Chapter 3
Transgenic Crops for Herbicide Resistance

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3.1 Introduction

A year after the introduction of the first commercial transgenic crop (Flavr Savr™ tomato with a longer shelf life) in 1994, transgenic, herbicide-resistant crops (HRCs) were introduced (Table 3.1) with the introduction of bromoxynil- (3,5-dibromo-4-hydroxybenzonitrile) resistant cotton and glufosinate- [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] resistant canola. Bromoxynil resistance had little market penetration during the years when it was available. The next year, 1996, marked the introduction of the first glyphosate- [*N*-(phosphonomethyl) glycine] resistant (GR) crop (soybean). Other GR and glufosinate-resistant crops were introduced in the subsequent years. GR crops now represent well over 80% of all transgenic crops grown worldwide (James 2008). Accordingly, this chapter will deal primarily with GR crops. Several reviews (e.g., Duke 2005; Duke and Cerdeira 2005; Cerdeira and Duke 2006) and two books (McClean and Evans 1995; Duke 1996) are available on the topic of HRCs, but this rapidly evolving topic requires timely updates.

Before the advent of transgenic crops, there was both controversy and optimism about their potential impact on farming, human health, and the environment (e.g., Goldberg et al. 1990; Duke et al. 1991). We have now (early 2009) had 14 years of experience with HRCs over vast areas in many parts of the world, providing a wealth of information on the utilization of this technology, as well as the impacts of HRCs on the environment. This review will deal, in part, with these topics as they apply to HRCs

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Table 3.1 Transgenic herbicide-resistant crops that have been deregulated (approved for sale) in North America

Herbicide	Crop	Year approved
Bromoxynil[a]	Cotton	1995
	Canola	2000
Glufosinate	Canola	1995
	Maize	1997
	Cotton	2004
	Rice[b]	2006
Glyphosate	Soybean	1996
	Canola	1996
	Cotton	1997
	Maize	1998
	Sugarbeet[c]	1999
	Alfalfa[d]	2005

[a]Removed from market
[b]Deregulated, but not commercialized
[c]Removed from market, but reintroduced in 2008
[d]Returned to regulated status in 2007 by court

3.2 Present HRCS and Their Impact

3.2.1 Commercially Available HRCs

In 2008, after 14 years of HRCs, there are only nine different HRCs being grown in the US (Table 3.1), and only a few of these are grown in other countries. From 1995 until 2000, one or two new HRCs were introduced to the market every year, after which the number of introductions has dwindled, with only a single new HRC in occasional subsequent years.

The adoption rate of GR soybean was rapid in the US (Fig. 3.1), currently representing more than 90% of the area planted in soybean. The adoption rate of GR soybean in Argentina was even more rapid, reaching almost 90% adoption within 4 years of introduction (Penna and Lema 2003). Adoption of this HRC has also been rapid in other parts of South America.

Both cotton and maize have varieties that are either stand-alone GR varieties or varieties that combine GR and transgenic *Bt* (*Bacillus thuriengensis* toxin) traits for insect resistance. In both the crops, there are also stand-alone *Bt* toxin varieties. To generate the data in Fig. 3.1, adoption rates of the two types of GR varieties must be added. GR cotton adoption was initially similar to that of soybean, but it has stabilized at about 70% (Fig. 3.1), partly because of the adoption of glufosinate-resistant cotton in places where it fits the weed problems better than GR cotton. The economics for GR maize was not quite as good as with existing weed management methods when it was first introduced, but its adoption in the US is now rising rapidly and has almost caught up with that of cotton (Fig. 3.1). Relatively little GR canola is grown in the US, but about 90% of the canola grown in Canada was GR canola in 2006 (Dill et al. 2008). Of the canola grown in the US, 62% was GR

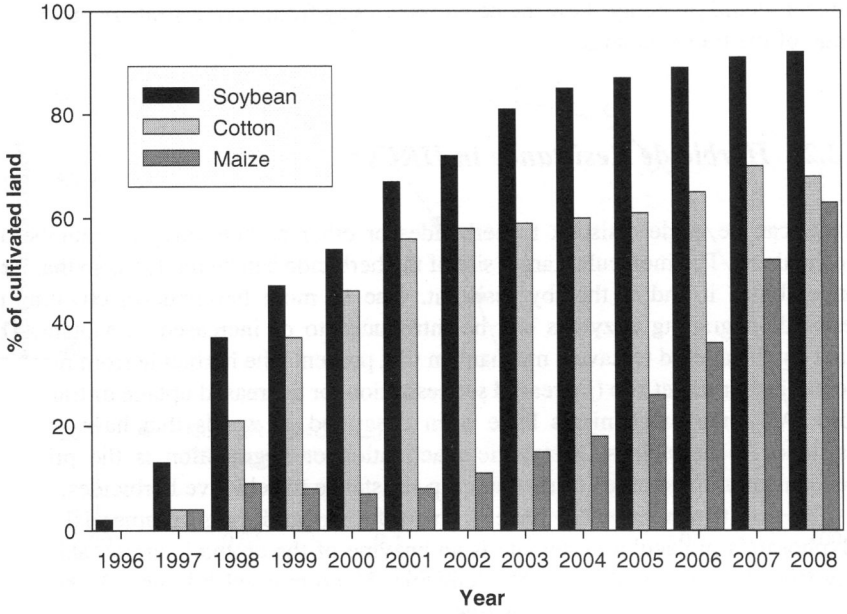

Fig. 3.1 US adoption rate of glyphosate-resistant crops. Data from: http://www.ers.usda.gov/Data/BiotechCrops

and 31% was glufosinate-resistant in 2005 (Sankula 2006). After a false start in 1999, GR sugar beet was reintroduced in 2008, with an unprecedented adoption rate of about 60% for the initial year of availability and an anticipated 95% adoption rate in 2009 (Thomas Schwarz, Beet Sugar Development Foundation, pers. comm). The adoption rate was limited by the availability of transgenic seed. GR alfalfa was introduced and well accepted by farmers in 2005, but deregulation was challenged in court by organic alfalfa growers in 2007, resulting in the removal of the product from the market.

Glufosinate-resistant crops are also available (Table 3.1), but they have garnered a much smaller fraction of the HRC market. Their biggest market penetration is with canola in the US. Glufosinate-resistant cotton has been adopted at high rates in the US state of Texas. Partly owing to the evolution of GR weeds, glufosinate-resistant crop adoption is increasing. Crops with both GR and glufosinate resistance are being made available.

The economics for the biotechnology industry with HRCs is also good. HRCs offer profits from both a "technology fee" added to seed costs and for the purchase of the herbicide. No other type of transgenic trait offers this opportunity for dual profits from the seed and a chemical upon which the value of the gene is dependent. There has been some consideration of linking expression of transgenic traits to a chemical inducer of transgene expression (e.g., Jepson et al. 1998), but farmers would be unlikely to pay much for such a chemical, and the cost of applying the

inducer would probably skew the economics away from such a strategy, unless the value of the trait was large.

3.2.2 Herbicide Resistance in HRCs

Plants can be made resistant to herbicides or other phytotoxins by a number of mechanisms. The molecular target site of the herbicide can be modified so that it no longer binds it and is thereby resistant. One or more herbicide-inactivating or herbicide-degrading enzymes can be introduced to or increased in a plant. The plant can be altered to have a mechanism that prevents the herbicide from reaching the molecular target site (increased sequestration, or decreased uptake or translocation). All three mechanisms have been described in weeds that have evolved resistance to herbicides. Metabolic inactivation or degradation is the principle mechanism in most cases of natural crop resistance to selective herbicides.

