Review

Received: 30 April 2017 Revised: 5 October 2017 Accepted article published: 10 October 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.4760

Overview of glyphosate-resistant weeds worldwide

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Abstract

Glyphosate is the most widely used and successful herbicide discovered to date, but its utility is now threatened by the occurrence of several glyphosate-resistant weed species. Glyphosate resistance first appeared in *Lolium rigidum* in an apple orchard in Australia in 1996, ironically the year that the first glyphosate-resistant crop (soybean) was introduced in the USA. Thirty-eight weed species have now evolved resistance to glyphosate, distributed across 37 countries and in 34 different crops and six non-crop situations. Although glyphosate-resistant weeds have been identified in orchards, vineyards, plantations, cereals, fallow and non-crop situations, it is the glyphosate-resistant weeds in glyphosate-resistant crop systems that dominate the area infested and growing economic impact. Glyphosate-resistant weeds present the greatest threat to sustained weed control in major agronomic crops because this herbicide is used to control weeds with resistance to herbicides with other sites of action, and no new herbicide sites of action have been introduced for over 30 years. Industry has responded by developing herbicide resistance traits in major crops that allow existing herbicides to be used in a new way. However, over reliance on these traits will result in multiple-resistance in weeds. Weed control in major crops is at a precarious point, where we must maintain the utility of the herbicides we have until we can transition to new weed management technologies.

Keywords: glyphosate resistance; glyphosate-resistant crops; herbicide resistance; mode of action; multiple resistance; weeds

1 INTRODUCTION

Glyphosate is a non-selective, systemic, post-emergence herbicide that controls more weed species than any other herbicide.[1–3] Glyphosate disrupts the shikimate pathway, resulting in the arrest of aromatic acid production via inhibition of the chloroplast enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS).[4] When first introduced in 1974, glyphosate was relatively expensive, limiting its use to high-value horticultural crops (orchards) and some non-crop situations.[3] As its price declined, it was adopted in many other situations, including weed control in pre-seeding and fallow situations, which enabled growers to convert their operations to minimal and zero tillage.

Glyphosate-resistant soybean was introduced in 1996, the first of several glyphosate-resistant crops,[5] and allowed glyphosate to be used as a selective post-emergence herbicide. In the same year, *Lolium rigidum* was identified as the first case of evolved glyphosate resistance in a weed. This first case was unrelated to glyphosate-resistant crops, as *L. rigidum* had evolved resistance after 15 years of multiple glyphosate treatments per year in an apple orchard.[6] Since then, there has been a steady increase in weed species evolving resistance to glyphosate worldwide (Fig. 1), in large part due to the increase in glyphosate usage in glyphosate-resistant crops. The effectiveness of glyphosate in glyphosate-resistant crops, combined with its broad spectrum of control at a relatively low cost, led to a rapid adoption rate in soybean, corn, cotton, canola and sugarbeet.[5,7] The reduction in the cost of weed control due to the introduction of glyphosate-resistant crops had an adverse effect on the profitability of introducing new herbicides, resulting in a subsequent reduction in herbicide discovery programs (Fig. 2).[8] If it was not for glyphosate-resistant weeds, this would not be a major issue; however, the appearance of glyphosate-resistant weeds and the lack of new herbicide sites of action over a period of more than 30 years leaves agronomic crops increasingly vulnerable to resistant weed infestations.

2 COUNTRY OF OCCURRENCE

The International Survey of Herbicide-Resistant Weeds (http://www.weedscience.org)[9] records the occurrence of herbicide-resistant weeds globally, and is the source of most of the data presented here. Data in the survey are collected from weed scientists that have confirmed herbicide resistance through replicated trials that follow the survey requirements posted on the site (http://www.weedscience.com/Documents/ResistanceCriterion.pdf). Undoubtedly, the survey underreports the true number of herbicide-resistant weeds because many cases are not scientifically confirmed, or are not submitted to the site, particularly cases from less-developed countries. Thirty-eight weed species have evolved resistance to glyphosate, 18 monocots and 20 dicots (Fig. 1). About half of these have evolved resistance in glyphosate-resistant crop systems and the other half in orchards, plantations, cereals, fallow and non-crop situations. Although

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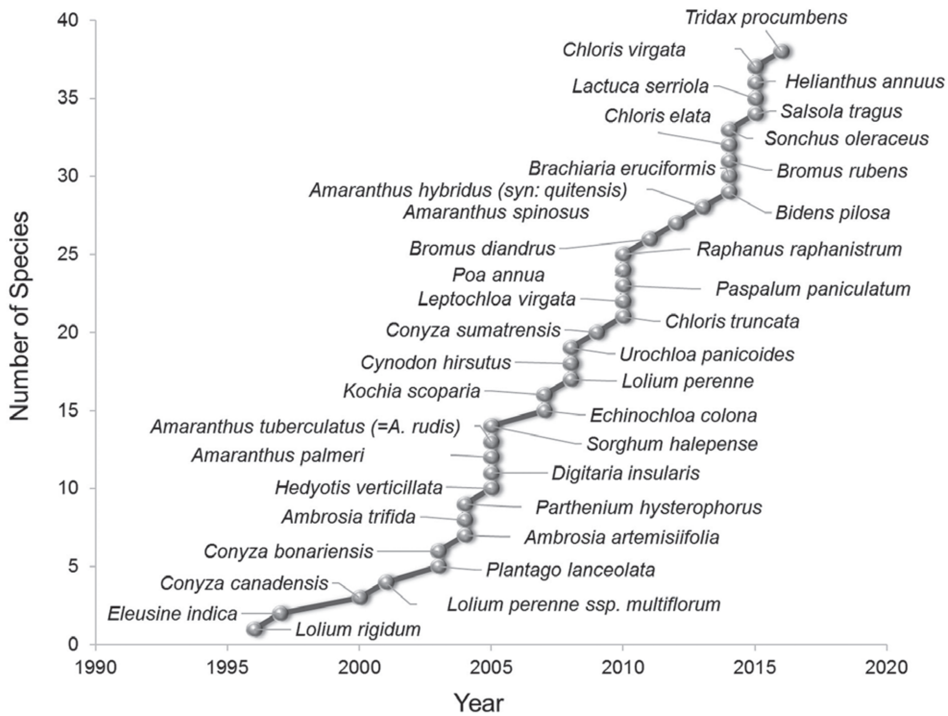

Figure 1. Chronological increase in weed species evolving resistance to glyphosate worldwide. Data from www.weedscience.org (accessed 20 April 2017).

