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 effects of glyphosate on nitrogen metabolism in GR soybeans. Furthermore, glyphosate is preferentially translocated to nodules of soybeans (Reddy and Zablotowicz, 2003). Related research (King et al., 2001; Reddy et al., 2000), summarized by Zablotowicz and Reddy (2004), indicated that such effects as reduction in nodulation, nodule size, and leghemoglobin content of nodules can be caused in GR soybeans sprayed with glyphosate. However, the effect of glyphosate on nitrogenase activity in nodules from GR soybeans in field studies was inconsistent. Greenhouse studies indicated that adverse effects should be maximal under moisture stress. Sensitivity of *B. japonicum* strains varied. In the field, effects are transient, and there is no evidence that crop yield is affected. In GRCs, amino acids from the plant may prevent significant effects on *B. japonicum*, since aromatic amino acids added to *B. japonicum* grown in culture can reduce growth inhibition by glyphosate (dos Santos et al., 2005).

Kremer et al. (2005) reported that root exudates from GR soybean treated with glyphosate contained glyphosate at concentrations higher than 1000 ng per plant and about twice the amino acid content of non-treated plants over a period of 16 d after treatment. Carbohydrate content of the root exudate of GR plants was unaffected by glyphosate treatment, although carbohydrate root exudation of GR soybean was about twice that of non-transgenic soybean. In vitro bioassays showed that root exudates of glyphosate-treated GR soybean stimulated growth of selected rhizosphere fungi (*Fusarium* spp. and *Pseudomonas*

spp.) that could adversely affect plant growth and biological processes in the soil and rhizosphere. With *Fusarium* spp., microbial growth was generally better with glyphosate-treated GR soybean root exudate than with exudate from non-transgenic soybean either treated or not treated with glyphosate. These results would predict that there could be more problems with *Fusarium* spp. in GR soybeans than non-transgenic soybeans. In a study in which glyphosate was sprayed on soil, the bacterial endophyte community associated with subsequently planted soybeans was altered (Kuklinsky-Sobral et al., 2005).

Motavalli et al. (2004) concluded in a review that there is so far no conclusive evidence that those GRCs and other transgenic crops, which have been deregulated and used in many cropping situations, climates, and soil types over the past 10 yr, 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 GRCs 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 removal or appearance of certain microbes (e.g., rhizobacteria or plant pathogens) could affect soil health and ecosystem function. Of course, other herbicides could also cause such minor effects, and soil-applied herbicides might cause more than minor temporary effects. 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. So far, however, no agriculturally significant effect of glyphosate on soil microorganisms has been documented.

Soil Loss and Compaction

A positive impact of the use of GRCs is that they facilitate reduced- or zero-tillage 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 GRCs on tillage practices and associated environmental effects. Loss of top soil due to tillage is perhaps the most environmentally de-

structive effect of row crop 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 to replace.

A survey by the American Soybean Association (2001) found that 53% of U.S. soybean farmers made an average of 1.8 fewer tillage passes per yr through their soybean fields since GR soybeans were introduced. This translates to a savings of \$385 million per yr in reduced tillage costs. In a 5-yr period in the U.S.A., during which the planting of GR soybeans increased from only a few percent to about 70% (Fig. 2), there was a dramatic increase in the adoption of no-tillage and reduced-tillage management (Fig. 5). Most of this change was associated with the growing of GR soybeans (Fig. 6). We have not found documentation as to whether this trend has continued; however, weed changes (as discussed later in this review) in GRCs 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 GR soybeans, where there is a yearly loss of 10 tons of topsoil per ha in soybeans produced with conventional tillage, compared to 2.5 tons annually with no-tillage agriculture in GR soybeans (Penna and Lema, 2003).

A lesser studied effect is that of GRCs on soil compaction. Use of GRCs has generally resulted in fewer 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 GRCs have made has not been studied in the context of soil compaction. Reduced tillage in non-transgenic crops has generally reduced soil compaction (Koch et al., 2003; Shukla et al., 2003), but reduced compaction by reducing tillage has not always been the case (Schwab et al., 2002).

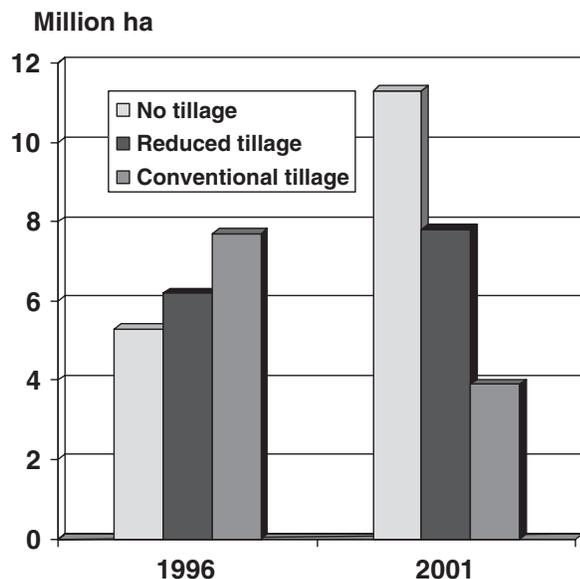


Fig. 5. Soybean tillage methods by hectares farmed in the U.S.A. in 1996 and 2001. In 1996 and 2001, there were 19.2 and 23 million ha, respectively, of soybeans grown (American Soybean Association, 2001).

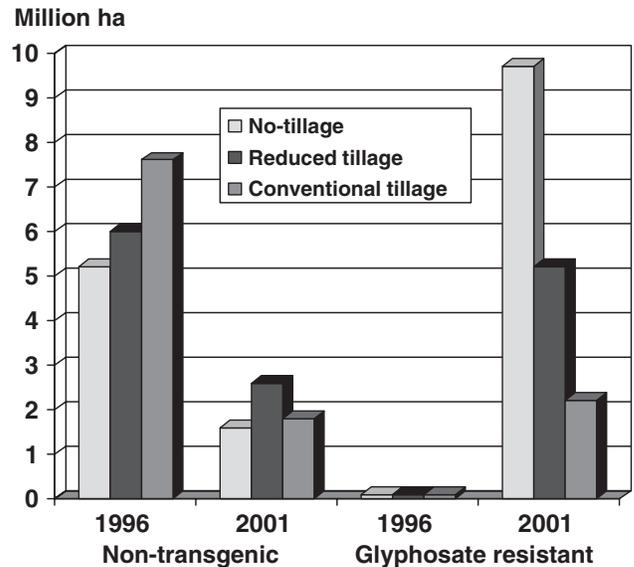


Fig. 6. Tillage practices and glyphosate-resistant soybean use by hectares in the USA in 1996 and 2001. (American Soybean Association, 2001).