The first two approaches have been useful in producing commercial HRCs. Glyphosate's molecular target site is an enzyme of the aromatic amino acid pathway (the shikimate pathway). This enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), is highly sensitive to glyphosate (Duke 1988), and there are apparently no other good inhibitors of the enzyme. All plant EPSPS is sensitive to glyphosate, making it a nonselective herbicide that can be used to kill almost all weed species. Some fungi and bacteria also have EPSPS, and there are bacterial versions of the enzyme that are very resistant to glyphosate. A gene encoding *Agrobacterium* sp. EPSPS, the *CP4* gene, has been used as a transgene for almost all GR crops (Cerderia and Duke 2007). In some maize varieties, a maize EPSPS gene modified by site-directed mutagenesis has been used as the transgene in GA21 varieties (Dill 2005). In canola, a gene from the soil bacterium *Ochrobactrum antropi* that encodes a glyphosate-degrading enzyme (glyphosate oxidoreductase, GOX) has been used with the CP4 EPSPS. This enzyme catalyzes the degradation of glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate, both relatively innocuous compounds.

The CP4 EPSPS alone makes soybean approximately 50-fold less sensitive to glyphosate (Nandula et al. 2007) (Fig. 3.2). *CP4* and *GOX* genes together provide about the same level of resistance to canola (Nandula et al. 2007). Despite a generally high resistance factor, there can be problems if the transgene promoter is not sufficiently active in reproductive tissue, as appeared to be case in some of the first GR cotton varieties (Pline et al. 2002a,b; Pline-Srnic 2005; Yasuor et al. 2006; Dill et al. 2008). This problem was solved by using a promoter that is stronger in reproductive tissues. A similar, but lesser problem, has been reported in some maize varieties (Thomas et al. 2004).

Under some environmental circumstances, glyphosate can cause leaf damage in GR soybean (e.g., Reddy and Zablotowicz 2003), although this is a rare occurrence from which the crop eventually recovers. The degradation product of glyphosate, AMPA, is a weak phytotoxin (Hoagland 1980). Reddy et al. (2004) correlated

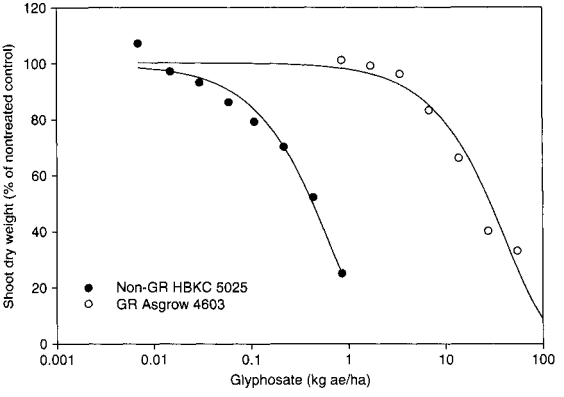

Fig. 3.2 Response of glyphosate-resistant (GR) Asgrow 4603RR and nonGR HBKC 5025 soybean in the one- to two-trifoliolate leaf (22-day-old, 45-cm tall) growth stage to glyphosate-potassium 3 weeks after treatment. Mean values of nine replications are plotted. From Nandula et al. (2007)

mild phytotoxicity in glyphosate-resistant (GR) soybeans to high levels of AMPA formed from high-glyphosate-dose applications. Applications of AMPA alone that resulted in the same endogenous AMPA concentrations caused the same level of phytotoxicity.

Papers have been published supporting the view that glyphosate impairs growth of GR crops under some conditions because of effects on micronutrient status (e.g., Jolley et al. 2004; Bott et al. 2008). Glyphosate chelates divalent metal cations (Duke 1988), which could limit their availability when the glyphosate levels are very high and the divalent metal cation concentration is very low. Conversely, binding of Ca^{2+}, Mn^{2+}, or Fe^{2+} can reduce the efficacy of glyphosate (e.g., Schonherr and Schreiber 2004; Bernards et al. 2005). The pH can strongly influence how tightly each cation species is chelated to glyphosate. There have been exceedingly few reports of nutrient deficiencies in the huge areas in which GR crops are grown. Considering the very disproportionate abundance between the number of molecules of glyphosate that would reach the soil and the number of divalent cations available for binding, it seems implausible that glyphosate would have a significant effect on the availability of essential minerals in GR crops. However, if a plant had a borderline mineral deficiency, chelation in vivo might cause a problem. The proper experiments have not been conducted to determine if this possibility could cause a significant problem in the field. Ozturk et al. (2007) reported that glyphosate inhibits ferric reductase in plant roots, suggesting that this effect would cause

iron deficiency in GR crops. However, this work was carried out with glyphosate-sensitive sunflower and the assay was an in vivo assay performed hours after treatment with high concentrations of glyphosate so that there was no way to determine if the effects were direct or indirect. These studies should be repeated with GR crops to answer this question.

Root-associated microbes are involved in plant mineral nutrition (e.g., Jakobsen et al. 2002). Some of these microbes are glyphosate-sensitive (e.g., Moorman et al. 1992). Some of the glyphosate applied to foliage is exuded by roots (e.g., Coupland and Caseley 1979; Laitinen et al. 2007). Thus, mineral nutrition could be altered by adverse effects on root-associated microflora. However, considering that nutrient deficiency problems have not been a commonly reported problem with GR crops after more than 10 years of adoption over vast areas, the potential mineral nutrition problems reported by only very few laboratories are of questionable impact in the field.

Glyphosate is toxic to *Bradyrhizobium japonicum* (Moorman et al. 1992). Several studies have indicated that there is potential for reduced nitrogen fixation in GR soybeans, but yield reductions due to such an effect have not been documented in the field when glyphosate is used at the label rate (Zablotowicz and Reddy 2004, 2007).

The no longer used bromoxynil-resistant crops owed their resistance to a transgene of microbial origin (*Klebsiella ozaenae*) that converts the benzonitrile to a nonphytotoxic benzoic acid derivative (Stalker et al. 1996). This gave the crops a more than tenfold level of resistance. Bromoxynil is an older category of selective herbicide that inhibits photosynthesis by binding the D1 protein of photosystem II (Devine et al. 1993).

Glufosinate is a synthetic version of the natural product, phosphinothricin. It is not accepted by organic farmers, partly because it is chemically synthesized as a racemic mixture, and the D enantiomer of the racemic mixture is not a natural compound. It acts by inhibition of glutamine synthetase, thereby causing accumulation of toxic levels of ammonium ion and indirectly stopping photosynthesis (Lydon and Duke 1999). It is considered a broad-spectrum herbicide, but is not quite as effective in some situations as glyphosate. One of the microbes that produce phosphinothricin, *Streptomyces hygroscopicus*, has an enzyme that inactivates phosphinothricin and glufosinate by acylating it. The gene (*bar*) encoding this enzyme, phosphinothricin *N*-acetyltransferase (PAT), is used as a transgene for glufosinate-resistant crops (Vasil 1996). In addition to the HRC use, the *bar* gene has been used extensively as a selectable marker.