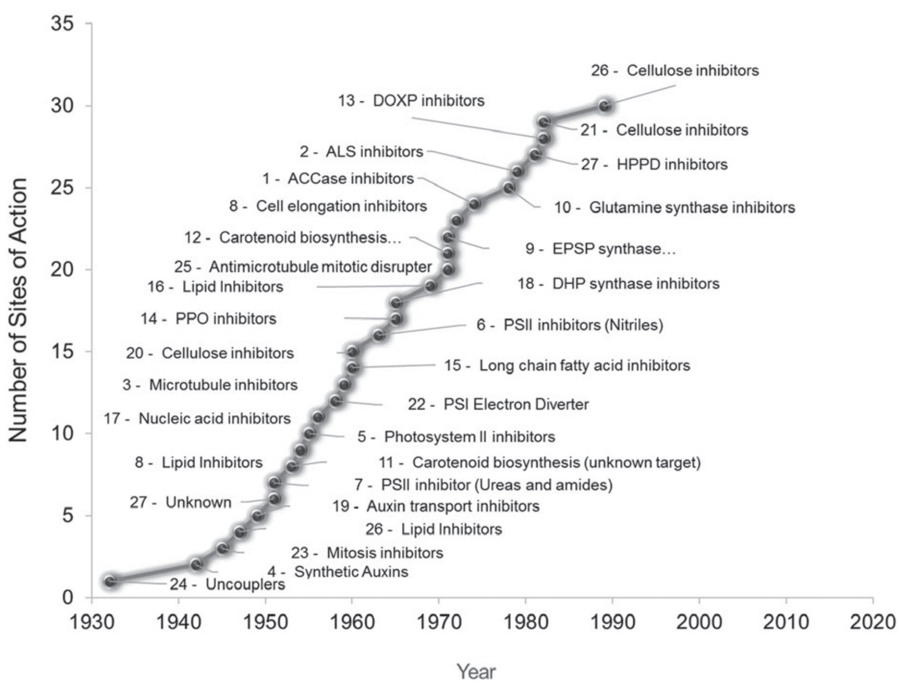

Figure 2. Introduction time of new herbicide-sites of action (WSSA Codes). Data from www.weedscience.org (accessed 20 April 2017).

this is a relatively even split in terms of the number of resistant species originating in glyphosate-resistant crops versus other crop systems, glyphosate-resistant weeds in glyphosate-resistant crop systems account for >90% of the area infested and the economic damage caused by glyphosate-resistant weeds globally. The USA, Argentina, Brazil and Canada rapidly adopted glyphosate-resistant crops, and these four are in the top five countries with the most glyphosate-resistant weed species (Fig. 3) and have the greatest area infested with glyphosate-resistant weeds. Because glyphosate-resistant crops were first introduced in the USA, where they were overwhelmingly adopted, glyphosate-resistant weed problems have been greatest in the USA.

2.1 USA

Seventeen weed species have evolved resistance to glyphosate in the USA (Table 1). Thirteen of them have been found in glyphosate-resistant crops, seven glyphosate-resistant species have been found in both glyphosate-resistant crops and other

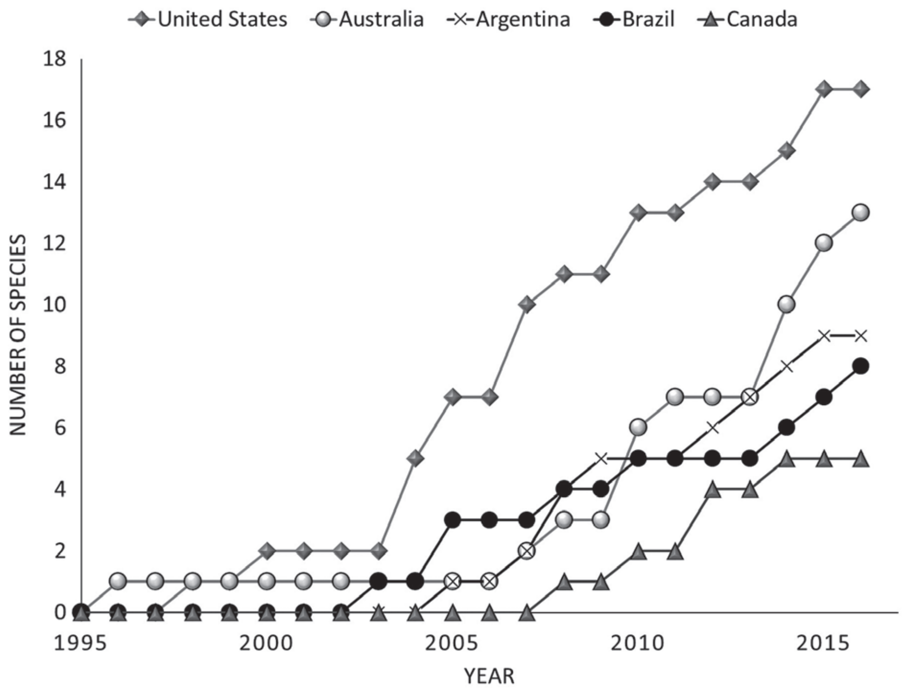

Figure 3. Chronological increase in glyphosate-resistant weed species for the five countries with the largest number of glyphosate-resistant weed species. Data from www.weedscience.org (last accessed 20 April 2017).