EFFECTS ON AIR

One study has focused on impacts of GRCs on the air. Bennett et al. (2004) used 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 GR sugarbeet were grown. The results suggested that growing this GRC 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, toxic impacts on aquatic water ecosystems, and acidification and nutrification of soil and water, were much lower for the GRC 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 HRC (Fig. 3). Herbicides can pollute the air by drift (air movement of sprayed herbicide-containing droplets to unintended sites) or volatility. Glyphosate is essentially not volatile at 25°C (Vencill, 2002) and has not been reported as an atmospheric contaminant (Van Dijk and Guicherit, 1999).

Most herbicides are applied by spraying, resulting in movement to non-target sites and organisms through the air. Herbicide drift effects on unintended crops and other vegetation have been a problem since the use of potent, synthetic herbicides began. After GR soybeans were introduced, Owen (1998) reported that complaints of herbicide drift problems increased in Iowa. Growing a GRC next to a non-GRC of the same species may exacerbate such problems, as there is no visual difference between the two crops to the herbicide applicator. Furthermore, with GRCs, the herbicide can be used during

later crop development by aerial application, further increasing the risk of drift.

EFFECTS ON WATER

Water Contamination

Glyphosate is strongly adsorbed to soil particles, and, even though it is highly water soluble, it does not leach into ground water in most soils. Soil and sediments of bodies of water are the main sinks for glyphosate residues from surface water, greatly reducing further transport (Franz et al., 1997; Goldsborough and Brown, 1993). Once in surface water, it dissipates more rapidly than most other herbicides. Various studies have shown that glyphosate appears in surface water less than several alternative herbicides (Carpenter et al., 2002). For example, in long-term studies conducted in Canada, no residues of glyphosate were found in groundwater (Miller et al., 1995). Several studies have found no glyphosate in ground water in the U.S.A. where it is applied in no-tillage cropping systems (Kolpin et al., 1988) and in Brazil in various cropping systems (Bonato et al., 1999; Cerdeira et al., 2005; Lanchote et al., 2000; Paraiba et al., 2003). Similar results were found for surface waters (Clark et al., 1999). Even in a worst case scenario of preferential flow, where compounds that bind soil strongly might leach more readily, glyphosate at recommended use rates did not leach past 2.4 m at concentrations approaching environmental concern (Malone et al., 2004).

A recent study in Denmark found that both glyphosate and AMPA can leach to a depth of 1 m through structured soils with pronounced macropore flow (Kjaer et al., 2005). Considering the results of many other studies, this must be an unusual situation. Spraying onto vegetated soil vs. bare soil in vineyards reduced the potential for movement of both glyphosate and AMPA to ground water (Landry et al., 2005). Kolpin et al. (2006) detected AMPA almost four times as often as glyphosate downstream from waste water effluent from treatment plants treating water from urban environments where glyphosate was used.

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 herbicides to maize and other crops (Wauchope et al., 2002). A model was used to predict maize herbicide concentration in reservoirs as a function of herbicide properties, comparing broadcast surface pre-plant atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide] applications with glyphosate or glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] with both GR 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 use of transgenic crops, would dramatically reduce herbicide concentrations in vulnerable watersheds. Similarly, Estes et al. (2001) predicted 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 GR maize, thereby reducing risk to drinking water and related ecosystems. By modeling, Peterson and Hulting (2004) predicted less risk to groundwater and aquatic plants by a GR wheat/herbicide system than from a non-transgenic wheat/conventional herbicide system.

Effects on Aquatic Life

Because of its relative safety, glyphosate is one of only nine synthetic herbicides approved for use in aquatic sites in the U.S.A. (Getsinger et al., 2005). Glyphosate was not found 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 $< 4 \text{ kg ha}^{-1}$ and only slightly greater at application rates of 8 kg ha^{-1} . As we mentioned earlier, glyphosate is less likely to pollute ground and surface waters than many of the herbicides that it replaces. Cedergreen and Streibig (2005) found glyphosate to be the least toxic herbicide of ten tested to the aquatic plant *Lemna minor* L. and the third least toxic of the ten herbicides to the green alga *Pseudokirchneriella subcapitata* (Korschikov) Hindak. The formulated version of glyphosate was four-fold more phytotoxic than unformulated glyphosate, but this did not change the rankings. A life-cycle assessment technique used to compare conventional sugarbeet agricultural practices with risks that might be expected if GR sugarbeet were grown suggested that growing this GRC would be less harmful to the ecology of water for the GRC than for the conventional crop (Bennett et al., 2004). Relyea (2005) reported that a commercial formulation of glyphosate sprayed directly into aquatic mesocosms (in 1200-L polyethylene tanks) caused a 22% reduction in species diversity with particularly severe impacts on amphibians. The control mesocosms were not sprayed with a formulation blank, so whether the effects were due to glyphosate or the other formulation ingredients is unclear.

Finally, glyphosate was recently found to reduce toxicity of some metal ions (Ag, Cd, Cr, Cu, Ni, Pb, and Zn), but not ions of Hg or Se, to a freshwater claderceran by binding their ionic forms and reducing their bioavailability (Tsui et al., 2005). Thus, under circumstances of metal ion toxicity, glyphosate could benefit some organisms. Such effects would be transient due to the relatively short environmental half-life of glyphosate. One would assume that these results would extrapolate to other aquatic organisms, but further work should be done. In general, what we know about glyphosate's movement to surface water suggests less impact of GRCs on aquatic vegetation than conventionally-grown crops.