3.2.3 Impacts on Weed Management

Nothing has had more impact on weed management in such a short time period as GR crops, except perhaps the introduction of synthetic, selective herbicides. Several factors have contributed to the strong acceptance of GR technology. A strong

argument can be made that glyphosate is the most effective and useful herbicide available (Duke and Powles 2008b). It is a slow-acting, highly translocated, foliarly applied product that kills weeds by inhibiting a molecular target site that is ubiquitous to all plants, EPSPS (Duke et al. 2003a). Its slow action allows translocation to meristematic tissues, ensuring that all growing points, including subterranean ones, are killed. It has very good environmental safety characteristics (see Sect. 3.2.5) in that it is highly nontoxic, does not easily move to ground or surface water, and has a relatively short half-life in soil. Before GR crops, it could only be used in places without crops or with methods that avoided crop contact. GR crops opened the door to widespread utilization of this exceptional herbicide directly on field crops.

In addition to the generally profound economic advantages to using GR crop/glyphosate technology (Gianessi 2005, 2008; Clewis and Wilcut 2007), this technology simplifies weed management (Bonny 2008). The farmer can often rely on only glyphosate applied once or twice during a growing season for weed control, rather than using a complicated strategy of both soil-incorporated and foliarly applied herbicides, involving multiple herbicides with different molecular target sites. Since glyphosate is used only as a postemergence herbicide, the farmer can wait to see what kind of weed problem emergences before spraying. Tillage can often be reduced or eliminated, creating both environmental (see below) and economic benefits. The benefits of this technology are not farm size dependent. A small farmer who farms only on weekends might derive more benefits, as this technology is more forgiving than traditional methods of weed management. Thus, one does not have to hire pest management consultants for prescription recommendations in order to obtain excellent weed control at a reasonable cost. This technology is also useful to farmers who grow multiple GRCs, in that they can apply one herbicide to multiple crops. Prior to GRCs, a particular herbicide could rarely be used on more than one crop.

Initially, weed management with GR crop technology was excellent, as indicated by the rapid adoption. But, the specter of the evolution of GR weeds is jeopardizing reliance on this weed control method (Powles 2008a). Although Bradshaw et al. (1997) gave reasons why the evolution of GR resistance was implausible, the first report of an evolved GR weed occurred the same year as their paper (Heap 1997). Since then, the number of cases of evolved GR resistance has grown at a steady pace all over the world (Table 3.2, Fig. 3.3), and about half of the cases have occurred in GR crops, where the selection pressure is intense (Fig. 3.3). In most of the other cases, resistance evolved in vineyards or orchards in which glyphosate was sprayed several times a year for several consecutive years.

The levels of evolved resistance of weeds to glyphosate are much lower than that of GR crops, usually in the range of two- to tenfold. The most common mechanism of resistance in these evolved biotypes is reduced translocation (e.g., *Lolium* spp., *Conyza* spp.) (Preston and Wakelin 2008), although mutations in EPSPS that provide marginal resistance have also occurred in *Eleucine indica* (Baerson et al. 2002) and some populations of *Lolium* spp. (Wakelin and Preston 2006; Perez-Jones et al. 2007). In at least some evolved GR *Conyza*, increased numbers of EPSPS transcripts may also contribute to resistance (Dinelli et al. 2008).

Table 3.2 Occurrence of evolved GR weeds by species, year, and country

Species	Year	Location
Amaranthus palmeri	2005	USA
Amaranthus rudis	2005	USA
Ambrosia artemisifolia	2004	USA
Ambrosia trifida	2004	USA.
Conyza bonariensis	2003	South Africa
	2004	Spain
	2005	Brazil
	2006	Columbia
	2007	USA
Conyza canadensis	2000	USA
	2005	Brazil
	2006	China, Spain
	2007	Czech Republic
Digitaria insularia	2006	Paraguay
	2008	Brazil
Echinochloa colona	2007	Australia
Eleusine indica	1997	Malaysia
	2006	Colombia
Euphorbia heterophylla	2006	Brazil
Lolium multiflorum	2001	Chile
	2003	Brazil
	2004	USA
	2006	Spain
	2007	Argentina
Lolium rigidum	1996	Australia
	1998	USA
	2001	South Africa
	2005	France
	2006	Spain
Parthenium hysterophorus	2005	Columbia
Plantago lanceolata	2003	South Africa
Sorghum halepense	2005	Argentina
	2007	USA
Urochloa panicoides	2008	Australia

Source: From the International Survey of Herbicide-Resistant Weeds: http://www.weedscience.org/In.asp and Vila-Aiub et al. (2008)

Nature abhors a vacuum. In addition to the evolution of GR weeds, when farmers rely on glyphosate year after year, other species of weeds can fill the ecological niches vacated by the species that are easily managed with glyphosate. The process of weed species shifts in GR crop fields has been documented (e.g., Reddy 2004; Owen 2008). Some of these species have a low level of natural (not evolved) resistance, and others can avoid glyphosate by germinating later in the growing season or having a broad germination pattern throughout the season. One potential mechanism of some species that are naturally resistant is enhanced conversion of glyphosate to AMPA (Reddy et al. 2008).

The advent of evolved GR weeds and shifts to naturally GR or glyphosate-avoiding weeds has made the GR crop/glyphosate weed management package less

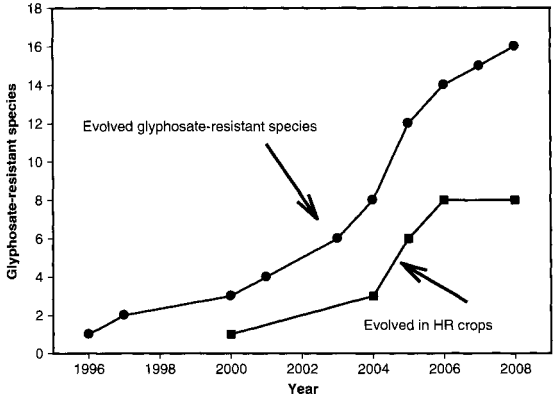

Fig. 3.3 Incidence of species that have evolved resistance to glyphosate by year

effective in some locales. Application rates and number of applications of glyphosate in GR crops have increased. Herbicides, in addition to glyphosate, are being used with GR crops. New herbicide-resistant crops are being developed to have resistance to glyphosate, plus resistance to another herbicide or class of herbicides that might be effective on weeds that are not controlled by glyphosate. Other options for coping with GR weeds and weed shifts are resistance management strategies that involve approaches such as alternation of herbicides, utilization of alternative/or residual herbicides in conjunction with glyphosate, and use of cultivation (e.g., Gustafson 2008; Neve 2008; Werth et al. 2008).

The effects of HRCs on weed management is in a constant state of flux because of weed dynamics, economics, and changes in the availability of both old and new weed management tools. GR crops have been a valuable asset for weed management and will probably continue to be, even as GR weeds evolve and weed species shift. The effective longevity of this technology will depend, in part, on how wisely it is used (Powles 2008a).

3.2.4 Impacts on Food and Feed

One of the concerns of those opposing transgenic crops is that the transgene will alter the quality and/or safety of the consumable part of the plant. This could occur through the protein from the transgene being toxic, through a metabolic product of

the enzyme encoded by the transgene being toxic, through pleiotropic effects of the transgene, through alteration of expression of nontransgenic genes by the position of the transgene in the genome or through more indirect effects. For regulatory approval, transgenic crops are scrutinized to a far greater level than conventional crops using analytical, nutritional, and toxicological methods (Atherton 2002; Malarkey 2003; König et al. 2004), although some have proposed that even more extensive tests be done by metabolomic, proteomic, and transcriptomic analysis to detect potential unintended effects of the transgene and its insertion on food safety and quality (Kuiper et al. 2002; Cellini et al. 2004).