situations, and four in other situations alone (primarily orchards, roadsides, golf courses and fallow). The first glyphosate-resistant weed discovered in a glyphosate-resistant crop system was *Conyza canadensis*, found in glyphosate-resistant soybeans in the US state of Delaware in 2000.[10] *Conyza canadensis* remains the most widespread glyphosate-resistant weed, found in 11 countries and 25 US states; however, it is not the most economically important because there are effective and inexpensive alternative control options. Glyphosate-resistant *Amaranthus palmeri* was first identified in a cotton field in Georgia in 2004[11] and is now present in 27 US states. It is the most economically damaging glyphosate-resistant weed globally and is commonly found in glyphosate-resistant cotton, corn and soybeans. *Amaranthus tuberculatus* is the next most important glyphosate-resistant weed, being a problem in 18 US states. *Amaranthus palmeri* predominates in southern US states, and glyphosate-resistant *A. tuberculatus* is more commonly seen in northern US states, where it the greatest threat to corn and soybean production in the Upper Midwest. Both *Amaranthus* spp. are particularly worrisome because they have already evolved resistance to herbicides belonging to most of the other herbicide site of action groups used in these major crops, and there are a number of examples in the International Survey of Herbicide-Resistant Weeds database where individual plants have multiple resistance to herbicides representing four or more sites of action.[9]

Glyphosate-resistant *Kochia scoparia* was first identified in Kansas in 2007[12] and is now present in 10 US states. Its rate of spread has been particularly rapid due to its 'tumbleweed' dispersal mechanism. Glyphosate-resistant *Ambrosia artemisiifolia* and *A. trifida* have been found in 15 and 12 US states, respectively, and although not as widespread as *Conyza* spp., *Amaranthus* spp. or *Kochia scoparia*, they can devastate yield in corn and soybean where they occur. It is likely that their large seed size and fecundity limit their rate of dispersal in comparison with *C. canadensis*, *Amaranthus* spp. or *K. scoparia*.

Table 1. Glyphosate-resistant weed species in the USA by number of states and number of crops (e.g. corn, soybean, rice) or situations (e.g. roadside, railway, industrial site)

USA	No. of states	No. of crops/situations
Amaranthus palmeri	27	7
Conyza canadensis	25	12
Amaranthus tuberculatus (= *A. rudis*)	18	6
Ambrosia artemisiifolia	15	3
Ambrosia trifida	12	3
Kochia scoparia	10	8
Lolium perenne ssp. multiflorum	7	6
Poa annua	3	3
Sorghum halepense	3	1
Eleusine indica	2	2
Salsola tragus	2	2
Echinochloa colona	1	6
Conyza bonariensis	1	4
Parthenium hysterophorus	1	4
Helianthus annus	1	1
Lolium rigidum	1	2
Amaranthus spinosus	1	1

Data from www.weedscience.org (accessed 20 April 2017).

2.2 Australia

Lolium rigidum was the first glyphosate-resistant weed to evolve in Australia, in an apple orchard in New South Wales. Unlike the USA, Australia does not have a long history of glyphosate-resistant crops, so glyphosate has primarily been used non-selectively. Despite this, 13 weed species (Table 2) have evolved resistance to glyphosate, second only to the USA. Part of the reason why

Table 2. Glyphosate-resistant weed species by country

Country	No. of glyphosate-resistant weed species	Country	No. of glyphosate-resistant weed species
USA	17	Israel	2
Australia	13	Malaysia	2
Argentina	9	Mexico	2
Brazil	8	New Zealand	2
Canada	5	Bolivia	1
Spain	5	Chile	1
Colombia	3	Costa Rica	2
Greece	3	Czech Republic	1
Italy	3	Indonesia	1
Japan	3	Paraguay	1
Portugal	3	Poland	1
South Africa	3	Switzerland	1
China	2	Venezuela	1
France	2		

Data from www.weedscience.org (accessed 20 April 2017).

so many herbicide-resistant weeds have been identified in Australia may be the relatively large number of qualified weed scientists searching for them. In most other countries, many of these cases might have been overlooked. For example, five glyphosate-resistant species in Australia have been identified along fence lines and roadsides. Undoubtedly, there are numerous instances of glyphosate-resistant weeds along US roadsides that have gone undetected. Another reason for the disproportionate number of glyphosate-resistant weeds in Australia, considering the relatively small amount of farmland compared with the USA, may be that glyphosate has been used in Australia at a lower rate than in North and South America, which allows for the selection of low-level resistance mechanisms that evolve more readily than higher levels of resistance (see Section 4.2).

2.3 Argentina

Argentina rapidly adopted glyphosate-resistant crops in the late 1990s,[13] and nine weed species have evolved glyphosate resistance, primarily in glyphosate-resistant soybean. Glyphosate-resistant *Sorghum halepense* is widespread in the soybean-growing regions in northern Argentina. What is somewhat surprising about this is that *S. halepense* is a common weed in the Southern USA, yet there have been only a few cases of glyphosate-resistant *S. halepense* reported in the USA. It would be interesting to determine if agronomic practices that differ between the countries have contributed to the difference in the rate of spread of resistance. Has glyphosate-resistant *S. halepense* been spread in Argentina through contaminated soybean seed and/or through movement of harvesting machinery? In addition to glyphosate-resistant *S. halepense*, other glyphosate-resistant grasses (*Digitaria insularis*, *Echinochloa colona* and *Eleusine indica*) are also of significant economic importance in glyphosate-resistant soybean in Argentina. Two glyphosate-resistant *Amaranthus* species (*A. hybridus* subsp. *quitensis* and *A. palmeri*) are becoming widespread in glyphosate-resistant corn and soybean. *Amaranthus palmeri* is thought to have been introduced to soybean fields in Argentina as a contaminant of seed imported from the USA.