EFFECTS ON OTHER NON-TARGET ORGANISMS

In Europe there has been some controversy about the effects of HRCs on biodiversity in farm-scale evaluation studies. There is a desire in Europe 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 shelter and a food supply for these animals. Marshall (2001) speculated that the currently available HRCs, including GRCs, 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 GR fodder maize than with conventional weed management. Dewar et al. (2003) devised a strategy to use band spraying in a GRC to increase biodiversity within the crop while providing habitat for birds and wildlife. Their method of leaving weeds between crop rows could, in some cases, be used without compromising crop yield. Petersen and Röver (2005) used such a method in GR sugarbeet and found no loss in yield. In a similar study using such a strategy, May et al. (2005) found that GR sugarbeets offered more flexibility in devising management strategies to enhance wildlife than conventional weed management methods.

Some have suggested that biodiversity would be increased with HRCs that use a broad-spectrum, foliar-applied herbicide like glyphosate, since the farmer can wait to spray weeds after there has been some weed growth, providing habitat and/or food for birds, arthropods, or other herbivores. However, Freckleton et al. (2004), using weed phenologies and a population model, predicted that such effects would probably be transient. The study suggested that if herbicide application could be ceased earlier, a viable population of late-emerging weeds could be maintained. In a multi-year study in Argentina on GR soybeans in which glyphosate was used yearly, 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 glyphosate is the effect of tillage on vegetation. No-tillage agriculture, which is used more with GRCs, results in weed species shifts (as discussed later in this review) and more vegetation on the field before and after the period of crop production, which in turn results in improved habitat for other organisms.

Non-Target Plants

For most farmers, the goal of using herbicides is to kill all unwanted vegetation with as little damage as possible to the crop. Even a small infestation of weeds can reduce crop yield by competing for resources, allelopathy, and/or by harboring pests and diseases. The farmer often 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 crop land. Thus, 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, drift to non-transgenic crops of the same species is a new problem with HRCs, although effects of herbicides on non-target crops of a different species are not a new problem. There are several studies on such effects with glyphosate.

Ellis and Griffin (2002) evaluated the response of non-transgenic soybean and cotton to simulated drift of glyphosate. 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. Injury by 12.5% of the recommended rate was minimal when applied at first flower and was up to 35% injury at 14 d after application when applied at the 2- to 3-trifoliolate stage (Table 2). However, by 28 d after application, glyphosate did not cause injury, regardless of application rate and timing, except for the highest rate at the early application time, and, even then, the injury was only 8%. A similar study was done with rice (*Oryza sativa* L) and maize with similar results (Ellis et al., 2003). Injury to both crops was observed, particularly when glyphosate was applied early at the highest rate (12.5% of the recommended rate for weed management).

Glyphosate, sprayed on non-GR cowpea (*Vigna unguiculata* L. Walp) 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 up to 890 g ai ha⁻¹ sprayed near seed maturity, seven showed a significantly adverse effects of

Table 2. Injury of non-transgenic soybean after simulated drift rates of glyphosate at two application timings (Ellis and Griffin, 2002).

Rate g ai ha ⁻¹ §	Soybean injury	
	Early timing	Late timing
	%	
	<u>7 DAT†</u>	
140	29(21)‡	25(7)
70	18(8)	3(5)
35	3(4)	0(0)
9	0(0)	0(0)
	<u>14 DAT</u>	
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)
	<u>28 DAT</u>	
140	8	0
70	0	0
35	0	0
18	1	0
9	0	0

† Days after treatment.

‡ Application timings correspond to 2 to 3 trifoliolate (early timing) and first flower (late timing). Data averaged across years. Data are for 1998 and 1999. The 1999 data are in parentheses.

§ Rates correspond to 12.5, 6.3, 3.2, and 0.8% of the labeled rates of 1.12 kg ai ha⁻¹ glyphosate.

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 significant ecological changes could occur in some cases of glyphosate drift.

Temperature and water stress can affect the injury due to glyphosate application to plants. Higher temperatures, light intensity, and water stress can decrease the resistance of GR soybean to glyphosate (Pline et al., 1999; King et al., 2001). Glyphosate translocates from source to sink tissue, such as reproductive tissues. In GR cotton, accumulation of glyphosate in reproductive tissues and insufficient expression of the CP4 EPSPS can affect the boll retention under some conditions (Pline et al., 2002; Pline–Srnic, 2005). Impact on yield and fruit set varies greatly with the environment and timing of glyphosate applications. In general, applications made near the time of pollen development in GR crops cause more reproductive damage than early applications (Pline–Srnic, 2005). These effects and others are summarized by Pline–Srnic (2005).

Glyphosate-resistant turf grass may present another method of affecting non-target plants. Grass clippings from GR creeping bentgrass that had been sprayed with glyphosate for weed control possessed enough residual glyphosate for 3 d after treatment to cause injury to other species when used as mulch (Goss et al., 2004). The indirect effect of glyphosate on plants through their influence on plant pathogens is discussed later in this review. Potential subtle effects of sublethal concentrations of glyphosate on non-target vegetation through this mechanism have not been studied in the field.

At subtoxic concentrations, glyphosate can be a growth stimulant (Schabenberger et al., 1999; Wagner et al., 2003; Belz et al., 2006). Thus, at low concentrations glyphosate drift could stimulate growth of non-target plant species. This type of effect (hormesis) has been a controversial aspect of environmental toxicology (Calabrese and Baldwin, 2003). Sugarcane farmers in several parts of the world use glyphosate at low doses as a ripener to increase sugar content (McDonald et al., 2001).

Plant Pathogens

The effect of pesticides on plant pathogens that affect crops has been an inadequately studied and controversial topic (Altman, 1993). Glyphosate inhibits the biosynthesis of the aromatic amino acids affecting biosynthesis of proteins, auxins, phenolic phytoalexins, folic acid, lignins, flavonoids, plastoquinone and hundreds of other phenolic and alkaloid compounds and might be expected to increase the susceptibility of sensitive plants to pathogens or other stresses (Pline–Srnic, 2005).