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

The potential allergenic properties of the protein products of transgenes must be determined before approval. These data are provided to regulatory agencies, but publications on this topic are scarce. However, there are a few published studies showing no allergenic properties of transgene products associated with HRCs. Sten et al. (2004) in a study with soybean-sensitized patients, found that the allergenicity of 10 GR and eight nonGR soybean cultivars were not different. Chang et al. (2003) found no significant allergenicity to rats of the *CP4 EPSPS* gene product conferring glyphosate resistance. On the basis of both an analysis of published literature and experimental studies, Herouet et al. (2005) concluded that there is a reasonable certainty of no harm resulting from the inclusion of the gene for glufosinate resistance in human food or in animal feed.

Most of the tests have simply examined HRCs for equivalence in food quality to nonHRCs. Health Canada's review of the information, presented in support of the food use of refined oil from glufosinate-resistant canola line HCN92, concluded that such refined oil does not raise concerns related to safety. Health Canada is of the opinion that refined oil from canola line HCN92 is as safe and nutritious as refined oil from the current commercial varieties (http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_nf7web00.html). The nutritional properties of glufosinate-resistant sugar beets and maize grains were found to be essentially equivalent to nontransgenic cultivars in feeding studies with swine and ruminants (Daenicke et al. 2000; Bohme et al. 2001); similar results have been produced with glufosinate-resistant rice in swine feeding studies (Cromwell et al. 2005).

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 analyzes 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 16 field sites over two growing seasons. No significant differences were found. Similarly, Tutel'ian et al. (2001) found no compositional differences between conventional maize and maize line GA21. The nutritional safety of maize line GA21 was also evaluated by Sidhu et al. (2000) in a

poultry feeding study. Results from the poultry feeding study showed that there were no differences in growth, feed efficiency, adjusted feed efficiency, and fat pad weights among chickens fed with GA21 grain or with parental control grain. These data taken together demonstrate that GR GA21 maize is as safe and nutritious as conventional maize for food and feed use.

The occurrence of mycotoxins, specifically aflatoxin, contamination is of concern with maize cultivation in warm climates subject to preharvest moisture stress. Studies by Reddy et al. (2007) indicated increased incidence of aflatoxin contamination in GR compared to conventional maize in 1 year out of four. But, in 1 year out of four, fumonisin levels were higher in nonGR maize than in GR maize. The observed effects were thought to be due to differences in how the crops were grown, rather than due to glyphosate or the transgene.

Several other studies have found no substantial difference in the nutrient content of GR and nontransgenic crops. These studies include maize (Ridley et al. 2002; Autran et al. 2003), soybean (Padgette et al. 1996), wheat (Obert et al. 2004), and cotton (Nida et al. 1996). In the Autran et al. (2003) study, the characteristics of glyphosate- and glufosinate-resistant maize in different foods (e.g., beer, hominy, oil, grits) were compared and found not to be substantially different from the respective, nontransgenic parental lines.

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. Padgette et al. (1996) found no effects of the transgene on isoflavone content of soybean. 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. 2002a,b), 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, no significant effects of glyphosate on isoflavones were found at the highest and latest legal application rates (Duke et al. 2003b).

Table 3.3 summarizes most of the published results of animal feeding studies with GR crops. All the studies support the view that food from GR crops is substantially equivalent to nontransgenic crops. In addition to these studies, no evidence of the *CP4* gene or its protein product could be detected in pork from swine fed with 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).

HRCs, being highly resistant to the herbicide to which they have been made resistant, could also contain levels of the herbicide or its metabolite(s) that exceed the legal tolerance levels. Surprisingly, little has been published on herbicide residues in HRC foods. Most of what we know is from studies with nonHRC crops. However, herbicide residue data must be supplied for regulatory approval of HRCs.

Glyphosate acid and its salts are moderately toxic compounds in EPA toxicity class II. Glyphosate (either the anion or the isopropylamine salt) is practically

Table 3.3 Some results of animal feeding studies with glyphosate-resistant crops

Crop	Animal	Result	Reference
Maize	Rat	No effect	Hammond et al. (2004)
		No effect	Healy et al. (2008)
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
Soybean	Rat	No effect	Zhu et al. 2004
		No effect	Hammond et al. (1996)
		No effect	Appenzeller et al. (2008)
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	Catfish	No effect	Hammond et al. 1996
Soybean	Poultry	No effect	Hammond et al. (1996)
		No effect	Taylor et al. (2007)
Canola	Rainbow trout	No effect	Brown et al. (2003)
Canola	Poultry	No effect	Taylor et al. (2004)
Alfalfa	cattle	No effect	Combs and Hartnell (2008)
Sugarbeet	Sheep	No effect	Hartnell et al. (2005)

nontoxic by ingestion, with a reported acute oral LD_{50} of >5,000 mg kg^{-1} in the rat (Vencill 2002). The trimethylsulfonium salt of glyphosate is more toxic, with an oral LD_{50} of about 705 mg kg^{-1}. It is not a restricted use pesticide and is a best-selling weed killer for home use. Animals do not contain the herbicide molecular target site (EPSPS) of glyphosate.

Occasional reports of severe effects of ingestion of formulated glyphosate occur (e.g., Stella and Ryan 2004); however, the glyphosate molecule itself is considered one of the most toxicologically benign herbicides available. Williams et al. (2000) extensively reviewed the toxicology literature on glyphosate and its metabolites and concluded that under present and expected conditions of use, glyphosate does not pose a significant health risk to humans.

In a testing program to detect whether GR soybeans had been sprayed with glyphosate or not, Lorenzatti et al. (2004) found glyphosate and AMPA in green, immature seeds. Both glyphosate and its degradation product, AMPA, were found in mature, harvested seeds of different GR soybean varieties grown in widely separated geographical regions (Duke et al. 2003b). Even though the glyphosate applications were at legal, but at relatively high rates and late timing, the residues were within the established tolerance levels. We were surprised to find higher AMPA than glyphosate levels since at that time plants were thought to degrade glyphosate very little, if at all (Duke 1988; Duke et al. 2003a). Subsequently, we found that some legume plant species readily convert glyphosate to AMPA (Reddy et al. 2008). In a study conducted earlier, but published later (Arregui et al. 2004), similar levels of glyphosate were found in harvested seed of GR soybeans, but these

scientists found much lower AMPA levels. We have found no publications on glyphosate residues in GR crops other than soybean.

Glufosinate is not a restricted use pesticide and is sold for home weed control in the USA. Its acute oral toxicity in rats is an LD_{50} of ca. 2.2 g kg^{-1}. Glufosinate chemically resembles glutamine, a molecule used to transmit nerve impulses in the brain. Ebert et al. (1990) concluded in an extensive review that glufosinate is safe under conditions of recommended use. Similarly, Hack et al. (1994) also concluded from their studies that glufosinate is unlikely to cause health effects of either users or consumers when used as directed, although the herbicide target site, glutamine synthetase, is also found in animals.

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

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

3.2.5 Environmental Impacts

There are numerous possible environmental effects of HRCs. These can be either positive or negative. They can be associated with the transgene or with the herbicide to which the transgene is linked. But, ultimately, potential environmental impacts of HRCs must be compared with the impacts of the technologies that they replace. All the published studies and analyses of this type have found that the environmental benefits of substituting HRCs for conventional crops are usually substantial (e.g., Wauchope et al. 2002; Nelson and Bullock 2003; Bennett et al. 2004; Amman 2005; Brimner et al. 2005; Brookes and Barfoot 2006; Cerdeira and Duke 2007; Cerdeira et al. 2007; Kleter et al. 2007, 2008; Devos et al. 2008; Gardner and Nelson 2008; Shiptalo et al. 2008). Of course, the potential benefits vary with the HRC, the geographic location of use, the way the farmer uses the HRC, and different components of environmental impact. Since nature is not static, the environmental impact will change with time as farmers using HRC technology adjust their methods to deal with changing weed and other problems.

