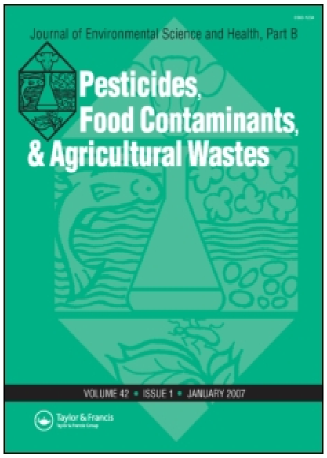

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Review of potential environmental impacts of transgenic glyphosate-resistant soybean in Brazil

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Transgenic glyphosate-resistant soybeans (GRS) have been commercialized and grown extensively in the Western Hemisphere, including Brazil. Worldwide, several studies have shown that previous and potential effects of glyphosate on contamination of soil, water, and air are minimal, compared to those caused by the herbicides that they replace when GRS are adopted. In the USA and Argentina, the advent of glyphosate-resistant soybeans resulted in a significant shift to reduced- and no-tillage practices, thereby significantly reducing environmental degradation by agriculture. Similar shifts in tillage practiced with GRS might be expected in Brazil. Transgenes encoding glyphosate resistance in soybeans are highly unlikely to be a risk to wild plant species in Brazil. Soybean is almost completely self-pollinated and is a non-native species in Brazil, without wild relatives, making introgression of transgenes from GRS virtually impossible. Probably the highest agricultural risk in adopting GRS in Brazil is related to weed resistance. Weed species in GRS fields have shifted in Brazil to those that can more successfully withstand glyphosate or to those that avoid the time of its application. These include *Chamaesyce hirta* (erva-de-Santa-Luzia), *Commelina benghalensis* (trapoeraba), *Spermacoce latifolia* (erva-quente), *Richardia brasiliensis* (poaia-branca), and *Ipomoea* spp. (corda-de-viola). Four weed species, *Conyza bonariensis, Conyza Canadensis* (buva), *Lolium multiflorum* (azevem), and *Euphorbia heterophylla* (amendoim bravo), have evolved resistance to glyphosate in GRS in Brazil and have great potential to become problems.

Keywords: GMO; environment; glyphosate; transgenic crops; Brazil.

Introduction

Glyphosate [*N*-(phosphonomethyl)glycine]-resistant crops (GRC) are the transgenic crops most extensively grown worldwide, with soybean being the major GRC.[1] Soybean was introduced in Brazil in early 1900s, but its commercial importance dates to the 1940s in Rio Grande do Sul State. Soybean varieties introduced from the USA and varieties from early introductions in Brazil were part of the Brazilian soybean-breeding program which spread the crop from high to low latitudes, allowing production in tropical acidic soils with lime and phosphorus supplements.[2] In the year of 2004/2005, Brazil was the second largest world soybean producer with 50 million metric tons, about 25% of world production[3] with a worldwide total area of 22,895,300 ha.[4]

The topic of herbicide-resistant crops has been extensively reviewed[5–13] and has been the topic of one edited book.[14] Dill[15] briefly covered the current status of GRC products. None of these publications have focused solely on an assessment of the potential environmental impacts of GRCs in tropical areas.

After years of debate, transgenic soybean (*Glycine max*), is now legally grown in Brazil. Glyphosate-resistance is the only available transgenic herbicide resistance trait used in Brazil. Glyphosate-resistant soybeans (GRS) and their environmental impact have been covered in depth in a review on GRC[16] but this review had little comment on tropical areas such as in Brazil.

The purpose of this review is to discuss the potential environmental impacts of GRS in Brazil, with emphasis on the effects of this relatively new technology as a weed control method. Since human toxicology data is not as geography dependent, it will not be discussed in detail. The viewpoints in this analysis are those of the authors and are not meant

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to reflect those of our employers. Where there are few data from Brazil, comparisons have been made with data from other geographic areas.

Some data from temperate areas regarding the behavior of pesticides can be extrapolated to tropical soils. Comparing the fate of pesticides on tropical and temperate conditions, Racke et al.[17] found no evidence of unique behavior of the pesticides in the tropics. However, they concluded a greater rate of pesticide degradation generally occurs under tropical conditions. The authors also concluded that since soil microbial activities are strongly modulated by temperature, pesticide degradation would be expected to be greater in tropical soils, which experience higher year-round temperatures, than in temperate soils. This explanation would be consistent with observations of the elevated rates of soil organic matter turnover that characterize udic and ustic (rainy season) tropical environments. A study and review on glyphosate effects in a tropical environment in Colombia found no evidence of a unique behavior of glyphosate in the tropics.[18,19] A proposed 5- year study is being started in Brazil to supply information to the Biosafety Committee of the Ministry of Science and Technology (CTNBio) involving eight ecological regions in the states of Mato Grosso, Mato Grosso do Sul, Goiás, Baia, Paraná, and Rio Grande do Sul.[20] The purpose of this study is to determine effects on physical, chemical and biological attributes of soil where GRS is being sown. There are no data available from this study yet.