Glyphosate is toxic to many microorganisms, including plant pathogens (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 glyphosate. For example, Morjan et al. (2002) found that glyphosate alone was not fungi-

cidal to the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* Metschnikoff, *Nomuraea rileyi* (F) Samson, and *Neozygites floridana* F. 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. In a laboratory study, growth of *Pythium ultimum* Trow and *F. solani* could be stimulated or inhibited, depending on glyphosate concentration (Kawate et al., 1992).

The influence of glyphosate on plant diseases in GR crops is variable, sometimes reducing and other times increasing disease. In GR cotton, glyphosate application at the four-leaf stage reduced disease on hypocotyls in field studies (Pankey et al., 2005). Glyphosate was recently reported to inhibit rust diseases in both glyphosate-resistant soybean and wheat (Anderson and Kolmer, 2005; Feng et al., 2005). Glyphosate inhibits the growth of the plant pathogen that causes red crown rot [*Calonectria crotalariae* (Loos) Bell] on non-GR soybean (Berner et al., 1991). Field trials showed a reduction in red crown rot incidence with preplant applications of low rates of glyphosate.

Kremer et al. (2001) compared the effects of glyphosate, a conventional herbicide mix of pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] plus imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid}, and glyphosate plus the conventional herbicide mix on soil microbes in four GR soybean varieties at eight sites. Frequency of *Fusarium* spp. on roots increased 0.5–5X at 2 or 4 wk after application of glyphosate or glyphosate plus conventional herbicides compared with the conventional herbicide alone. This effect was due to both higher root exudate levels of GR than non-GR soybeans, as well as to glyphosate stimulation of root exudation of microbial substrates (Kremer et al., 2005). 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).

Dead or dying weeds can provide a good microenvironment for plant pathogens. *P. ultimum* and *F. 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-GR plants, glyphosate can make both crops and weeds more susceptible to plant pathogens (Johal and Rahe, 1988; Liu et al., 1997; Sharon et al., 1998), largely or at least partly by inhibiting the production of defense-related compounds derived from the shikimate pathway, such as phytoalexins and lignin. Low doses of glyphosate can sometimes make pathogen-resistant cultivars susceptible to plant disease (Brammall and Higgins, 1988). Colonization of root tissues in tomato seedlings genetically resistant to *Fusar-*

ium oxysporum F. occurred following exposure to a sublethal concentration of glyphosate (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 GRCs. Nevertheless, there have been reports of increased susceptibility of GRCs to plant pathogens. Farmers in Michigan have reported increased susceptibility of GR soybean to *S. sclerotiorum* (Lee et al., 2003). Glyphosate, its formulation components, and the glyphosate resistance transgene were not 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, Harikrishnan and Yang (2002) concluded that GR and susceptible soybeans reacted similarly to most herbicide treatments with respect to root rot and damping off diseases caused by *Rhizoctonia solani* Kuehn. Similarly, the response of GR soybeans to *F. solani*-caused sudden death syndrome (SDS) was not different than that of conventional soybeans in which disease symptoms were increased by application of glyphosate (Sanogo et al., 2000, 2001). Njiti 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 GR soybean cultivars and application of different herbicides to these cultivars with respect to susceptibility to *S. sclerotiorum*-caused stem rot. Thifensulfuron {3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid} treatment resulted in lower disease severity in isogenic glyphosate-susceptible cultivars than with GR cultivars. Sulfonylurea herbicides, such as thifensulfuron, have been reported to stimulate production of products of the shikimate pathway (Suttle et al., 1983), from which some phytoalexins are derived.

When 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 GR volunteer wheat could increase wheat diseases such as wheat streak mosaic virus and *Rhizoctonia* root rot. Field observations in Ohio suggested a possible interaction between soybean cyst nematode (*Heterodera glycines* Ichinohe SCN) and glyphosate in a transgenic GR 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 GR soybean 8 to 29% across all experiments, but there was no interaction of glyphosate and SCN in GR soybean.

One unexpected aspect of the interactions between GRCs 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 GRC itself may influence plant pathogens either negatively or positively.

Arthropods

Glyphosate has not been reported to have insecticidal or other activities against terrestrial 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 show no significant direct effects of glyphosate on arthropods. 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 trifurcate* Forster, adult *Spisistilus festinus* Say, larvae of *Plathypena scabra* F., and the caterpillar of *Anticarsia gemmatalis* Huebner were unaffected by GR soybeans or by glyphosate at recommended or delayed doses. But, adult *Geocoris punctipes* Say populations were decreased by the herbicide. The authors concluded that this effect was due to reduced weed densities after glyphosate treatment.

No effects of glyphosate resistance transgenes in plant material have been found on insects. Host plant suitability to green cloverworm (*Hyponomeuta scabra* F.) was evaluated on two conventional soybean varieties and two GR varieties, with and without exposure to glyphosate (Morjan and Pedigo, 2002). Treatments did not affect 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*. Huang et al. (2004) found that pollen from GR canola had no adverse effects on honeybees.

Weed management systems that allowed more weeds generally had higher insect population densities (Buckelew et al., 2000). However, some species did not fit this generalization, as systems with fewer weeds appeared to be preferred by potato leafhoppers (*Empoasca fabae* Harris). Bean leaf beetles (*Cerotoma trifurcate* Forster) and potato leafhoppers preferred certain soybean varieties, but these effects were attributed to soybean plant height. Their findings (Table 3) indicate that although the GR soybean varieties did not strongly affect insect populations, weed management systems can affect insect populations in soybean.

In summary, there is little evidence of any direct effect of glyphosate on arthropods in the field or in natural

Table 3. Mean number of insects per 20 sweeps collected from transgenic (glyphosate resistant) and non-transgenic soybean varieties in two weed management systems (Buckelew et al., 2000).

Soybean variety/management system	Bean leaf beetle	Potato leafhopper	Tarnished plant bug	Grasshopper nymphs	Insidious flower bug	Damsel bug
Glyphosate-resistant	3.96a†	5.94c	0.49a	1.13a	1.11a	1.17a
Glyphosate-resistant‡	3.87ab	7.29b	0.44a	1.14a	0.92a	1.05ab
Glufofenate-resistant	3.53b	6.02bc	0.57a	1.28a	1.65a	0.89b
STS§	3.48b	6.14bc	0.53a	1.10a	0.82a	1.10ab
Jack‡	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 ($p > 0.05$).