2.4 Brazil

As in the USA and Argentina, glyphosate-resistant weeds have evolved in Brazil primarily in response to widespread use of glyphosate in glyphosate-resistant soybeans. Glyphosate-resistant soybeans have been grown (initially from seed illegally transported in from Argentina) since 1999 in Brazil, and as a result eight glyphosate-resistant weed species have been identified in Brazil. The most serious in glyphosate-resistant crops are *Conyza* spp. (*C. canadensis*, *C. bonariensis* and *C. sumatrensis*), *D. insularis*, *E. indica* and more recently, *A. palmeri*.

2.5 Canada

As in Australia, Canada has long relied upon glyphosate as a burndown application prior to crop emergence in minimal tillage systems. Thus, there has been significant selection pressure for glyphosate resistance. In addition, glyphosate-resistant crops have been used in Canada, particularly glyphosate-resistant soybean and corn in Ontario, and glyphosate-resistant canola in the Prairie provinces. Currently, five weed species are reported to have evolved resistance to glyphosate in Canada, the first, *A. trifida*, being found in glyphosate-resistant crops in Ontario, followed by *C. canadensis*, *A. artemisiifolia* and *A. tuberculatus* in the same crop system. Glyphosate resistance has been slow to evolve in glyphosate-resistant canola in the Prairie provinces, perhaps because growers have often rotated glyphosate-resistant with imidazolinone- and glufosinate-resistant canola, and even bromoxynil resistant canola when it was available. Glyphosate is used extensively in the Prairie provinces to enable zero tillage crop systems and is often used in fallow as well as a burndown treatment prior to crop emergence. This has led to the selection of glyphosate-resistant *K. scoparia*, which is rapidly spreading and causing significant economic impact in the Prairie provinces of Canada.

2.6 Other countries

All the countries mentioned above grow significant areas of glyphosate-resistant crops except for Australia. But, 22 other countries have glyphosate-resistant weeds. Most of these do not grow glyphosate-resistant crops, and those that do either grow very little of these crops or/and have grown them for a short time (e.g. Bolivia). Paraguay has the largest area of glyphosate-resistant crops, growing 3.7 million ha in 2015. Thus, the number of glyphosate-resistant weed species in Paraguay is the most likely increase of these 22 countries, especially as it borders both Argentina and Brazil which have growing glyphosate-resistance problems.

Several cases of glyphosate-resistant weeds from these 22 countries are notable. For example, the second reported case of a glyphosate-resistant weed was glyphosate-resistant *E. indica* from an orchard in Malaysia in 1997 (Fig. 1).[14,15] No glyphosate-resistant crops are grown in Malaysia, and no more cases of glyphosate resistance have been reported from this country.

3 WEED FAMILIES

Holm *et al.*[16,17] published *The World's Worst Weeds*, which allows us to tabulate which weed families contain the highest number of principal weed species (Table 3). We have also tabulated the number of glyphosate-resistant weed species in each family (Table 3). It is apparent from a comparison of these lists (Table 3) that four weed families (Poaceae, Asteraceae, Amaranthaceae

Table 3. Weed families with glyphosate resistance. The numbers of distinct species, countries and crops (e.g. corn, soybean, rice) or situations (e.g. roadside, railway, industrial site) for glyphosate-resistant weeds by weed family and the percentage of species considered principal weeds by Holm *et al.*[16,17] for each of these families

Family	No. of species	No. of countries	No. of crops/situations	Glyphosate resistant (% of total)	Weed species (% of world's principal weeds)
Poaceae	18	23	34	47	25
Asteraceae	11	17	20	30	16
Amaranthaceae	4	4	9	11	3
Chenopodiaceae	2	2	11	5	2
Plantaginaceae	1	1	2	3	<1
Brassicaceae	1	1	1	3	4
Rubiaceae	1	1	1	3	<1

Data from www.weedscience.org (accessed 20 April 2017).

and Chenopodiaceae) have been more prone to glyphosate resistance than would be expected when compared with their prominence as principal weeds (Table 3).

3.1 Poaceae

Glyphosate generally has a higher activity on the Poaceae than for other major weed families. It is interesting that more grasses have evolved resistance to glyphosate than weeds of any other family (Table 3). Whereas grasses account for 25% of the world's principal weeds,[16,17] glyphosate-resistant grasses account for 47% of all glyphosate-resistant weeds. Thus, the glyphosate doses that grasses normally receive are higher on their dose–response curve than the doses on non-grass species. The disproportionate number of glyphosate-resistant Poaceae species might be due to a predisposition of grass species for evolving glyphosate resistance, a greater number of grass weeds exposed to glyphosate, and/or more effective selection for glyphosate-resistant individuals at relatively high dose rates. This success in selecting for glyphosate-resistant grasses at relatively high dose rates is contrary to the argument that low doses are more effective in selecting for resistance (Section 4.2). Within Poaceae, the genera of *Lolium*, *Chloris* and *Bromus* appear to be prone to glyphosate resistance, accounting for 8 of the 17 cases. Glyphosate-resistant *Lolium* sp., *D. insularis*, *E. indica*, *S. halepense* and *E. colona* have the widest distribution and greatest economic impact.

3.2 Asteraceae

Glyphosate-resistant weeds in the Asteraceae family are also unusually prevalent, as 16% of the world's worst weeds belong to the Asteraceae family (Table 3), yet they account for 30% of the glyphosate-resistant weeds. The genera of *Conyza* and *Ambrosia* are particularly prone to glyphosate resistance as they account for nearly half of the glyphosate-resistant weeds in the Asteraceae family.