Whether HRCs have increased or decreased herbicide use in terms of the amount of material used per hectare of crop can be argued either way, depending on many factors. However, in terms of environmental impact this factor is not useful, as the

relative toxicity and environmental fate of glyphosate or glufosinate compared with the herbicides that they replace in HRCs can be large. Glyphosate and glufosinate have relatively low mammalian toxicity (Ebert et al. 1990; Giesy et al. 2000; Williams et al. 2000), compared to many of the herbicides that they can replace when used with transgenic crops. Glyphosate, in particular, has lower acute toxicity than aspirin or many other commonly ingested compounds.

In a study, using acute mammalian toxicity data, Gardner and Nelson (2008) compared the number of LD_{50} doses per unit area that were decreased by GR crops in the United States. Depending on the crop and the location, they calculated that conventional weed management with other synthetic herbicides could result in as much as 3,000 more LC_{50} doses per hectare with maize, more than 375 with cotton, and more than 90 with soybean. An example of some of their data with cotton is provided in Fig. 3.4. They concluded that GR technology has a positive environmental effect. In a European study, Devos et al. (2008) found that use of GR maize and glufosinate-resistant maize would lower the pesticide occupational and environmental risk or weed management. The main benefits were due to the lower potential of these chemicals to contaminate groundwater and their lower acute toxicity. Glyphosate gave slightly greater reductions than glufosinate. Their calculations were done with the assumption that other herbicides would not be used with glyphosate nor glufosinate. However, glyphosate is increasingly used with other

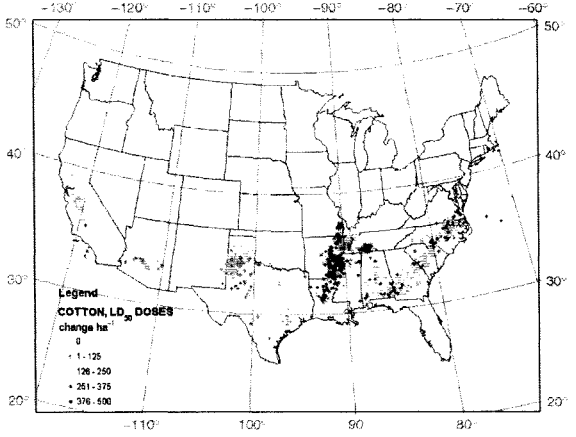

Fig. 3.4 Increase in LD_{50} doses per hectare of herbicides needed if GR cotton were not available in the US. From Gardner and Nelson (2008)

herbicides in GR crops because of evolved glyphosate resistance and shifts to naturally resistant weed species.

Neither glyphosate nor glufosinate are problematic pesticides in the context of surface and groundwater contamination of the herbicides that they replace (reviewed by Duke and Cerdeira 2005; Cerdeira and Duke 2006; Borggaard and Gimsing 2008). Glyphosate does not move well in soil because of its strong sorption to soil minerals (e.g., Mamy and Barriuso 2005). Furthermore, in most soils, it degrades more rapidly than the herbicides that it replaces (e.g., Mamy et al. 2005). In addition to microbial degradation, both glyphosate and AMPA can undergo nonbiological degradation in soil (Barrett and McBride 2005), although the relative contribution of the two types of processes is unknown.

Concern over AMPA contamination of groundwater has recently been expressed (Mamy et al. 2005, 2008). AMPA is more persistent than glyphosate in the environment, and it more readily leaches in soil (Mamy et al. 2008; Simonsen et al. 2008). Both glyphosate and AMPA are leached from soil containing high phosphate levels than soil with low levels (Simonsen et al. 2008). The relative toxicities of AMPA versus contaminants from conventional herbicide use have not been adequately analyzed.

Fuel utilization associated with weed management has been reduced by GR crops because of reduced tillage and fewer trips across the field to spray herbicides (reviewed by Cerdeira and Duke 2006). Bennett et al. (2004) estimated that there would be a 50% fossil fuel savings in growing sugar beet in Europe by switching to GR crops. Brookes and Barfoot (2006) estimated that GR crop use in 2005 worldwide reduced carbon emissions approximately the same as the removal of four million family automobiles from the road.

Effects of the herbicides used with HRCs on nontarget organisms are a concern. Obviously, since glyphosate is a herbicide, glyphosate drift to nontarget plants can be harmful (e.g., Bellaloui et al. 2006). This is not a new problem, as there have been problems with herbicide drift since the advent of herbicides that are sprayed. Concentrations reaching nontarget plants are generally only a small fraction of the recommended dose for weed control. At very low doses that one might expect with drift, herbicides can often stimulate growth, activate host defense systems against pathogens, or enhance nitrogen utilization, depending on the herbicide (reviewed by Duke et al. 2006). The phenomenon of a stimulatory effect at subtoxic concentration of a toxin is termed hormesis. Glyphosate clearly enhances growth of plants at very subtoxic application rates (e.g., Schabenberger et al. 1999; Wagner et al. 2003; Cedergreen et al. 2007; Velini et al. 2008). But, at least in barley, the effect is not sustained over time (Cedergreen 2008). The mechanism(s) of herbicide-caused hormesis is poorly understood and may well differ between herbicides with different molecular target sites.

Perhaps, the greatest damage done by conventional agriculture, other than taking land out of its natural state, has been caused by tillage. The primary reason for tillage has been weed management. Glyphosate use in GR crops has resulted in significantly reduced tillage, especially in soybean and cotton (Fig. 3.5) (American Soybean Association 2001; Penna and Lema 2003; Dill et al. 2008; Locke et al. 2008;

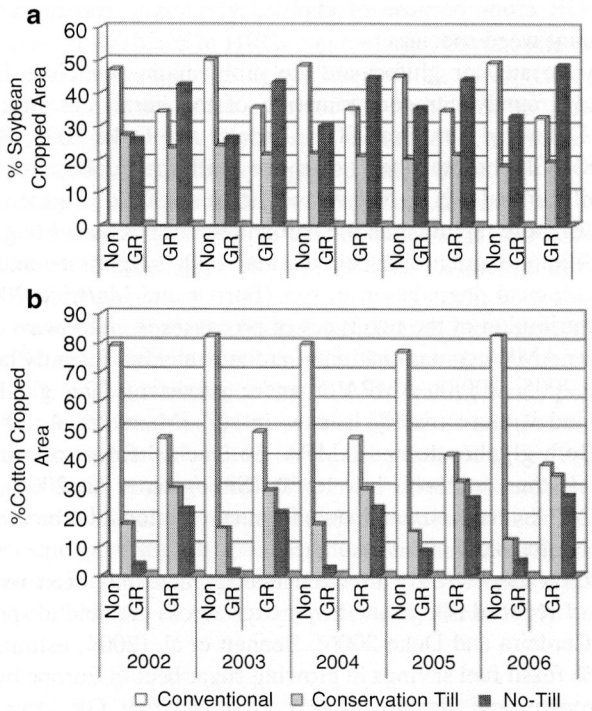

Fig. 3.5 Comparison of US tillage practices in glyphosate-resistant (GR) and nonGR soybean (**a**) cotton (**b**) from 2002 through 2006 as a percentage of hectares planted. From Dill et al. (2008)

Powles 2008b). Even in some nonGR crops, reductions in the price of glyphosate and the increases in the cost of diesel fuel have favored conservation tillage rather than traditional tillage (Nail et al. 2006). Reduced tillage sometimes reduces soil compaction (Koch et al. 2003; Shukla et al. 2003). To our knowledge, this aspect of the influence of HRCs has not been studied. However, as both evolved and naturally resistant GR weeds increase, some farmers of GRCs are returning to occasional tillage for more complete weed management.