‡ Denotes a variety that is also resistant to soybean cyst nematode.

§ Denotes a non-transgenic variety that is resistant to sulfonyleurea herbicides.

environments. Effects of GRCs and associated cultural practices can affect arthropods indirectly.

Birds and Wildlife

The environmental effect of the use of GR soybeans was compared to the effects with non-transgenic soybeans for over 1400 midwestern U.S. farms by Nelson and Bullock (2003). They used the LD₅₀ doses of glyphosate and other herbicides for rats in making their environmental effect estimates. Unlike most previous studies, this one considered the relative toxicity of herbicides available to farmers. The simulation model results suggested that glyphosate resistance soybean technology is more environmentally friendly, especially with regard to mammalian toxicity, than other herbicide technologies for all farms in the midwestern USA. 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 GR wheat with those associated with 16 other herbicides used in spring wheat in the northern U.S. Great Plains. 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 to the herbicides. They found that the ecological risks for the 15 herbicides relative to glyphosate were highly variable, with glyphosate having less relative risk to non-target terrestrial and aquatic plant life and groundwater than than most other active ingredients. The study predicted that glyphosate use in GRCs will be less toxic to terrestrial and aquatic wildlife than several of the herbicides which they replace.

GRCs can affect birds and wildlife indirectly by altering habitat and food sources through 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 GRCs on wildlife through effects on habitat. Glyphosate can be applied to GRCs later in the growing season

because it is 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, and would benefit wildlife. Strandberg (2004) studied such possibilities in GR 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 use reduces weed seed production, with possible eventual negative consequences on wildlife. He concluded that long-term investigations of cropping systems with each GRC should be conducted to understand the full effects (both positive and negative) on farm land wildlife.

We discussed earlier that glyphosate formulations can be toxic to some amphibians when sprayed directly on them (Relyea, 2005). 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 GRCs.

FOOD AND FEED SAFETY

There are two components of food safety associated with GRCs. Glyphosate might directly alter food safety if it or its metabolic products are found in the edible portions of the crop at levels above tolerance levels. 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 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).

Glyphosate Residues

A fundamental consideration in evaluation of the potential impact of a pesticide in the food supply is its toxicity. Glyphosate is classified as a category E pesticide (evidence of noncarcinogenicity for humans) by the USEPA (Franz et al., 1997). The toxicities of glyphosate, its isopropylamine salt, and formulations have been well-studied in mammals with short- and long-term toxicity, and bioaccumulation or elimination properties,

and data suggest that glyphosate has a very low level of toxicity in mammals and is not retained to a significant extent in animal tissues (Franz et al., 1997). Glyphosate (either the anion or the isopropylamine salt) is practically nontoxic by ingestion, with a reported acute oral LD₅₀ of > 5000 mg kg⁻¹ in the rat (Vencill, 2002). The trimethylsulfonium salt of glyphosate is more toxic, with an oral LD₅₀ of about 705 mg kg⁻¹. It is not a restricted-use pesticide and is a best-selling weed killer for home lawn and garden use. Animals do not contain the herbicide molecular target site (EPSPS) of glyphosate. Intestinal flora of some animals do contain the EPSPS, but in studies with sheep, glyphosate had no significant effect on rumen fermentation parameters and in sacco degradation of grass hay and corn grain (Huther et al., 2005).

Occasional reports of severe effects of ingestion of formulated glyphosate occur (Sorensen and Gregersen, 1999; Stella and Ryan, 2004). Most reported cases of severe glyphosate poisoning involve ingestion of greater than 85 mL of a concentrated formulation containing adjuvants, surfactants, and/or other additives (Bradberry et al., 2004). 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. Considering the limited toxicity of the compound, it seems likely that most or all of the reports of acute toxicity after ingestion are due to formulation ingredients.

Surprisingly little has been published on herbicide residues in GRC foods. Most of what we know is from studies with non-GRCs. However, herbicide residue data must be supplied for regulatory approval of GRCs. In a testing program to detect whether GR soybeans had been sprayed with glyphosate or not, Lorenzatti et al. (2004) found glyphosate and AMPA in green, immature seeds. Duke et al. (2003b) found both glyphosate and AMPA in harvested seeds of different GR soybean varieties grown in widely separated geographical regions. At legal glyphosate application rates that were relatively high and late, glyphosate residues were within established tolerance levels. Higher AMPA than glyphosate levels were surprising, 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 GR soybeans, apparently due to herbicide drift. These findings led to a study that indicated the occasional phytotoxicity caused by glyphosate in GR soybeans is due to AMPA accumulation (Reddy et al., 2004), a compound known to be weakly phytotoxic (Hoagland, 1980). We have found no publications on glyphosate residues in GRCs other than soybean.

Glyphosate and/or its degradation products can be expected to be found in edible parts of plants at levels below regulatory thresholds (Arregui et al., 2004; Duke et al., 2003b; Feng and Chiu, 2005). Little is known about how food processing might affect such residues. Low et al. (2005) found that baker's yeast (*Saccharomyces*

cerevisiae) rapidly reduced glyphosate levels in wheat flour during bread making, but the degradation products were not identified.

Transgene-Related Effects on Food and Feed

A transgene might pose a food safety risk for two basic reasons. First, the transgene protein product itself could be toxic, due to direct toxicity, anti-nutritive 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 GRCs, 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. Gene expression and metabolite 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 (i) non-toxic to mice when consumed at doses thousands of times higher than potential human exposure, (ii) readily degraded by digestive fluids, and (iii) not structurally or functionally related to any known protein allergens or toxins, based on amino acid sequence homology searches.

Studies with GR 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 significant 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 this study showed that there were no significant 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 indicate that GR GA21 maize is as safe and nutritious as conventional maize for food and feed use.