3.3 Amaranthaceae

Of the 163 genera of the Amaranthaceae family, the only genus to evolve glyphosate resistance is *Amaranthus*. Four *Amaranthus* species have evolved glyphosate resistance (*A. palmeri*, *A. tuberculatus*, *A. hybridus* and *A. spinosus*). Glyphosate-resistant *A. palmeri* has the greatest economic impact of any glyphosate-resistant weed, and *A. tuberculatus* is rapidly reaching a similar impact, in part because both these species have evolved resistance to most of the major herbicide sites of action used to control them.

3.4 Chenopodiaceae

Glyphosate-resistant *K. scoparia* and *S. tragus* are the two weed species of Chenopodiaceae that have evolved resistance to glyphosate. At present glyphosate-resistant *K. scoparia* is a major economic problem on the Great Plains of the USA and in the Prairie provinces of Canada, and is spreading rapidly due to its efficient tumbleweed seed dispersal mechanism. *Salsota tragus* is also a tumbleweed, and glyphosate-resistant *S. tragus* has only recently been identified in isolated populations in Montana and Oregon.[18,19] It will be interesting to see if its dispersal mechanism will provide it with rapid spread like that of *Kochia*.

4 FACTORS AFFECTING OCCURRENCE AND SPREAD OF GLYHOSATE RESISTANCE

4.1 Spread of resistant weeds versus *in situ* selection for resistant weeds

Researchers have found Acetyl CoA Carboxylase (ACCase) and Acetolactate Synthase (ALS) inhibitor-resistant weeds to be relatively common, in part because there are several mutations at the target site of each of these sites of action that confer high levels of resistance. For both these target sites, there are many effective herbicide chemistries. The target site can be considered promiscuous or plastic in nature, making selection for resistance relatively rapid. Most of the ACCase and ALS inhibitor-resistant weed cases are due to one base pair mutations that result in high (> 10) resistance factors. By contrast, there are relatively few 1 bp mutations in the *EPSPS* gene that confer even low levels of glyphosate resistance (see Section 5.1), which makes the initial frequency of glyphosate-resistant weeds much lower, by a factor of 10 or more. Glyphosate is the only commercial herbicide that acts by inhibition of EPSPS, and there are no other compounds that are as effective EPSPS inhibitors, as the binding site is very specific. The practical significance of the rarity of glyphosate-resistant weeds is that spread of resistance is much more important as a source of new infestations than new cases arising *in situ* from the existing weed population. For ACCase and ALS inhibitor resistance cases, the high initial resistance gene frequency meant that strict quarantine rules (cleaning incoming machinery, trucks, livestock, etc.) was not as effective a method of avoiding resistance. It was highly likely that resistant individuals already existed in the native weed population in the field or would soon because of the frequency of the occurrence of the right mutation. However, weed seed hygiene is very important in limiting the selection

Table 4. Glyphosate-resistant weed species worldwide by number of countries and number of crops (e.g. corn, soybean, rice) or situations (e.g. roadside, railway, industrial site)

Species	No. of countries	No. of crops/situations
Conyza canadensis	11	15
Eleusine indica	10	11
Lolium perenne ssp. multiflorum	9	13
Conyza bonariensis	9	11
Lolium rigidum	7	15
Echinochloa colona	4	9
Conyza sumatrensis	4	5
Amaranthus palmeri	3	7
Lolium perenne	3	5
Digitaria insularis	3	4
Kochia scoparia	2	10
Amaranthus tuberculatus (= A. rudis)	2	6
Parthenium hysterophorus	2	5
Ambrosia artemisiifolia	2	3
Ambrosia trifida	2	3
Sorghum halepense	2	1
Poa annua	1	3
Amaranthus hybridus (syn: quitensis)	1	2
Bromus diandrus	1	2
Chloris truncata	1	2
Chloris virgata	1	2
Cynodon hirsutus	1	2
Plantago lanceolata	1	2
Salsola tragus	1	2
Sonchus oleraceus	1	2
Urochloa panicoides	1	2
Amaranthus spinosus	1	1
Bidens pilosa	1	1
Brachiaria eruciformis	1	1
Bromus rubens	1	1
Chloris elata	1	1
Hedyotis verticillata	1	1
Helianthus annus	1	1
Lactuca serriola	1	1
Leptochloa virgata	1	1
Paspalum paniculatum	1	1
Raphanus raphanistrum	1	1
Tridax procumbens	1	1

Data from www.weedscience.org (accessed 20 April 2017).

of glyphosate-resistant weeds because the very low initial gene frequency of glyphosate resistance may mean that resistance does not already exist at some level in the native population and is unlikely to occur in any particular field over a relatively long time frame, even when exposed to glyphosate year after year, a point made by Bradshaw *et al.*[20] in their discussion of why evolution of glyphosate resistance was unlikely.

4.2 Selection of glyphosate-resistant weeds: low versus high rates

For many years, weed scientists debated whether high rates (implying a high selection pressure) or low rates (implying a low selection pressure) would result in more rapid evolution of herbicide resistance. The answer appears to be yes and yes. For resistance mechanisms that confer very high levels of resistance (as is often the case for target site resistance to inhibitors of ALS and ACCase), and when the resistance trait is dominant, high application rates can eliminate susceptible individuals quickly and result in a quicker build-up of resistance in the weed population. For mechanisms that do not confer a high level of resistance, where a high rate of herbicide would result in death or significant reduction in growth of individuals carrying the trait, low application rates are more likely to result in resistance. In addition, low herbicide rates allow the possibility for outcrossing and combining of low-level glyphosate resistance traits into one individual that would then be able to survive higher glyphosate rates (creeping resistance).[56] The majority of glyphosate-resistance mechanisms fall into the low-level resistance category, and, as such, the strategy for avoiding or delaying glyphosate resistance is to maximize the percent kill by using the highest recommended rate of glyphosate under ideal application conditions. In this way, many of the individuals with low-level resistance mechanisms may be killed before they have a chance to amalgamate different resistance genes through outcrossing, resulting in individuals with a higher level of glyphosate resistance. This rationale has apparently not been the case for grasses, as discussed above (Section 3.1), even though glyphosate application rates have been relatively high compared with most other species, the number of resistant grass species has been high.