Glyphosate-resistant soybeans in Brazil

Glyphosate is a very effective, non-selective, post-emergence herbicide. Prior to introduction of GRS, it was used in non-crop situations and in crop situations before planting, or with specialized application equipment to avoid contact with the crop.[21,22] Glyphosate inhibits the shikimate pathway by inhibiting 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). This results in reduced aromatic amino acids and deregulation of the shikimate pathway causing massive flow of carbon into the pathway, with accumulation of high levels of shikimic acid and its derivatives (reviewed by Duke et al.[22]). Glyphosate 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.

A gene (*CP4*) encoding a glyphosate-resistant form of EPSPS from *Agrobacterium* sp. was found to effectively bypass the effect inhibiting the native enzyme, producing a GRC.[15,23] Most commercial GRS varieties have the CP4 EPSPS gene.[24] This is the transgene in commercially available GRS in Brazil.

Adoption of GRS has been rapid and substantial in the United States and Argentina. Its use in Brazil, where it was only recently approved, has increased rapidly. In Brazil, the acreage planted in the 2004/2005 growing year was estimated to be 56% of total soybean area.[25] In Argentina, the adoption of GRS was rapid, reaching almost 90% within 4 years of introduction.[26] In 2006, 89% of the soybeans planted in the USA were GRS, where rapid adoption has been due to relatively inexpensive, excellent, simplified, and more flexible weed control.[27,28]

Glyphosate-resistant soybean responses to glyphosate

Higher temperatures, light intensity and water stress can decrease the resistance of some GRS varieties to glyphosate[29,30] (Table 1). However, no adverse effects of glyphosate on GRS have been reported in Brazil. Temperatures during the crop season are not very different than those in the United States, and generally there is no water deficit in Brazil during the soybean cropping season, although it can happen occasionally.

Glyphosate is preferentially translocated from source to sink tissues, such as reproductive tissues and nodules of soybeans.[22,31] Nodules are the site of the nitrogen-fixing symbiont *Bradyrhizobium japonicum*, which possesses a

Table 1. Glyphosate-resistant (GR) soybean effects and interactions (Adapted from Pline-Srnic. [24])

Vegetative and reproductive resistance	Chlorosis with higher off-label rates at high temperatures
	Biomass reduction with higher off-label rates
Interactions with plant pathogens and pests	Increased incidence of *F solani* infection in treated GR soybean
Interactions with symbiotic micro-organisms	Early glyphosate applications temporarily delayed nitrogen fixation, decreased biomass
	Nodule number similar in GR and non-GR, but nodule dry mass reduced in non-GR
	No glyphosate effect on nodule number, but reduction in nodule biomass and leghemoglobin
	Glyphosate reduced nodule number and yield in treated vs. non-treated GRS
Interactions with environmental factors	GR cultivars more susceptible to high temperatures than non-GR (reduced biomass, chlorophyll, stem splitting)
	Chlorosis with off-label rates at high temperatures
	Glyphosate × water stress reduced biomass yields compared to glyphosate × no water stress

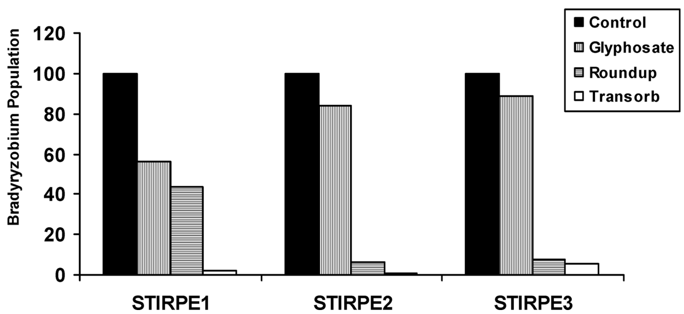

Fig. 1. *In vitro* effects of glyphosate and two commercial formulations of glyphosate (Roundup® and Transorb®) (43.2 μg/L a.e.of glyphosate in each treatment) on three Brazilian *Bradyrhyzobium stirpes* isolates (Drawn from Dos Santos et al.[34])

glyphosate-sensitive EPSPS.[32] Thus, there are potential effects on nitrogen metabolism in GRS. A study has shown that these effects were transient, and crop yield was unaffected.[33] Laboratory studies made in Brazil by Dos Santos et al.[34,35] have shown a direct effect of glyphosate formulations on *Bradyrhizobium stirpes* (Fig. 1). Glyphosate plus the surfactant seems to affect the *Bradyrhizobium stirpes* much more than glyphosate alone. However, there is no evidence that any of the formulation ingredients other than glyphosate would be translocated to the nodules of soybeans in the field. Another recent study conducted in Brazil has shown no effect of glyphosate on soybean nodulation and mycorrhyzae colonization.[36] Overall, there are no indications that an effect of glyphosate on *Bradyrhizobium stirpes* could have any impact on soybean yield in the field.