There is concern about the potential of HRCs to create new weed problems, either themselves becoming a weed or the HR transgene escaping to relatives, either feral crops or related species, to create new weed problems. Gene flow to native populations of species with which the HRC can crossbreed could result in unwanted agricultural and/or environmental effects.

The HR transgene confers no advantage where the matching herbicide is not sprayed, so the HR crop is no more likely to invade a natural habitat than the nonHR crop. None of the crops that have been made herbicide resistant with transgenes are crops that become weeds outside of agricultural fields. However, HRCs are sometimes problems in agricultural fields in which the same herbicide is used in

subsequent years with a different HRC. This has already been a problem with GR crops (e.g., York et al. 2004; Soltani et al. 2006; Beckie and Owen 2007), requiring the use of herbicides other than glyphosate to control the "volunteer" GR crop.

Gene flow to nontransgenic crops of the same species has been a commercial and political problem, but not an environmental threat. Organic farmers cannot retain the organic status of their crops if transgene presence is above a set limit, nor can crops be sold to markets that require the product to be nontransgenic if transgenic occurrence is above the level set the regulatory jurisdiction. For crops like soybean, outcrossing is not a problem, but considerable outcrossing can occur with maize, rice, sugar beet, and canola. Substantial gene flow can occur between HR and nonHR canola (e.g., Hall et al. 2000; Reiger et al. 2002; Mallory-Smith and Zapiola 2008). Outcrossing may account for the contamination of nontransgenic rice with the glufosinate-resistant gene (Vermij 2006), even though glufosinate-resistant rice has only been grown experimentally. To our knowledge, no herbicide-resistant transgenes have been found to be a problem in nonherbicide-resistant maize, although there is significant potential for this to happen (Allnutt et al. 2008). There has been considerable controversy about whether Mexican maize landraces are contaminated with transgenes (reviewed by Cerdeira and Duke 2006). No gene flow from transgenic to nontransgenic cotton has been reported in cultivated fields, but it should occur because of insect pollination. Gene flow from GR alfalfa to organic alfalfa was the ostensible reason for its re-regulation. Flow of a GR transgene from bentgras being evaluated for commercial use to nontrangenic bent grass occurred rapidly, and mitigation efforts have not been effective (Mallory-Smith and Zapiola 2008; Zapiola et al. 2008).

A much bigger concern is the potential effect of gene flow from HRCs to weedy relatives. The only environmental aspects of transgenic crops that are not also associated with nontransgenic crops are those associated with the transgenes. HR transgenes offer no advantage in natural ecosystems where the herbicide is not used, but when coupled with transgenes imparting traits that would improve fitness in a natural ecosystem (e.g., insect or drought resistance), the HR trait would improve the likelihood of introgression of the gene into the unintended recipient species (see below). Once a transgene escapes to another species, it is unlikely that it could be eliminated in the population by human efforts. Indeed, removal of a GR transgene from a nontransgenic population of bentgrass has not been accomplished with the mitigation methods used (Zapiola et al. 2008).

Gene flow or introgression involves the movement of a gene or genes into a sexually compatible species. This type of gene flow is also termed vertical gene flow. The first generation is generally not very fit (e.g., Scheffler and Dale 1994), but subsequent backcrosses to the noncrop species will eventually fully introgress the transgene into this species. Yearly spraying of the herbicide to which the transgene confers resistance should facilitate the process by selecting only crosses with the transgene and removing competing weeds to give the unfit F_1 and usually the F_2 generations a significant survival advantage.

Transmission of HR transgenes could make weedy relatives of the HRC much more problematic for the farmer. This has not happened with soybean, cotton, and

maize, presumably because there are few or no weedy species with which they are sexually compatible in the places in which they are grown.

Canola outcrosses with several weedy related *Brassica* species, including related *Brassica* crops (e.g., cabbage, cauliflower, broccoli, etc.). These crosses are generally quite unfit, but introgression of a GR transgene from canola to its weedy relative, bird rape (*Brassia rapa*) has occurred in field situation, and the introgressed gene appears to be stable in the population, even in the absence of spraying glyphosate (Warwick et al. 2008). A potential exists for a transgene to move from through compatible relative (a bridge species) to a third *Brassica* species to increase the means by which a GR gene could spread (Brown and Brown 1996).

Work by Darmency et al. (2007) indicates that gene flow from sugar beets to weedy beets is sufficient to cause problems for the farmers growing HR beets within a few years if herbicides are not rotated. However, the degree of outcrossing that they measured varied considerably between lines of transgenic beets, years, and field locations.

Rice outcrosses readily with feral rice (e.g., red rice), as well as weedy related species. Nontransgenic, imidazolinone (IMI) herbicide-resistant rice created by selection in cell culture (Tan et al. 2005) was commercialized in the USA in 2002. Outcrossing to produce IMI-resistant weeds occurs (e.g., Shivrain et al. 2007). The incidence of IMI-resistant weeds in rice is growing in locations where IMI-resistant rice is grown (Valverde 2007), although whether this is due to evolved resistance or gene flow is unclear in most cases, because IMI resistance evolves quickly in some species when exposed to this herbicide class (Heap 2008). We can expect that gene flow from rice to feral rices and to sexually compatible species will occur with any transgene, including those imparting herbicide resistance.

Keeping HRCs out of areas in which sexually compatible species exist is a daunting task. For example, transgenic, GR and glufosinate-resistant canola are not approved as crops in Japan, but the seed can be imported for processing. Saji et al. (2005) found these HRCs growing along routes to processing plants in Japan.

There is considerable literature on strategies and technologies for mitigating or eliminating vertical gene flow (reviewed by Gressel 2002; Cerdeira and Duke 2006). Much of this literature deals with simply reducing movement of the genes to plants other than the transgenic crop (e.g., Devos et al. 2005). However, if the gene would pose a potential problem in a natural environment, a fail-safe approach would be preferable. Strategies coupling one or more technologies such as genetic use restriction technology genes to make transmission of a functional transgene impossible (Oliver et al. 1998) or linking the HR transgene to one that would confer unfitness to a wild plant (e.g., genes that prevent shattering or dormancy; Al-Ahmad et al. 2004; Gressel and Al-Ahmad 2004) have the potential for fulfilling this need. In the latter approach, the HR gene(s) should be in the same construct as the unfitness gene(s) to keep the genes from segregating.

Lastly, some have voiced concern that there could be gene flow from HRCs and other transgenic crops to totally unrelated organisms (horizontal gene transfer), especially soil or gut microbes (e.g., Bertolla and Simonet 1999; Giovannetti 2003), from degraded HRCs in the field or through ingestion of food composed of GRCs.