Several other studies have found no significant differences in the nutrient content and composition of GR and non-transgenic crops. These studies include maize (Ridley et al., 2002; Aufran et al., 2003), soybean (Padgett et al., 1996b; McCann et al., 2005), wheat (Obert et al., 2004), and cotton (Nida et al., 1996). The McCann et al. (2005) study found a consistent lack of significant effect of the CP4 gene on composition of several GR soybean varieties over 3 yr. In the Aufran 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. Similarly, Filipe et al. (2005) found no significant effect of glyphosate resistance on trypsin inhibitor activity of soybeans, and the effects of cooking and processing on trypsin inhibitor activity was the same in both GR and non-transgenic soybeans.

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 GR soybeans, a reduction of the content of these compounds could occur. In a well-replicated field study at two sites, hundreds of kilometers apart, we found no significant effects of glyphosate on isoflavones in harvested seed (Duke et al., 2003b). Earlier, Lappe et al. (1999) reported reductions of isoflavone levels on GR 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. Padgett et al. (1996b) found no effects of the transgene on isoflavone content of soybean.

Most of the peer-reviewed, published results of animal feeding studies with GRCs are summarized in Table 4. All of these studies support the view that food from GRCs 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 GR soybean meal (Jennings et al., 2003). No effects on GR 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. There are a few published studies that show no allergenic properties of transgene products associated with GRCs. Sten et al. (2004), in a study with soybean-sensitized patients, found that the allergenicity of ten GR 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.

Table 4. Results of animal feeding studies with GRCs.

Crop	Animal	Result	Reference
maize	rat	no effect	Hammond et al., 2004
maize	swine	no effect	Hyun et al., 2004
maize	cattle	no effect	Erickson et al., 2003
maize	dairy cattle	no effect	Donkin et al., 2003
		no effect	Ipharraguerre et al., 2003
		no effect	Grant et al., 2003
maize	poultry	no effect	Sidhu et al., 2000
		no effect	Taylor et al., 2005
soybean	rat	no effect	Zhu et al., 2004
		no effect	Hammond et al., 1996
soybean	mice	no effect	Brake and Evenson, 2004
soybean	swine	no effect	Cromwell et al., 2002
soybean	dairy cattle	no effect	Hammond et al., 1996
soybean	caffish	no effect	Hammond et al., 1996
soybean	poultry	no effect	Hammond et al., 1996
canola	rainbow trout	no effect	Brown et al., 2003
canola	poultry	no effect	Taylor et al., 2004
sugarbeet	sheep	no effect	Hartnell et al., 2005

A last, but almost unstudied aspect of food quality and GRCs is their influence on contamination of food with poisonous weed seeds. Weed seeds can be sources of toxic compounds (Powell et al., 1990). GRCs 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).

GLYPHOSATE-RESISTANT WEEDS WITHOUT GENE FLOW

This section will deal with weed problems for farmers that may or will occur because of the use of GRCs. Although glyphosate is a non-selective, broad-spectrum herbicide, it 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 GRC cropping systems. Weeds evolve resistance to herbicides, and this process is accelerated when they are used year after year. GRCs can become feral in some situations, creating a weed that cannot be controlled by the herbicide to which it has been engineered to be resistant. The gene(s) conferring herbicide resistance to the crop can move to weedy relatives by outcrossing, causing a more problematic weed. Thus, to combat the weed problems associated with these developments, farmers are beginning to apply other herbicides with glyphosate (Grichar et al., 2004). In a modeling study that analyzed 1356 potential interactions between biological, chemical, and physical factors for potential hazards associated with herbicide-resistant canola, Hayes et al. (2004) predicted 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 GRC use at this time are problems for farmers, but not for the general public.

Feral Crops

Feral versions of some crop species exist (Gressel, 2005), and gene flow to these biotypes is a strong concern. This topic is dealt with later in this review. However, GRC technology may contribute to current weed problems if GRCs themselves become 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. Additionally, the existence of an HR gene cannot provide any fitness advantage in the wild, where the herbicide is not used. This problem can be exacerbated if two crops used in rotation are both GRCs. For example, York et al. (2004) found GR cotton to be a problem in subsequent crops of GR soybean. Feral cotton in soybeans can be a problem in harboring undetected cotton boll weevils. Volunteer GR canola and wheat could pose problems in weed control in conservation tillage systems in the U.S. Pacific Northwest. (Rainbolt et al., 2004). These systems currently rely on glyphosate for weed control during fallow and before planting. Thus, con-

tinued use of only glyphosate with a GRC will make this problem worse.

Evolved Resistance

An excellent web site exists that catalogs all verified cases of evolved resistance to herbicides (Heap et al., 2005). After a long lag phase, the cases of evolved herbicide resistance to all herbicides have grown linearly since the late 1970s, reaching more than 300 resistant biotypes of 182 weed species by 2006. Although several species of weeds have evolved resistance to glyphosate, only three of these cases have been associated with GRCs, but more are expected to evolve due to the increased use of GRCs and to the increasing dose and frequency of glyphosate use within HRCs.

Because of the complex manipulations that were required to produce an effective transgene for imparting glyphosate resistance, it was considered unlikely that a similar type of resistance would evolve quickly in weeds (Bradshaw et al., 1997). However, since this publication, several weed species have evolved resistance to glyphosate (Heap et al., 2005; Nandula et al., 2005) (Table 5), 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* Gaud. 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 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* (L.) Gaertn. is due to a resistant form of EPSP (Baerson et al., 2002b). This gene has been patented for use in producing GRCs (Baerson et al., 2004).

All of the cases of evolved glyphosate resistance took place in non-transgenic crop situations except those of *Coryza canadensis* L., *Ambrosia artemisiifolia* L., and *Amaranthus palmeri* S. Wats. In the U.S.A., *C. canadensis* evolved resistance to glyphosate largely or entirely in

GR soybeans (VanGessel, 2001). However, as this weed is largely treated before planting, it should be noted that exposure to glyphosate began before introduction of GR soybeans, and attributing this resistance solely to the introduction of GR soybeans may not be completely accurate (Dill, 2005). Its mechanism of resistance is apparently reduced translocation of the herbicide (Feng et al., 2004; Koger and Reddy, 2005). With the continued extensive use of GRCs, more cases of evolved GR weeds are expected to emerge.