Effects on Brazilian soils and on herbicide use

In general, most Brazilian soils have characteristics of tropical and humid subtropical climate regions, including high acidity and high exchangeable aluminum content, sometimes associated with low fertility. These constraints to agricultural production have been overcome in part by liming and phosphate fertilizer application.[37] Glyphosate is rapidly adsorbed and tightly complexed by most soils and is rapidly degraded by soil microbes.[21,22,38] Brazilian soil contains microbes that degrade glyphosate.[39] The pH 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. One would expect a lower mobility in Brazilian soils because of the lower pH of those soils.[37] Inactivation of glyphosate through adsorption is of critical importance. Leaching is nearly negligible, and glyphosate is not volatile.[40] A study conducted with Brazilian soils has shown similar behavior.[41]

There are no specific data from Brazil, but there has been controversy about whether GRS increase herbicide use or not. Some studies have claimed that the volume of herbicide use is greater with GRS.[42] Others studies have concluded that no significant change in the overall amount of herbicide has been observed with the adoption of GRS, but GRS use leads to the replacement of some herbicides that are more toxic, and that persist nearly twice as long as glyphosate.[43] Thus, the effects of herbicide use may be more environmentally friendly with GRS adoption. Overall, GRS use is expected to have less adverse effects in Brazil than the herbicides that it replaces (Table 2). Other herbicides used such as chlorimuron, lactofen and haloxyfop were not mentioned on this list.[3] In Argentina, where GRS has been used for a longer period, an overall cost savings of US$ 30/ha and a reduction of 41.4 million of kg of pesticide use has been obtained, as well as a desirable effect on carbon sequestration.[44]

In conclusion, glyphosate would replace herbicides used to control the most common weeds in Brazil (Table 3) that are generally more toxic (Table 2). These herbicides have higher persistence in the environment and more potential to leach into ground water than glyphosate (Table 2).

Table 2. Some herbicides used in soybean in Paraná state in Brazil (Adapted from Inoue et al.[45])

Herbicide	*%*[1]	K_{oc}^{2}	$T_{1/2}(Days)^{3}$	*GUS*[4]	$LD_{50}(mg/kg)^{5}$
Glyphosate	29.85	24,000	47	NL	5600
2,4-D[7]	10.85	60	10	IN	370
Trifluralin	3.6	7,000	45	NL	5000
Metolachlor	2.54	200	195	L	2000
Paraquat[7]	1.54	1,000,000	1,000	NL	50
Alachlor	1.29	103	80	L	1000
Bentazon	0.82	34	20	L	400
Imazaquin	0.78	20	60	L	5000
Cloransulam-metil	0.58	454	9	L[6]	2000[6]
Fomesafen	0.57	60	100	L	2000[6]
Clethodin	0.56	16	3	NL	1360
Pendimethalin	0.55	17,200	44	NL	1050

% [1]of total herbicide of total crops, [2]Adsorption coefficient (mg/g^{-1}), [3]Half-life, [4] Ground-water Ubiquity Score, NL=Not leach, IN=Intermediate, L=Leaches easily, [5]Lethal dose data from Extoxnet,[46] [6]Data from Fluorideaction.org,[47] [7]Used as desiccant in No-till.

Table 3. Principal weeds in soybean in Brazil.[3]

Weeds	*Common name*[1]
Acanthospermum australe	Carrapicho rasteiro
Acanthospermum hispidum	Carrapicho de carneiro
Amaranthus hybridus	Caruru
Amaranthus viridis	Caruru de mancha
Bidens pilosa	Picão preto
Brachiaria plantaginea	Capim marmelada
Cenchrus echinatus	Capim carrapicho
Commelina benghalensis	Trapoeraba
Cyperus rotundus	Tiririca
Desmodium tortuosum	Carrapicho beiço de boi
Digitalis horizontalis	Capim colchão
Echinochloa crusgalli	Capim arroz
Eleusine indica	Capim pé de galinha
Emilia sonchifolia	Falsa serralha
Euphorbia heterophylla	Amendoim bravo
Galinsoga parviflora	Picão branco
Ipomoea grandifolia	Corda de viola
Parthenium hysterophorus	Losna branca
Portulaca oleracea	Beldroega
Raphanus raphanistrum	Nabiça
Richardia brasiliensis	Poaia branca
Senna obtusifolia	Fedegoso
Sida rhombifolia	Guanxuma
Solanum americanum	Maria pretinha
Sorghum halepense	Capim massambará
Spermacoce latifolia	Erva quente
Tagetes minuta	Cravo de defunto

[1]Portuguese (Brazilian) common names.