4.3 Selfing versus outcrossing

Because most glyphosate-resistance mechanisms (translocation, sequestration and gene amplification) provide a low level of resistance,[21] it is of great advantage to a weed species in having at least some degree of outcrossing as it enables mechanisms to combine, resulting in higher levels of glyphosate resistance. Individuals containing single low-level mechanisms may survive, but are likely to be severely suppressed by glyphosate applications and will produce less seed than individuals containing two or more glyphosate-resistance mechanisms. For this reason, outcrossing plants are likely to continue to evolve towards higher levels of glyphosate resistance if glyphosate usage is continued after resistance has been identified, as it often used to control other weed species.

4.4 Multiple resistance

Glyphosate resistance alone would not be a major issue, as older herbicides are available for control of glyphosate-resistant weeds and could be used in mixture or sequence with glyphosate, enabling broad spectrum weed control by glyphosate, and specific control of glyphosate-resistant weeds by older chemistries. However, this is rarely the case. Growers rapidly adopted glyphosate-resistant crops, in part to control weeds that had evolved resistance to other herbicide chemistries, in particular ALS-, ACCase- and triazine-resistant weeds. Initially, growers dealt with herbicide resistance by using newly introduced herbicide chemistries. Triazine- and dinitroaniline-resistant weeds that evolved in the 1970s and 1980s were controlled by addition or replacement with ALS and ACCase inhibitors in the 1980s and 1990s. ALS and ACCase inhibitor-resistant weeds were, in turn, controlled by the addition of Protoporphyrinogen Oxidase (PPO) inhibitors, or glyphosate in glyphosate-resistant crops. Today, there are no new herbicide modes of action and few new chemistries which makes the increasing number of glyphosate-resistant weeds a particularly difficult problem.

5 MECHANISMS OF RESISTANCE

5.1 Target-site resistance

Several types of target-site resistance to glyphosate have been identified. Target-site resistance, as the name implies, is due to an alteration of the target enzyme (EPSPS) which confers glyphosate resistance. Target-site resistance can be conferred by a single base pair alteration, a multiple base pair alteration, codon deletion or gene amplification. To date, glyphosate-resistant weeds have been identified with each of these mechanisms, with the exception of codon deletion.

5.1.1 Single base pair alteration

Certain single base pair changes in the *EPSPS* gene can make the enzyme slightly resistant to glyphosate, resulting in a low level of glyphosate resistance (usually 2–6×) for the weed. However, it is not uncommon to have the single base pair mechanism combined with a non-target site mechanism, resulting in a higher fold resistance. All single base pair mutations of *EPSPS* that provide resistance result in changes in Pro106 or Ser, Ala, Thr or Leu.[21] These mutations have been reported in *E. indica*, *L. rigidum*, *L. multiflorum*, *D. insularis*, *A. tuberculatus* and *E. colona*, all summarized by Sammons and Gaines.[21] More recently, both a Ser and Leu substitution of Pro106 has been reported in *C. virgata*.[22] In this study, plants with the Pro106Leu mutation were approximately three- to fivefold more resistant than those with the Pro106Ser mutation, in keeping with the relative *in vitro* resistance of the resulting EPSPS.[21] No other mechanisms of resistance were found. Similarly, two different *EPSPS* mutations at the Pro106 codon were found in *E. colona* from California.[23] This species has two *EPSPS* genes, and the two mutations occurred in different genes. The Pro106Ser mutation was less resistant than the Pro106Thr mutation, in keeping with the relative in vitro resistances.[21] But, other mechanisms may have been involved in this population, as one *E. colona* biotype reported in this paper had no EPSPS mutation, but still had a low level of glyphosate resistance.

5.1.2 Multiple base pair alteration

Two base pair alterations of the *EPSPS* gene that produces two amino acid changes in EPSPS can result in a much higher level of glyphosate resistance than that provided by the Pro106 mutations. A transgenic form of EPSPS with both a Thr102Ile and a Pro106Ser change in wild-type EPSPS (the TIPS double mutation) has been used in commercial transgenic, glyphosate-resistant maize varieties.[24] The TIPS form of EPSPS has evolved in glyphosate-resistant *E. indica*.[25] Plants with the double mutation have a 180-fold resistance factor, a level that makes as much or more resistant than glyphosate-resistant crops[26] and comparable with target site resistance levels of ALS- and ACCase-resistant weeds. Since the Thr102Ile mutation alone produces a very inefficient EPSPS,[21] the Pro106Ser mutation occurred first, with the Thr102Ile coming later. This type of sequential evolution of a high level of resistance was not envisioned when Monsanto provided the rationale for why it would be virtually impossible for a double mutant to occur.[20] With continued glyphosate selection pressure on the single mutation glyphosate-resistant biotypes, TIPS mutants should eventually occur in resistant populations of these species.