Weed Shifts

Although glyphosate is a broad-spectrum, non-selective herbicide, different species and different biotypes within species have different levels of natural resistance to it (Nandula et al., 2005). Levels of natural resistance can also vary dramatically within growth stages. For example, the following weeds in Brazil are not weeds that have evolved resistance, but are difficult to control with glyphosate due to their natural resistance: *Chamaesyce hirta* (L.) Millsp Peelegreeir, *Commelina benghalensis* (L.), *Spermacoce latifolia* Aubl, *Euphorbia heterophylla* (L.), *Richardia brasiliensis* Gomes, and *Ipomoea* sp. (A. Brighenti, personal communication, 2004). Genetic variation in glyphosate resistance exists in weedy morning glory [*Ipomoea purpurea* (L.) Roth] (Baucom and Mauricio, 2004), bermudagrass (*Cynodon dactylon* L.) (Bryson and Wills, 1985), and field bindweed (*Convolvulus arvensis* L.) (Duncan and Weller, 1987). Many GR morning glory species were found to produce fewer seeds, so this trait may be a fitness disadvantage in the absence of glyphosate. However, these authors did not determine whether there is a linkage of the seed production and glyphosate resistance traits. In Argentina, the naturally resistant weed *Parietaria debilis* G. Foster appeared and increased during several years of GR soybean monoculture and GR soybean/maize rotation (Puricelli and Tuesca, 2005). This effect was much more pronounced in no-tillage than conventional tillage agriculture. The populations of naturally resistant species were expected to increase in GRCs 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) seem to be coming true.

Waterhemp [*Amaranthus rudis* Sauer and *A. tuberculatus* (Moq.) Sauer] biotypes possess extremely variable levels of susceptibility to glyphosate (Patzoldt et al., 2002). Waterhemp and velvetleaf (*Abutilon theophrasti* Medik.), which are not effectively controlled by glyphosate, became a greater problem in GR soybean in Iowa after a short period (Owen, 1997). A Kansas study showed that ivyleaf morning glory (*Ipomoea hederacea* Jacq) and large crabgrass [*Digitaria sanguinalis* (L.) Scop] that were not controlled well by glyphosate dominated the weed community in a maize-soybean rotation using glyphosate applications for weed control

Table 5. Weed species that have evolved resistance to glyphosate (Heap et al., 2005).

Species	Year first reported	Countries
<i>Amaranthus palmeri</i> S. Wats.	2005	USA
<i>Ambrosia artemisiifolia</i> L.	2004	USA
<i>Coryza bonariensis</i> L.	2003	South Africa
	2004	Spain
<i>Coryza canadensis</i> (L.) Cronq.	2000	USA
<i>Eleusine indica</i> (L.) Gaertn.	1997	Malaysia
<i>Lolium multiflorum</i> L.	2001	Chile
	2003	Brazil
	2004	USA
<i>Lolium rigidum</i> Gaud.	1996	Australia
	2001	South Africa
	1998	USA
<i>Plantago lanceolata</i> L.	2003	South Africa

(Marshall et al., 2000). In a similar study, Coble and Warren (1997) reported that morning glory species increased in abundance with the continuous 3-yr use of glyphosate compared with other herbicide programs. Nandula et al. (2005) lists Asiatic dayflower (*Commelia communis* L.), birdsfoot trefoil (*Lotus corniculatus* L.), Chinese foldwing [*Dicliptera chinensis* (L.) Juss], common lambsquarters (*Chenopodium album* L.), and tropical spiderwort (*Commelina benghalensis* L.) as weeds that are naturally resistant to glyphosate. Other weed species that possess some level of natural resistance to glyphosate include nutsedge species (*Cyperus* spp.), horseweed [*Conyza canadensis* (L.) Cronq], and hemp sesbania [*Sesbania exaltata* (P. Mill.) McVaugh] (Shaner, 2000). The relatively high level of natural resistance of horseweed to glyphosate could have aided in its evolution of an even higher level of resistance.

In an extensive, six-site, 4-yr study in which GR wheat was rotated with canola and peas, Harker et al. (2005), found shifts from weeds that have commonly been associated with wheat production to more resistant weed species and/or volunteer canola and wheat, depending on the site. Overall, the study showed that use of GR wheat in the rotations of this study, did not significantly increase short-term weed management risks.

A field study that was conducted from 1999 through 2001 in Mississippi, to determine the effects of bromoxynil (3,5-dibromo-4-hydroxybenzotrile)-resistant and GR 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 GR 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 avoidance of glyphosate by germination and/or 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 morning glory (*Ipomoea hederacea* Jacq.) to both better survive the herbicide after emergence and to avoid it by late emergence. Shattercane [*Sorghum bicolor* (L.) Moench] avoided glyphosate applications by late emergence.

In the U.S.A., there have already been increasing populations of naturally resistant species and biotypes in GRCs, requiring other herbicides to be used frequently with glyphosate in GRCs (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 equally effective and affordable herbicides 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 GRCs (Fig. 5 & 6) also

causes shifts in weed species (Swanton et al., 1999), although this has not been well documented in the literature.

Potential Effects on Invasive Plant Species

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; Duncan et al., 2004). Part of the spread of these species is due to contamination of crop seeds with those of weeds. In Canada, harvested GRCs are more weed-free than conventional canola, resulting in less contamination of harvested seed with weed seed (Canola Council of Canada, 2001). Foreign matter of representative GR and conventional soybean was determined by evaluating elevator receipts collected from soybean producers in the southern and midwestern United States (Shaw and Bray, 2003). A total of 16535 ha were represented, of which 13903 were from GR soybean and 2632 were from conventional soybean. The average foreign matter content from the GR soybean was 1.9%, compared with 2.5% from the conventional soybean. The authors concluded that the glyphosate-resistant program reduced foreign matter, an indication of reduced weed seed. Whether similar results would be found for other GRCs is unknown. This aspect of GRCs needs further study.