Soil erosion and no-tillage

A beneficial impact from the use of GRS in Brazil is that they facilitate reduced or zero tillage systems, which contributes to reductions in soil erosion from water and wind, fossil fuel use, air pollution from dust, soil moisture loss, and soil compaction.[48] Reduced tillage also improves soil structure, leading to reduced risk of runoff and pollution of surface waters with sediment, nutrients, and pesticides. Loss of top soil due to tillage is perhaps the most environmentally destructive effect of row crop agriculture. However, the selection for natural or evolved glyphosate resistant weeds in GRS could make the farmers use more plowing for weed control purposes. A survey by the American Soybean Association found that there was a dramatic increase in the adoption of no-tillage and reduced tillage management in the United States and that most of this change was associated with the growing of GRS.[49] Similarly, there has been a rise in no-tillage agriculture in soybeans in Argentina with the adoption of GRS.[26] Dramatic reductions in soil erosion were documented where no-tillage, GRS were grown in Argentina, and we believe this may happen with the GRS adoption in Brazil also. With the adoption of no-tillage systems in Brazil, an acceleration of glyphosate mineralization is expected according to a study conducted by Prata et al.[50] in a Rhodic Oxisol soil, in fields under no-till and conventional management systems in Ponta Grossa, Paraná state, in soybean production for 23 years. They found reduced glyphosate persistence under no-tillage systems in Brazil. However, no-tillage and reduced tillage agriculture with glyphosate will result in strong selection pressure for weed species shifts and evolution of glyphosate resistance (see below). Some of these new problem weeds might be best managed with tillage, resulting in a permanent or occasional return to tillage.

Effects on soil biota and microorganisms

The potential direct effects of GRS and their management on soil biota include changes in soil microbial activity due to direct effects of glyphosate, differences due to the amount and composition of root exudates of GRS versus non-GRS, changes in microbial functions resulting from gene transfer from the transgenic crop, and the effects of management practices for GRS, such as changes in other herbicide applications and tillage (reviewed in part by Dunfield and Germida[51]). Most of the available literature addresses direct effects of glyphosate.

Glyphosate can be toxic to many microorganisms, including plant pathogens found in soybean in Brazil, but not all fungi are susceptible to glyphosate.[52,53] Glyphosate has a half-life in soils with an average value of approximately 47 days, but reaching 174 days in some soils under some environmental conditions.[54,55] Studies conducted in Brazil have shown a half-life of about a month, which is shorter than in some temperate climates.[56] A study of the effect of glyphosate on microbial activity in typical Hapludult and Hapludox soils in Brazil measured by soil respiration (evolution of CO_2) and fluorescein diacetate (FDA) hydrolysis revealed a transient increase of 10-15% in the CO_2 evolved and a 9–19% increase in FDA hydrolysis in the presence of glyphosate.[57] This indicated glyphosate degradation by microorganisms in Brazilian soils and some transformation to AMPA (aminomethyl phosphonic acid), as shown on Figure 2. Their results have shown that after 32 days of incubation the number of actinomycetes and fungi had increased while the number of bacteria was reduced slightly.

In general, there is little or no effect of glyphosate on soil microflora within weeks or months of application. For example, Gomez and Sagardoy[58] found no effect of glyphosate on microflora of soils in Argentina at twice the recommended rates of the herbicide and detected AMPA, indicating glyphosate degradation by soil microorganisms. Motavalli et al.[59] and Kowalchuk et al.[60] concluded that there is no conclusive evidence that those GRS and other transgenic crops which have been used in many cropping situations in many climates and soil types over the past 10 years have had any significant effect on nutrient transformations by microbes. So far, no agriculturally significant