5.1.3 Gene amplification or duplication

Gene amplification or duplication increases gene copy numbers and consequently increases the production of the molecular target or enzymes involved in detoxification.[27] An increase in EPSPS would have the effect of increasing the amount of glyphosate needed to inhibit enough of the EPSPS to kill the plant. Since this resistance mechanism to glyphosate was first reported in *A. palmeri*,[28] duplication of the *EPSPS* gene has been associated with glyphosate resistance in several populations of *A. palmeri* throughout the USA.[21] Since this report, *EPSPS* amplification has now been reported for glyphosate-resistant biotypes of *A. tuberculatus*,[29] *A. spinosus*,[30] *A. rudis*,[31] *L. multiflorum*,[32] *K. scoparia*,[33] *B. diandrus*,[34] *E. indica*[35] and *C. truncata*.[36] In the case of *A. spinosus*, the amplification may be due to crossing of *A. spinosus* with *A. palmeri* in which EPSPS was amplified.[30] Amplification of the *EPSPS* gene generally gives a significantly higher resistance factor than a 1 bp mutation of *EPSPS*, although the number of extra *EPSPS* copies and levels of resistance are variable, with as few as three and sometimes >100 extra copies. The correspondence between *EPSPS* copies, amount of EPSPS enzyme and resistance at the whole-plant level is generally good. There is no apparent fitness cost associated with *EPSPS* amplification.[37]

All the target site mechanisms can exist or co-exist within the same species. For example, Chen *et al.*[35] found single- and double-mutated EPSPS, as well as gene amplification of *EPSPS* in *E. indica*. They also found amplification of a gene for phosphofructokinase in a glyphosate-resistant biotype of *E. indica* that also had *EPSPS* amplification.[38]

5.2 Non-target site resistance

In non-target site resistance, the EPSPS enzyme is still sensitive to glyphosate; however, a mutation(s) has conferred a mechanism that reduces the amount of glyphosate reaching the EPSPS enzyme, resulting in resistance. Many cases of glyphosate resistance are due to non-target site resistance. Non-target site resistance mechanisms usually to confer low levels of resistance over a range of 3–12-fold.

5.2.1 Enhanced metabolism

Glyphosate is metabolized to glyoxylate and aminomethylphosphonic acid (AMPA) by some weeds and crops.[39,40] Although seldom looked for, some plants may also metabolize glyphosate to sarcosine and inorganic phosphate.[39] AMPA is a very weakly phytotoxic compound,[41] and glyoxylate is a natural plant metabolite. Therefore, enhanced degradation of glyphosate to AMPA should not pose problem for a weed. In fact, the gene for the microbial enzyme, glyphosate oxidoreductase (GOX), that produces AMPA from glyphosate has been used in canola to help make it resistant to glyphosate.[42] However, this transgenic crop also contains a gene for a resistant microbial EPSPS (*cp4*), and it is unclear how much the GOX gene contributes to glyphosate resistance. The level of resistance (∼ 50×) is about the same for soybean with only *cp4* and canola with both *cp4* and *gox*.[26] The amount of AMPA found in some plant species after glyphosate application indicates that degradation could contribute to natural tolerance to glyphosate, but this has not been rigorously proven.[40] For example, a naturally glyphosate-tolerant weed, pitted morningglory (*Ipomoea lacunosa*) readily metabolizes glyphosate to AMPA, but there is no correlation between the amounts of glyphosate metabolism and the levels of tolerance between biotypes.[43] In our view, there is no robust evidence that enhanced degradation of glyphosate plays a role in the mechanism of resistance to any of the evolved glyphosate-resistant weeds. Considering that some plants have a gene to metabolize glyphosate and that glyphosate is a slow-acting herbicide, it is surprising that enhanced degradation has not been a common mechanism of resistance.

5.2.2 *Decreased absorption and/or translocation*

Resistance can occur when there is a decrease in the absorption or translocation of an herbicide to such an extent that it does not reach the site of action in sufficient concentrations to cause plant death. Reduced movement of glyphosate into plant cells from the plant epidermis (herbicide uptake) can contribute to weed resistance to glyphosate. This mechanism has been invoked for some glyphosate-resistant biotypes of *S. halepense*,[44] *L. multiflorum*,[45] *D. insularis*,[46] *C. eleta*[47] and *Leptochloa virgata*.[48] Reduced uptake can be the result of chemical and/or morphological changes in the leaf cuticle[49] or to changes in leaf shape or orientation that reduces the interception of herbicide spray.[45]

One reason that glyphosate is such an effective herbicide is that it is readily translocated from leaves to meristems.[4] Thus, reduced translocation to meristems will reduce the efficacy of glyphosate. Several studies have reported that glyphosate-resistant biotypes of weeds have reduced translocation of glyphosate.[47,50,51] However, the mechanisms by which this happens have only recently begun to be understood. It appears that enhanced sequestration of glyphosate into the vacuole of cells of the treated leaves is a cause of reduced glyphosate translocation in some glyphosate-resistant plants.[52–54]

5.2.3 *Sequestration*

Resistance can occur through the sequestration of herbicides into parts of the plant (vacuoles, or sorption onto cell walls) thus keeping the herbicide from being both translocated and getting to the chloroplast site of action in the cells in which it is found. Glyphosate resistance in *C. canadensis* and *Lolium* spp. is due to rapid sequestration of glyphosate into the vacuole.[21,52,53] In these cases, the activity and/or levels of an apparent tonoplast glyphosate transporter are elevated.[54] The transporter has characteristics of an ATP-binding cassette (ABC) transporter.[53] Two major ABC-transporter genes are upregulated in the resistant biotype of *C. canadensis* when the plant is exposed to glyphosate.[55] The rapid vacuolar sequestration mechanism does not function at lower temperatures in resistant *C. canadensis*, as vacuolar sequestration does not occur at cooler temperatures.[55,57]

5.2.4 *The 'Phoenix' phenomenon*

Glyphosate acts very slowly as a herbicide. However, sprayed leaves of some biotypes of *A. trifida* react very rapidly to glyphosate, withering and dying within hours after treatment.[58,59] As a result, the herbicide is not translocated from the dead tissues, and developing tissues and meristems can regrow, resulting in a 'Phoenix-like' recovery. The whole-plant level of resistance is 2.3–7.4-fold. The mechanism of the very rapid effect of glyphosate in this biotype is unknown, although it can be inhibited by exogenous aromatic amino acids, indicating that the effect is associated with inhibition of the shikimate pathway.[58] Furthermore, the reactive oxygen species accumulate rapidly in glyphosate-treated leaf discs of the resistant biotype. The rapid response requires either light or exogenous sucrose.