Almost all of the transgenes currently used for HRCs are from soil microbes. These genes are much more likely to be transferred to unrelated microbes from the natural, microbial sources than from HRCs (Kim et al. 2005). Levy-Booth et al. (2008) found that degradation of the *cp4-epsps* transgene from GR soybean leaf material in the soil was rapid, but could still be detected in soil after 30 days. Degradation of the transgene and a natural soybean gene in soil were similar. Dale et al. (2002) concluded that there is no compelling argument that there would be any more likelihood of such gene transfer transgenic crops than from nontransgenic crops. There has so far been no credible evidence of gene transfer from transgenic crops to microbes (Dunfield and Germida 2004), although some have criticized detection methods (e.g., Nielsen and Townsend 2004). Nevertheless, after 14 years of growing these crops on huge areas, there have been no reports of transgenes being transferred to microbes of any type.

3.2.6 Herbicide-Resistant Crops and Crop Disease

There are some untended benefits of GR crops and perhaps glufosinate-resistant crops. Both glyphosate and glufosinate are fungitoxic (reviewed by Duke et al. 2007). Thus, when used at full application rates, these herbicides may, under some circumstances, be providing sufficient protection from plant pathogens to prevent crop damage or to preclude spraying with a fungicide. Perhaps, the most carefully studied example is that of glyphosate effects of Asian rust in GR soybeans (Feng et al. 2005, 2008), in which glyphosate applications to GR soybeans reduced rust infection and damage, both as a preventative and a curative (Fig. 3.6) treatment. However, in many field situations, the optimal timing of glyphosate application for effective weed management is unlikely to also be most effective for rust control (Bradley and Sweets 2008). The effects of glyphosate on reducing this disease will

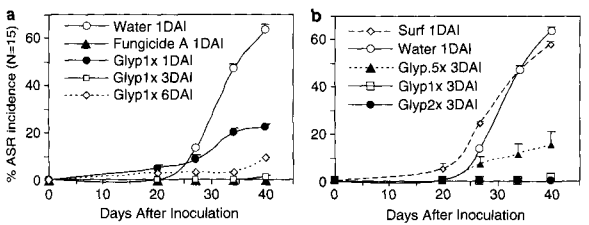

Fig. 3.6 (**a**) Curative activity for Asian rust in glyphosate-resistant soybeans sprayed with glyphosate (Glyp, 1× at 0.84 kg AE ha^{-1}) at 1, 3 or 6 days after inoculation (DAI), water and fungicide A (carbendazim + flusilazole) at 1 DAI. (**b**) Curative activity as a function of glyphosate dose (0.5×, 1× or 2×) from spray application at 3 DAI, surfactant (1 g L^{-1} MON 0818) or water alone at 1 DAI. From Feng et al. (2008)

Table 3.4 Reports of glyphosate interactions and lack of interactions with plant disease in glyphosate-resistant crops

Crop	Disease	Effect	References
Soybean	*Phakopsora pachyrhizi*	Reduces	Feng et al. (2005, 2008)
	Fusarium spp	Increases	Kremer et al. (2005)
	S. sclerotiorum	No effect	Lee et al. (2003)
		Increases	Nelson et al. (2002)
	F. solani	Increases	Sanogo et al. (2001), Nijiti et al. (2003)
Cotton	*Rhizoctonia solani*	Reduces	Pankey et al. (2005)
Wheat	*Puccinia triticina*	Reduces	Feng et al. (2008), Anderson and Kolmer (2005)
Sugarbeet	*Rhizoctonia solani*	Increases	Larson et al. (2005)
	Fusarium oxysporum	Increases	Larson et al. (2005)

probably increase as glyphosate use increases, both in application rates and number of applications, because of the evolution of GR weeds and weed species shifts to more naturally GR weeds.

On the other hand, glyphosate treatment is known to predispose nontransgenic plants to plant disease by more than one mechanism associated with the mechanism of action of glyphosate as a herbicide (reviewed by Duke et al. 2007), although these mechanisms should not exist in GR crops. There are many reported cases of exacerbation of plant disease symptoms with glyphosate, but these studies have generally been done with glyphosate-susceptible plants (reviewed by Duke et al. 2007). Nevertheless, there are a few reports of this phenomenon in GR crops (Table 3.4). Likewise, there are reports of reduced crop disease with glyphosate (Table 3.4). Thus, the interaction of glyphosate, fungal plant diseases, and GR crops is variable, depending on the crop, the disease, and perhaps the timing of herbicide application and infection. Also, differences in cultural practices between HRCs and nontransgenic crops can influence plant disease (e.g., Lee et al. 2005). Glyphosate effects on root exudates and soil moisture can influence root-borne soybean diseases (Kremer et al. 2005; Means and Kremer 2007; Means et al. 2007). Larson et al. (2005) found the that several factors, including the disease isolate, whether or not the crop was sprayed with glyphosate, and the variety of GR sugar beet determined whether there was increased disease with the GR crop. In a recent review, Powell and Swanton (2008) concluded that there was insufficient information from realistic field studies to determine whether glyphosate influences *Fusarium* spp. diseases in GR crops.

3.3 Coming Herbicide-Resistant Crops

3.3.1 Transgenes for Herbicide Resistance

Many transgenes have been reported and/or patented for the production of HRCs. Only a few of them are listed in Table 3.5. Technology is at a stage at which resistance to any herbicide can be imparted by transgene technology. Yet, only

Table 3.5 Some of the transgenes that have been used for making crops resistant to herbicides or classes of herbicides

Herbicide or herbicide class	Gene source and gene product	References
2,4-D	Microbial degradation enzyme	Llewellyn and Last (1996), Bisht et al. (2004)
Asulam	Resistant microbial dihydropteroate synthase	Surov et al. (1998)
Dalapon	Microbial degradation enzyme	Buchanan-Wollaston et al. (1992)
Dicamba	Microbial degradation enzyme	Behrens et al. (2007), Herman et al. (2005)
Hydroxyphenylpyruvate dioxidase (HPPD) inhibitors	Microbial, herbicide-resistant HPPD	Matringe et al. (2005)
Paraquat	Chloroplast superoxide dismutase	Sen Gupta et al. (1993)
Phenmedipham	Microbial degradation enzyme	Streber et al. (1994)
Phytoene desaturase (PDS) inhibitors	Genes from microbes and the aquatic weed Hydrilla encoding resistant PDS	Sandmann et al. (1996), Arias et al. (2005)
Protoporphyrinogen oxidase (PPO) inhibitors	Resistant microbial PPO and resistant *Arabidopsis thaliana* PPO	Li and Nicholl (2005)

three types of HRCs have been marketed; crops resistant to bromoxynil, glufosinate, and glyphosate, utilizing only five transgenes. Considering the huge economic impact of the GR crops, one would expect that other HRCs would have been marketed. Considerable effort and expense was put into development of HRCs that resist both hydroxyphenylpyruvate dioxygenase- and protoporphyrinogen oxidase-inhibiting herbicides (Li and Nicholl 2005; Matringe et al. 2005), yet the decision was apparently made to postpone or terminate plans to commercialize these HRCs. Devine (2005) attributed the few HRCs making it to the market place to the high cost of developing and getting regulatory approval of HRCs.

Further complications are international trade issues and the impact on the existing registration of the herbicide to which they are made resistant. Realistically, none of the herbicides on the market to which crops can be made resistant offer the advantages of glyphosate (Duke and Powles 2008b) or perhaps even glufosinate, both relatively safe, broad-spectrum herbicides.