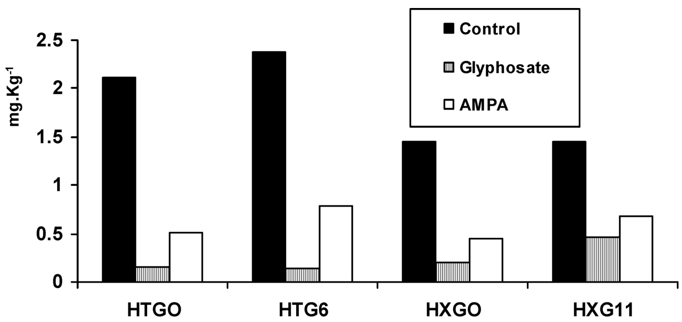

Fig. 2. Amounts (mg/kg) of glyphosate and aminomethyl phosphonic acid (AMPA) detected in different types of soil in Brazil before (control) and after incubation with glyphosate for 32 days. Typical Hapludult (HT) Brazilian soil with no reported application of glyphosate (HTG0) six years application of glyphosate (HTG6). Typical Hapludox (HX) Brazilian soil without reported application of glyphosate (HXG0) and with 11 years of application of glyphosate (HXG11) from a no-tillage soybean (not GRS) field. (Drawn from Araujo et al.[57])

effect of glyphosate on soil microorganisms has been documented worldwide.

In Brazil, Andréa et al.[61] found that earthworms (*Eisenia foetida*) did not influence the soil dissipation of glyphosate. Earthworms bioaccumulated glyphosate residues, and soil microbial activity was not altered, by either the earthworms or the treatments or time after treatments. Tuffi-Santos et al.[62] studying the root exudation of glyphosate by *Brachiaria decumbens*, its effects on eucalyptus, and microbial respiration in soil in Brazil, found no toxicity symptoms in eucalyptus and the microbial activity was greatest with the highest glyphosate rates applied to the *B. decumbens.* In a other study, Tuffi-Santos et al.[63] found an effect of glyphosate on eucalyptus only due to direct drift of sprayed glyphosate.

Entomopathogenic fungi present in soil are important to promote biological control. Among those microorganisms, *Metarhizium anisopliae* is important in Brazil for insect biocontrol, and no effect of glyphosate was found on it.[64] However, Andaló et al.[65] found glyphosate to reduce *in vitro* vegetative growth of the entomopathogenic fungus *Beauveria bassiana* found in Brazil.

Overall, no significant effect of GRS use on soil is reported in Brazil, other than that caused by adoption of reduced- or no-tillage agriculture or by switching from more environmentally toxic herbicides.

Water contamination and effects on aquatic life

Glyphosate is strongly adsorbed to soil particles, and, even though it is highly water soluble, it does not leach to 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.[39] Two extensive reviews about the topic have indicated a relatively low risk of ground and surface water contamination.[9,16]

Inoue et al.[45] ranking herbicides according to their leaching potential in Brazil, showed that acifluorfen-sodium, alachlor, atrazine, chlorimuron-ethyl, fomesafen, hexazinone, imazamox, imazapyr, imazaquin, imazethapyr, metolachlor, metribuzin, metsulfuron-methyl, nicosulfuron, picloram, sulfentrazone and tebuthiuron are potential leachers according to three theoretical criteria adopted. Although glyphosate represented 30% of the total herbicide use in Paraná State of Brazil (Table 2), it was not even listed in the ranking because it was virtually not leachable[45].

Glyphosate has little effect on aquatic life.[16] However, Relyea[66] reported that a commercial formulation of glyphosate sprayed directly into aquatic mesocosms caused a reduction in species diversity with particularly severe impacts on amphibians. The control mesocosms were not sprayed with a formulation blank. This effect may have been due to the surfactant. Glyphosate is even approved for use in aquatic weed control with the proper surfactant in the United States.

Effects on other non-target organisms

Birds, wildlife and arthropods

Comprehensive reviewshave concluded that no significant direct effect of GRC would be expected on birds and wildlife.[9,16] However, indirect effects of glyphosate in GRS could have effects on insects and wildlife. For example, no-tillage agriculture with GRS could result in weed species shifts and more vegetation in the field before and after the period of crop production, with an altered habitat for such organisms.

Glyphosate has not been reported to have insecticidal or other activities against arthropods. However, any herbicide can indirectly affect arthropod populations and species compositions in an area by its effects on vegetation. Changes in cropping systems (e.g., changing from tillage to no-tillage) can drastically influence arthropod populations. Virtually all studies on direct effects of glyphosate on arthropods show no significant effects in Brazil and in the rest of the world.[9,16,18–20,58,67]

There could be indirect effects through effects on host weeds and on insect pathogens. Commercially formulated glyphosate is toxic to the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae, Nomuraea rileyi*, and *Neozygites floridana*.[68]

Non-target plants

Drift of herbicides to non-target plants has been a problem since synthetic herbicides were introduced. Drift to non-transgenic crops of the same species is a new problem with GRS. At low doses that one might expect with drift, glyphosate can stimulate growth.[69]

Table 4. Principal fungi diseases in soybean in Brazil.[3]