GENE FLOW (INTROGRESSION) EFFECTS

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. Pollen can be carried for long distances by wind, water, insects, and animals, but viability decreases with time and environmental stresses. 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 problems with existing weed species in GRCs or to create a new weed problem with species that are normally not a problem.

Introgression of Glyphosate Resistance Transgenes to Weeds

Introgression is more likely for herbicide resistance transgenes than for other transgenes, in that the herbicide used with the crop 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 (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 herbicide resistance transgene into the wild population.

Crops are naturally resistant to 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., 2005), 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 typically also naturally resistant to the same selective herbicides. This is not the case with HRCs.

Gressel (2002a, b) and Kwon and Kim (2001) have reviewed the risks for introgression of transgenes from various crops into weedy relatives. Among the GRCs grown, only canola has a weedy relative (*Brassica rapa* L.) with which it can interbreed in North America (Légère, 2005). Gene flow from GR canola to *Brassica rapa* L. has been documented in commercial fields (Warwick et al., 2003); however, the genes were apparently not fully introgressed. 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 glyphosate, 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 to reduce the transfer of the transgene to wild *B. rapa* species. With some weedy relatives of canola such as *Sinapis arvensis* L., gene transfer from *B. napus* in the field is very rare or nonexistent (Moyes et al., 2002).

Maize genes could theoretically introgress into teosinte [*Euchlaena mexicana* (Schrud.) Kuntze], the species from which maize originated, since the two species can interbreed (Doebley and Stec, 1993). Teosinte is not a troublesome weed. 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).

In the case of wheat, crosses of non-transgenic, imidazolinone-resistant wheat and *Aegilops cylindrica* Host

were discovered after only 1 yr 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 delay the movement to *A. cylindrica* Host (Wang et al., 2001). However, genes of the D genome of wheat can readily introgress into *A. cylindrica*, which also has a D genome (Kroiss et al., 2004).

GR creeping bentgrass, a wind-pollinated perennial, is being tested as an 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 GRCs. There is good potential for introgression of transgenes from sunflower *Helianthus annuus* L. (Linder et al., 1998), sugarbeet (Desplanque et al., 1999), and rice (Messeguer et al., 2004), 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 (at the time) growing of GR soybean by some farmers in Brazil (Flaskerud, 2003).

Gene Flow to Non-Transgenic Crops

Gene flow to non-GRCs of the same species is much more likely than outcrossing with other species. The glyphosate resistance 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 glyphosate. Non-GRCs that contain 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.

Gene flow between fields of herbicide-resistant canola, including GR canola, and non-transgenic canola has resulted in herbicide resistance transgenes in several combinations being present in Canadian 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 landraces of maize, and at least one paper has claimed that this has already happened in Mexico (Quist and Chapela, 2001). There was considerable controversy over

this paper, and the journal concluded that “the evidence is not sufficient to justify the publication of the original paper” (Anonymous, 2002). A later report has again stated that there has been gene flow to maize landraces in Mexico (North American Commission for Environmental Cooperation, 2004). More recently, an extensive study found no transgenes in seed samples from landraces of maize in Mexico (Ortiz–Garcia et al., 2005).

Introgression of Transgenes and the Natural Environment

Relatively little is known about introgression of genes from crops into wild relatives and the potential impact of this on natural ecosystems. A persuasive argument can be made that an herbicide resistance gene should have no fitness advantage in a natural habitat where glyphosate is not used (Stewart et al., 2003), and glyphosate resistance might reduce fitness (Baucom and Mauricio, 2004), depending on the mechanism of resistance. Although the proper experiments do not appear to have been done, there is no evidence that the GR genes used in commercial crop varieties at this time have any fitness penalty. Thus, we would expect no effect of these genes on natural ecosystems if introgressed into wild plants. Simard et al. (2005) found little indication of reduced fitness of different canola populations resistant to two herbicides (all dual combinations of glyphosate, glufosinate, or imidazolinones), due to introgression of transgenes in Canadian fields. But, cropping situations are quite different than natural environments.

When a glyphosate 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. A gene conferring a fitness advantage, such as pest or stress resistance, could change the ecological balance between plant species within an ecosystem, which could be a potentially undesirable outcome. 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 glyphosate resistance transgenes are coupled in commercially available transgenic maize and cotton.

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 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 (Daniell et al., 1998). Advances are being made in plastid transformation (Zhang et al., 2003; 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 (Wang et al., 2004; Zhang and Sodmergen, 2003), and there is no information with large numbers of a lack of pollen transmission of plastome traits.
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 [*Cynodon dactylon* (L.) Pers.] for 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 into 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 less subject to introgression (Stewart et al., 2003). For example, in wheat, the A and B genomes of this triploid crop will not easily 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 proven.

We view development and use of “failsafe” methods for eliminating introgression as the most important step that could be taken to reduce the potential environmental impact of transgenic crops.

CONCLUSIONS

We have provided an abbreviated survey of the potential impacts (risks and benefits) of GRCs and glyphosate. Clearly, we and many of the authors who 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 apparent health and environmental benefits of the glyphosate/GRC combination are significant. Glyphosate is more environmentally and toxicologically benign than many of the herbicides that it replaces. In most cases, the effects on soil, air, and water contamination and on non-

target organisms appear to be relatively small. Soil erosion causes long-term environmental damage. Being a broad spectrum, foliarly applied herbicide, with little or no activity in soil, glyphosate is highly compatible with reduced- or no-tillage agriculture and has contributed to the adoption of these practices in the Western Hemisphere. This contribution to environmental quality by GRCs 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 transfer of many genes with both known and unknown functions. New crops and new 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 that utilizes 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 currently grown transgenic crops are as safe and nutritious as those derived from traditional crops. The lack of any adverse effects resulting from consumption of transgenic crops grown on hundreds of millions of cumulative hectares over the last 10 yr supports his conclusion.

All of the minimal environmental risks that we discuss above are reversible 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 glyphosate resistance transgenes if they introgress into wild populations, as they offer no advantage in the absence of glyphosate. However, when glyphosate resistance transgenes are 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 GRCs. Several methods could be used to prevent introgression, but more research should be done to discover and/or develop technology to prevent or mitigate it.

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