5.3 Multiple mechanisms

Even though glyphosate is a herbicide for which there is a comparatively low risk for evolution of resistance,[20,60] resistance is evolving at a rapid pace. But, the selection pressure with glyphosate has been over much greater land areas for extended periods, unlike that for any other herbicide class.[61] The mechanisms of resistance evolved for glyphosate are more diverse than for any other herbicide mode of action.[21]

Several mechanisms of glyphosate resistance have typically been reported in the more studied glyphosate-resistant weed species, with differences in combinations of mechanisms within the different resistant biotypes of the same species. As pointed out by Sammons and Gaines,[21] only the TIPS mutant glyphosate-resistant weeds have the very high levels of resistance (> 50×) typically found with target site resistance to ACCase and ALS inhibitor herbicides, making evolution of resistance to glyphosate effectively a selection process for low-dose resistance mechanisms in most situations. Over a sufficiently long selection period, mechanisms of resistance tend to accumulate within a species, increasing the overall resistance factor. Over time, this 'creeping' resistance due to accretion of mechanisms can produce robust resistance that provides protection from even the very highest glyphosate doses that a farmer could economically or legally apply.

6 THE FUTURE

Herbicides have provided the most effective and economic weed control method for almost 70 years, and if not for glyphosate-resistant weeds, the problem of weed control in major agronomic crops would be largely solved. The use of glyphosate in glyphosate-resistant crops was adopted rapidly, partly because growers faced herbicide-resistance issues with existing herbicide chemistries, particularly resistance to ALS, ACCase and Photosystem II (PSII) inhibitors. The very success of glyphosate-resistant crops is, in part, responsible for the lack of incentive for the agricultural chemical industry to identify and develop new herbicide sites of action. Glyphosate's widespread use has left us in a situation where glyphosate-resistant weed species are evolving at a steady pace and the more virulent glyphosate-resistant weeds are spreading rapidly. This problem is exacerbated for farmers because alternative herbicide choices are decreasing, largely because most of these weeds already have resistance traits for older herbicide chemistries and are evolving new resistances. So, it is a combination of multiple herbicide resistance (glyphosate plus others), along with the lack of new herbicide sites of action, that has left modern agriculture with a looming weed management crisis. In addition, non-target site multiple resistances, mostly metabolic to a wide array of herbicides other than glyphosate, in grasses like *L. rigidum*, and *Alopecurus myosuroides* add another layer of complexity to the herbicide-resistance problem. We expect that this problem will increase in importance over the next 30 years, with this type of multiple resistance expanding in broadleaf weeds. Management of non-target site resistance is difficult because it often presents unpredictable multiple resistance patterns that cross herbicide sites of action, and thus the advice of rotating herbicide sites of action may not be as effective against non-target-site resistance.

Despite all this, herbicides still provide the backbone of weed control in the developed world, and are likely to do so for the foreseeable future before they are slowly phased out by new technologies. However, to enable this to happen, herbicides need to be utilized in a more sustainable way, not only involving smart herbicide mixtures and rotations, but also by being part of a much more intensive integrated weed management program that includes mechanical and cultural strategies as well. Glyphosate-resistant weeds will continue to increase in terms of both species numbers and crop area infested, but this increase can be slowed by improved resistance management.

Even though herbicide discovery efforts have stalled, the current severity of multiple herbicide resistance in weeds and the lack of effective alternatives provide companies with strong economic incentives to allocate resources to new herbicide discovery programs. Unexploited herbicide modes of action exist that might yield products with sufficient research investment.[62] The development and introduction of new transgenic, mutation-bred and gene-edited herbicide-resistance crop traits may continue to allow old herbicides to be used in new ways. Stacked herbicide-resistance traits in crops will become standard where these crops can be grown, giving growers more flexibility in tailoring herbicide mixtures that slow the appearance of multiple resistance. RNAi technology could be useful in managing herbicide-resistant weeds; however, it apparently faces technological and economic issues that make the time of its commercialization hard to predict. A greater emphasis on maximizing the diversity of weed control techniques to reduce directed evolution will help extend the life of the herbicides that remain effective, perhaps buying us enough time to transition to new weed control technologies.

Eventually, herbicides will likely be replaced by new technologies. There may be advances in genetic engineering of microbial biological control agents and crop allelopathy to make these weed management options more effective, economical and selective. Rapid advances in computer power will increase the speed and accuracy of weed identification by image analysis and this, in combination with the rapid advances in robotics is likely to make both precision herbicide application and/or robotic mechanical weeding economical in many crop situations within the next 20 years. Further advancements in all these approaches will allow these technologies to integrate with existing weed control methods, including herbicides, to maximize weed control diversity and minimize directed evolution of resistance to any one weed control method.

7 CONCLUSIONS

Herbicides, once seen as the final solution to weed control problems in major crops, clearly have a limited lifespan because of herbicide-resistance and concerns about environmental issues. Glyphosate-resistant crops ushered in a short period during which growers abandoned complex weed control strategies in favor of simple, cheap and effective weed control through glyphosate that have saved growers billions of dollars in weed management.[63] Glyphosate-resistant weeds have resulted from this over-reliance on glyphosate, and we are at the precipice of widespread weed control failures because our weed control programs are not sufficiently diversified, and the chemical industry is not introducing herbicides with new sites of action. As multiple resistant weeds proliferate, growers will be forced towards more complex integrated weed management programs to maintain weed control. The extent to which they can implement complex weed control programs will determine how long they can delay the complete failure of herbicides. We hope that viable, new weed control technologies emerge and are effectively implemented before herbicide failures become catastrophic. This does not mean our guard can be let down once new technologies are implemented, as weeds will evolve around whatever weed control strategy is employed, and that is why it will be necessary to implement integrated weed management programs that destabilize directed evolution of resistance to any weed control method.

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