Leaves	*Pod, Steam, Seed*	*Roots*
Alternaria sp	*Cercospora kikuchii*	*Corynespora cassiicola*
Ascochyta sojae	*Colletotrichum dematium*	*Cylindrocladium clavatum*
Cercospora kikuchii	*Diaporthe phaseolorum*	*Fusarium solani*
Cercospora sojina	*Fusarium* sp	*Macrophomina phaseolina*
Corynespora cassiicola	*Meridionalis* sp	*Phialophora gregata*
Microsphaera diffuse	*Nematospora corily*	*Phytophthora megasperma*
Myrothecium roridum	*Phomopsis phaseofi*	*Rhizoctonia solani*
Peronospora manshurica	*Phomopsis* sp.	*Rosellinia* sp
Phakopsora meibomiae	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*
*Phakopsora pachyrhizi**		
Phyllosticta sojicola		
Rhizoctonia solani		
Septoria glycines		
Thanatephorus cucumeris		

*Asian soybean rust.

Plant pathogens

The main soybean pathogens in Brazil are shown in Tables 4 and 5.

The influences of glyphosate on plant diseases in GRS are variable, sometimes reducing and other times increasing disease (Table 6).

Glyphosate inhibits the biosynthesis of the aromatic amino acids, thereby reducing biosynthesis of proteins, auxins, pathogen defense compounds, phytoalexins, folic acid, precursors of lignins, flavonoids, plastoquinone and hundreds of other phenolic and alkaloid compounds.[22] These effects could, in theory, increase the susceptibility of glyphosate-sensitive plants to pathogens or other stresses.[22,24,80–82] Glyphosate causes lowered soybean phytoalexin levels and increased susceptibility to plant pathogens (Table 7). Low doses of glyphosate can sometimes make pathogen-resistant cultivars susceptible to plant disease.[83] Glyphosate was even patented as a synergist for a plant pathogen that controls weeds.[84]

Theoretically, there should be no effect of glyphosate on shikimate pathway-dependent disease resistance in GRS in Brazil or elsewhere, as the shikimic pathway is not blocked by the herbicide in these transgenic crops. However, as discussed in detail by Gressel[11] incomplete expression of the gene for the glyphosate-resistant EPSPS in certain tissues or under some environmental conditions could reduce shikimic acid pathway-mediated disease resistance mechanisms (*e.g.*, lignification, phytoalexins). Moreover, the sometimes fungicidal activity of glyphosate (Table 6) might prove beneficial to GRC. However, reports of both enhanced and reduced disease severity have been reported in GRC.[87]

The possible interactions of glyphosate with diseases found in soybean in Brazil are covered in detail according to the disease:

Asian soybean rust (*Phakopsora pachyrhi*). Recently, glyphosate was reported to have both preventative and curative properties on rust diseases in both glyphosate-resistant wheat and soybean.[70,71] Before the Asian soybean rust outbreak, about 80% of the area in Brazil was sprayed with fungicides at the end of the growing season mainly for control of diseases such as *Cercospora* spp, *Septoria glycines* and *Microsphaera diffuse* with fungicides of the inexpensive benzimidazoles group. With the Asian soybean rust outbreak, it is now necessary to use mixtures of triazole and strobirulin fungicide classes, with more frequent spraying. Before the Asian soybean rust, normally only one

Table 5. Principal bacterial, viral and nematode diseases in soybean in Brazil.[3]

Bacteria	*Virus*	*Nematodes*
Pseudomonas savastanoi	Vírus do mosaico comum da soja[1] (VMCS)	*Heterodera glycines*
Pseudomonas syringae	Vírus do mosaico amarelo do feijoeiro[1] (VMAF)	*Meloidogyne arenaria*
Xanthomonas axonopodis	Vírus da necrose branca do fumo[1](VNBF)	*Meloidogyne incognita*
	Vírus do mosaico da alfafa[1] (MVA)	*Meloidogyne javanica*
	Vírus da Necrose da haste da soja[1]	

[1]Portuguese common name.

Table 6. Reports of glyphosate interactions with Brazilian soybean diseases and nematodes

Disease	*Effect*	*References*
*Phakopsora pachyrhizi**	Reduces	Feng et al.[70]
		Anderson & Kolmer[71]
Fusarium spp.	Increases	Kremer et al.[72]
	No effect	Mendes et al.[73]
S. schlerotiorum	No effect	Lee et al.[74]
	Increases	Nelson et al.[75]
F. solani	Increases	Sanogo et al.[76]
		Njiiti et al.[77]
Rhizoctonia	Reduces	Black et al.[78]
Heterodera glycines	No effect	Yang et al.[79]

*Asian soybean rust

spraying was needed per growing season, but after soybean rust arrived, up to seven sprayings were required, making soybean production in Brazil much more expensive.[3]