In addition to the few number of herbicides used (only two), the number of crops represented among all the currently available HRCs is few (Table 3.1). This is partly because the cost of obtaining approval of HRCs is considered too great for minor crops (Devine 2005). However, this does not explain the absence of GR wheat and rice and glufosinate-resistant wheat, where the market could be very large, worldwide. GR wheat was almost marketed in North America, but concern over acceptance of wheat products from this source in Europe prevented it being marketed. A similar concern slowed the acceptance of GR sugar beets until 2008. The horrendous problems with outcrossing of rice with weedy feral and other species of may be related to GR rice being unavailable.

Table 3.6 Recently deregulated HRCs (http://www.aphis.usda.gov/brs/not_reg.html) that are not yet on the market and HRCs that have recent approval for field-testing in the USA (http://www.isb.vt.edu/cfdocs/fieldtests1.cfm)

Crop	Herbicide(s)	Company	Resistance gene(s)
Petition for deregulation			
Soybean[a]	Glyphosate and ALS inhibitors	Pioneer	Glyphosate acetyltransferase and a modified soybean ALS
Cotton	Glyphosate and glufosinate	Bayer Crop Sci.	Maize EPSPS modified by site-directed mutagenesis and *bar* gene
Maize	Glyphosate and ALS inhibitors	Pioneer	Glyphosate acetyltransferase and a modified maize ALS
Approval for field-testing			
Alfalfa	Glyphosate/ sulfonylureas	Pioneer	Glyphosate acetyltransferase and a modified plant ALS
Soybean	Glyphosate/dicamba	Monsanto	CP4 EPSPS and a demethylase
	Glufosinate/dicamba	Monsanto	CP4 EPSPS and a demethylase
	Glyphosate	Pioneer	Glyphosate acetyltransferase
	Glyphosate/isoxazole	MS	Technologies
	Not available		
	Glufosinate	MS	Technologies
Not	avaiable		
Maize	Glyphosate	Pioneer	Glyphosate acetyltransferase
Bentgrass	Glufosinate	HybriGene, LLC	Phosphinothrichin acetyl transferase

[a]Deregulation approved

Many types of HRCs have gotten to the point of being field-tested, but few have proceeded to be deregulated (approved for commercial use). Table 3.6 lists products for which deregulation has been applied, as well as recently approved applications for field-testing in the USA. This information on the USDA/APHIS website provides the best indication of what new products are in the offing. Considering that many of these crops have glyphosate resistance, we consider these products separately.

3.3.2 Glyphosate-Resistant Crops

Considering the enormous success of GR crops and the fact that glyphosate is now a generic herbicide, other companies have discovered or created new glyphosate-resistant transgenes and, in at least one case, are going forward with the commercialization process. This crop uses an artificially evolved glyphosate-resistant gene (Castle et al. 2004; Siehl et al. 2005). A gene from the soil bacterium *Bacillus licheniformis*, which encoded a weak glyphosate *N*-acetyltransferase (GAT) was put through 11 iterations of gene shuffling to increase its activity by almost four orders of magnitude. Properties of the resultant GAT are described by Siehl et al. (2005, 2007). Plants made resistant to glyphosate with this transgene were

ca. 100-fold more resistant to glyphosate than to nontransgenic lines (Green et al. 2008). Other glyphosate-inactivating enzymes are apparently encoded by genes of soil microbes, because there are other routes of degradation or inactivation. For example, a C-P lyase that converts glyphosate to inorganic phosphate and sarcosine is found in several bacteria, including *Arthrobacter* spp., *Rhizobium* spp., and *Pseudomonas* spp. (Kishore and Jacob 1987; Liu et al. 1991; Dick and Quinn 1995). Not all bacterial C-P lyases will degrade glyphosate (White and Metcalf 2004). A good C-P lyase transgene for use in GR crops has apparently not yet been developed. A microbial transgene-encoded EPSPS with some properties that might be superior to that used in commercialized GR crops is available (Vande Berg et al. 2008). Thus, transgenes for glyphosate resistance are available for companies that do not have GR crops.

3.3.3 Other Herbicide-Resistant Crops

Crops with both the *GAT* gene and a gene for resistance to acetolactate synthase inhibitors are in the final stage of development (Green et al. 2008), including testing for food safety (Table 3.6) (McNaughton et al. 2007). Monsanto is developing a dicamba (3,6-dichloro-2-methoxybenzoic acid) resistance trait (Table 3.6) that detoxifies this herbicide by demethylating it (Herman et al. 2005; Behrens et al. 2007). Dow Agroscience has developed HRCs with resistance to broadleaf-killing auxinic herbicides like 2,4-D [(2,4-dichlorophenoxy)acetic acid] and grass-killing aryloxyphenoxypropionate herbicides such as diclofop ((±)-2-[4-(2,4-dichlorophenoxy) phenoxy]propanoic acid). These crops are apparently being field-tested, but there is no specific information on the APHIS website as to what genes are being used. Thus, we have left them out of Table 3.6. A recent abstract mentions that a transgene encoding an α-ketoglutarate-dependent dioxygenase is used to confer resistance to these two herbicide classes (Simpson et al. 2008). A patent had been filed for such a unique bacterial (*Ralstonia eutropha*)-derived transgene by Dow Agroscience in 2005 (Wright et al. 2005).

3.4 The Future of HRCs

The future of HRCs will be influenced by the development of other approaches to weed management. Availability of acceptable new technologies that are economically competitive with HRCs may eventually stem their rapidly increasing adoption. There are other strategies for using transgenes for weed management (Gressel 2002; Duke 2003, 2006) including enhancing crop competitiveness and allelopathy, and enhancement of weed biocontrol agents. There is little likelihood of these approaches having a significant effect in the next decade, but ultimately they could have substantive impacts.

To some extent, regulatory requirements regarding environmental impacts of weed management methods will affect this scenario. The increasing costs of meeting the requirements for deregulation of transgenic crops and the conflicting regulatory processes in different parts of the world have hampered the adoption of HRCs. Eventual harmonization of this process could benefit this technology.

There are many other factors that could have an impact on the future of HRCs, such as the amount of biofuel crops that will be grown (Baylis 2008). Since these crops are not meant for human consumption, and, in some cases, could not be used for human or animal consumption, the regulatory requirements for deregulation of HRC biofuel crops may be simplified and less costly to fulfill. Indeed, GR sugarcane is being developed in Brazil (http://monsanto.mediaroom.com/index.php?s = 43&item = 656). Still, the issue of herbicide resistance management and gene flow will be as important for these crops as for those meant for human and livestock consumption.

Agriculture has clearly found HRCs to be of great value, but their utility is being eroded by the evolution of GR weeds and weed species shifts in GRCs. Both of these processes are the result of over reliance on this highly effective technology. Some of the new HRCs that are to be introduced will help cope with this situation, but we see overreliance on glyphosate continuing to undermine its effectiveness. The unique properties of glyphosate as a herbicide (Duke et al. 2003a; Duke and Powles 2008b), almost necessitate that its utility be preserved for future harvests (Duke and Powles 2008a; Powles 2008a). It will be shame to see the evolution of GR weeds continue unabated, as the strategies for preventing it are well known.

Finally, the future of HRCs has been and may continue to be affected by public opinion. For example, transgenic crops have made meager inroads in Europe for this reason. Even though Europeans grow almost no transgenic crops, they buy substantial amounts of transgenic crops grown elsewhere for animal feed. At the time of this writing, this situation does not appear to be changing significantly. In countries such as Australia and those of South America, resistance to transgenic crops has waned considerably and adoption is increasing rapidly. We expect available HRCs to be almost universal outside of Europe and perhaps a few small areas of resistance within a few more years.

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