Cercospora sp, Septoria glycines and Microsphaera diffuse. Except for the soybean Asian rust, these are the most important fungal diseases of soybean in Brazil.[3] No direct effect of glyphosate was found in the literature on these pathogens. Neither glyphosate nor its principle metabolic degradation product, AMPA, were fungitoxic to *Botrytis cinera, Colletotrichum acutatum, C. fragariae, C. gloeosporiodes, Fusarium oxysporum, Phomopsis obscurans*, and *P. vitcola* at concentrations up to 1 mM in an *in vitro* microtitre plate bioassay.[87]

Fusarium spp. and Pythium spp Kremer et al.[72,88] reported that GRS stimulated growth of selected rhizosphere fungi (*Fusarium* spp. and *Pseudomonas* spp.). These results would predict that there could be more problems in GRS than non-transgenic soybeans. 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.[89] Overall, the response of GRS 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.[76] Njiiti et al.[77] had similar results with *F. solani*-caused SDS in soybeans as influenced by glyphosate and the glyphosate resistance trait. Studies conducted in Brazil also did not detect an effect of glyphosate on *Fusarium* sp.[73] In a laboratory study, growth of *Pythium ultimum* Trow and *F. solani* could be stimulated or inhibited, depending on the glyphosate concentration.[90] 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.[91]

Table 7. Correlation of effects of glyphosate on reduced phytoalexin levels and increased susceptibility to a plant pathogen in soybean.

Phytoalexin	*Pathogen*	*Reference*
Glyceollin	*Phytophthora megasperma*	Ward[85]
	Pseudomonas syringae	Holliday & Keen[86]

Rhizoctonia spp. Smiley et al.[92] found that the incidence of *Rhizoctonia* spp. 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.

Sclerotinia spp. Nelson et al.[75] had mixed results with different GRS cultivars and application of different herbicides to these cultivars with respect to susceptibility to *S. sclerotiorum*-caused stem rot (Table 6). Farmers in Michigan have reported increased susceptibility of GR soybean to *S. sclerotiorum*.[74]

Nematodes. Field observations in Ohio suggested a possible interaction between soybean cyst nematode (SCN), *Heterodera glycines*, and glyphosate in a transgenic GR variety that also expresses SCN resistance derived from the 'PI88788' soybean line.[79] But, there was no conclusive interaction of glyphosate and SCN in GR soybean (Table 6). Four species of nematodes are common in soybean in Brazil (Table 5), and none of them seems to be affected by glyphosate.[3]

Glyphosate-resistant weeds and volunteer crops

The following weeds, with their Brazilian common names, are difficult to control with glyphosate, due to their natural resistance: *Chamaesyce hirta* (erva de Santa Luzia), *Commelina benghalensis* (trapoeraba), *Spermacoce latifolia* (erva quente), *Richardia brasiliensis* (poaia branca), and *Ipomoea* spp. (corda de viola), among others.[3,93,94] (Table 8). One would expect an increase of these weed species in GRS in Brazil. Vidal et al.[95] suggested measurement of shikimic acid accumulation in these resistant plants in response to glyphosate as a quick method to determine resistance.

Volunteer crops are those left over from the previous season that grow and compete with a subsequently planted crop such as GRS growing in glyphosate-resistant maize, which is not yet allowed in Brazil. Glyphosate is not yet allowed in Brazil. The popular practice of "safrinha," which is growing conventional maize or bean just after soybean in the same crop season without tillage, is also affected by using GRS because the farmers rely on glyphosate as a preplant desiccant, which does not work with volunteer GRS.[20] The other option, 2,4-D, is not legal in some areas of Brazil.[20] GRS have greater potential to become problems as volunteer crops than do conventional crops, but no other GRC than soybean is grown in Brazil, thus reducing the potential problem. The reduction in tillage

Table 8. Glyphosate-resistant or hard-to-control weeds and common names in soybean in Brazil.[93]

Weeds	*Common name*[1]
Chamaesyce hirt	Erva de santa luzia
Chloris polydacyla	Capim branco
Commelina benghalensis	Trapoeraba
*Conyza bonariensis**	Buva
*Conyza canadensis**	Buva
*Euphorbia heterophylla**	Amendoim bravo
Ipomoea spp.	Corda de viola
*Lolium multiflorum**	Azevem
Richardia brasiliensis	Poaia branca
Spermacoce latifolia	Erva quente
Synedrellopsis grisebachii	Agriãozinho
Tridax procumbens	Erva de touro

*Evolved resistance. [1]Portuguese (Brazilian) common names.

with GRS also could exacerbate certain weed problems, especially perennial weeds with some natural resistance to glyphosate.[93]

Eleven weed species have evolved resistance to glyphosate in the world (Table 9), and biotypes of eight of these evolved resistance in GRS.[96] In Brazil four species have evolved resistance in GRS, they are, *Euphorbia heterophylla* (amendoim bravo), *Conyza bonariensis and C. canadensis* (buva) and *Lolium multiflorum* (azevem).[93,97–103] *Lolium multiflorum*, was introduced as forage and cover crop in a no-till system, but became a serious weed in wheat and other winter cereals in Southern Brazil with a biotype resistant to glyphosate and probably will be a weed problem in GRS in Brazil[104,105] (Table 8).

Glyphosate-resistant *Sorghum halepense* was detected in neighboring Argentina[96] and may become a problem to GRS in Brazil, since the species is found throughout the country. Perhaps weed management and control is the most important potential problem with GRS in Brazil. Gazziero[20] has indicated that the majority of the area of soybean in Brazil is very different from that of the USA or Argentina. He also suggested the following actions to minimize the problem: a) rotate GRS with conventional soybeans in order to rotate herbicide modes of action, b) always follow the label recommendation and avoid lower than recommended rates c) keep the soil covered with a crop or legume at intercrop intervals, and c) use a preplant non-selective herbicide (glyphosate or paraquat) to eliminate early weed interference with the crop and to minimize escapes from later applications of glyphosate due to natural resistance of older weeds and/or incomplete coverage with the post-emergence application(s) of glyphosate. Unfortunately, there is a tendency of farmers in Brazil to increase herbicide rates to overcome weed resistance. This might exacerbate the problems of glyphosate off-rates mentioned in Table 1.[93]

Table 9. Weeds with evolved resistance to glyphosate in the world.[96]

Weed	*Country*	*Year First Detected*
*Amaranthus palmeri**	USA	2005
*Amaranthus rudis**	USA	2005
*Ambrosia artemisiifolia**	USA	2004
*Conyza bonariensis**	S. Africa, Spain and Brazil	2003
*Conyza canadensis**	USA and Brazil	2000
Eleusine indica	Malaysia	1997
*Euphorbia heterophylla**	Brazil	2005
*Lolium multiflorum**	Chile, Brazil and USA	2001
Lolium rigidum	Australia, USA and S. Africa	1996
Plantago lanceolata	South Africa	2003
*Sorghum halepense**	Argentina	2005

*Some or all resistant biotypes evolved resistance in glyphosate-resistant soybeans (GRS).

Gene flow (introgression) effects

Glyphosate resistance transgenes in soybeans are highly unlikely to be a risk to wild plant species in Brazil. According to Riches and Valverde,[106] soybean is a non-native crop without wild relatives in Brazil, making introgression of transgenes into wild relatives impossible. Soybean is a predominantly self-pollinated plant species with an outcrossing rate of about 1%. Thus, a very low rate of gene flow to non-GR soybean varieties might be possible, but this has not been reported in any place where GRS are grown.

Conclusion

GRS is now grown in Brazil, with an area planted in the 2004/2005 crop year estimated to be 56% of the total soybean area. High temperatures, light intensity and water stress can decrease the resistance of GRS to glyphosate. Up to now, no yield effect of glyphosate or effects on soybean nodulation and mycorrhyza colonization have been confirmed in Brazil. Glyphosate with GRS would replace herbicides that are generally more toxic, with higher persistence in the environment and potential to leach into ground water. Leaching of glyphosate is nearly negligible and glyphosate is not volatile. GRS facilitates reduced or zero-tillage systems, which contribute to reductions in soil erosion, soil moisture losses, and soil compaction[107] and even greenhouse gas emissions.[108]

In general, there is little effect of glyphosate on soil microflora, birds and wildlife and arthropods. Drift to non-transgenic crops of the same species is a new problem with GRS, although effects of herbicides on non-target crops of a different species are not a new problem. Recently, glyphosate was reported to have both preventative and curative properties on GRS Asian rust diseases. The influence of glyphosate on plant diseases in GRC is variable, sometimes reducing and other times increasing disease. Glyphosate resistance transgenes in soybeans are highly unlikely to be a risk to wild plant populations in Brazil. Perhaps the most important problems with GRS in Brazil are glyphosate-resistant weeds and volunteer crops. *Conyza bonariensis and C. canadensis*; *Euphorbia heterophylla*, and *Lolium multiflorum* have evolved resistance to glyphosate in GRS in Brazil. Glyphosate-resistant *Sorghum halepense* is a potential problem. Other Brazilian weeds such as *Chamaesyce hirta*, *Commelina benghalensis*, *Spermacoce latifolia*, *Richardia brasiliensis*, and *Ipomoea* spp. are naturally resistant to glyphosate, and are thus likely to become problems in GRS in Brazil. A good weed management program can overcome these problems.

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