

# Herbicide resistant weeds: the case of resistance to glyphosate

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## Abstract:

Glyphosate has become the most widely used herbicide worldwide since 1974 with a global use of 8.6 billion kg (glyphosate active ingredient) between 1974 and 2014. This study reports on glyphosate resistant (GR) weeds and their resistance mechanisms based on global scientifically reported cases. Forty-nine different weed species have evolved resistance to glyphosate in 29 countries with a total of 318 identified cases worldwide. Fifty percent of these resistance cases were found in glyphosate-resistant cropping systems. There were 255 identified cases (80.2%) of glyphosate resistance in the top five countries (in terms of number of cases and species), namely USA, Australia, Argentina, Brazil, and Canada. The five most popular weed species (in terms of number of cases) found to be resistant to glyphosate were *Conyza canadensis*, *Amaranthus palmeri*, *Amaranthus tuberculatus*, *Lolium perenne* ssp. *Multiflorum*, and *Ambrosia artemisiifolia* with 42, 42, 29, 26, and 21 reported cases, respectively. Out of 49 weed species, 19 GR weed species were found to not only be resistant to glyphosate but also to other herbicide sites of action (multiple herbicide resistance). Glyphosate resistance mechanisms in weeds include (1) target-site alterations: target-site mutation and target-site gene amplification; and (2) non-target-site mechanisms involving different modes of exclusion from the target site: reduced glyphosate uptake, reduced glyphosate translocation, and enhanced glyphosate metabolism. It is essential to have an integrated weed management program that includes not only smart herbicide mixtures and rotations, but also cultural, manual, mechanical, and crop-based weed management methods.

**Keywords:** crop, glyphosate resistance, herbicide, mechanism, resistance, tolerance, weed.

**Classification number:** 3.4

## Introduction

Glyphosate is the most widely used non-selective herbicide in the world [1]. There are several main attributes that make glyphosate a valuable herbicide. For example, it provides simple, inexpensive, flexible, and effective control of a broad spectrum of weeds in a wide variety of agronomic situations [2]. The use of this herbicide has continued to increase as its patent has expired and price declined. When the price of glyphosate declined, it was not only used in high-value crops [1] but also in many other crop or non-crop situations, such as weeds with burndown application, fallow situations, along roadsides, around structures, and in parks [3]. Importantly, when GR crops such as GR soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.),

and maize (*Zea mays* L.) became available for planting in the U.S in 1996, 1997, and 1998, respectively, and later in other countries, more glyphosate has been used in GR cropping systems. These GR cropping systems made it possible to use glyphosate as a broadcast, post-emergence herbicide by controlling all emerged weeds over a wide range of application timings.

Glyphosate (N-phosphonomethyl glycine) is a phosphonomethyl derivative of the amino acid glycine and has a unique mode of action. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [4], which is present in plants, fungi, and bacteria, but not in animals [5]. EPSPS is the sixth enzyme of the shikimic acid pathway, in which phosphoenolpyruvate (PEP) and erythrose 4-phosphate are converted to chorismate, the precursor

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of the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and many aromatic secondary metabolites (e.g., auxins, phytoalexins, anthocyanins, and lignin) [6]. EPSPS catalyses the transfer of the enolpyruvyl moiety from PEP to shikimate-3-phosphate (S3P) [7]. Glyphosate is a transition state analogue of PEP and inhibits EPSPS through the formation of an EPSPS-S3P-glyphosate ternary complex, only binding to the enzyme after the formation of EPSPS-S3P binary complex [8]. Therefore, glyphosate acts as a competitive inhibitor with PEP as it occupies its binding site [9]. EPSPS inhibition by glyphosate prevents the biosynthesis of aromatic amino acids [7]. Glyphosate is the only commercial herbicide that targets EPSPS in all higher plants [1].

Herbicide resistance is defined by the Weed Science Society of America [10] as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by techniques such as genetic engineering or selection of variants produced by tissue culture or mutagenesis. Similarly, Powles, et al. (1996) [11] defined herbicide resistance as “the inherited ability of a weed population to survive a herbicide application that is normally lethal to a vast majority of individuals of that species”. To develop effective herbicide resistance management strategies, it is essential to understand the processes and means by which weeds withstand labelled herbicide treatments. Thus, this document reports GR weeds based on scientifically reported cases globally and their resistance mechanisms to glyphosate are discussed.

### Occurrence of GR weeds

In 1996, the first evolved resistance to glyphosate in weed species was reported in rigid ryegrass (*Lolium rigidum* Gaudin) in Australia [12]. Since then, there has been a sharp increase in the number of GR weeds because of the decades long over-reliance on glyphosate for pre-sowing weed control and the introduction and rapid adoption of

GR transgenic crops in the mid-1990s [13]. To date, 318 cases of glyphosate resistance have been identified in 49 different weed species in 29 countries worldwide [14, 15]. Among these cases, about 50% were found in GR cropping systems [3] such as GR cotton, maize, soybean, and canola (*Brassica* spp.). Between 1974 and 2014, the global use of glyphosate active ingredient was 8.6 billion kg and 19% (over 1.6 billion kg) of glyphosate used in USA. Glyphosate use in the GR cropping systems accounted for 56% of total global glyphosate usage in 2012 [16]. Heap and Duke (2018) [3] stated that both high rates (implying a high selection pressure) and low rates of glyphosate application (implying a low selection pressure) would result in more rapid evolution of herbicide resistance. Therefore, half of glyphosate resistance cases were found in these GR crop systems.

There were 255 identified cases (80.2%) of glyphosate resistance in the top five countries (in terms of number of cases and species). There were 169 cases in 17 species in the United States of America (USA), 36 cases in 19 species in Australia, 19 cases in 15 species in Argentina, 17 cases in 10 species in Brazil, 14 cases in 6 species Canada (Table 1). These four countries (except Australia) were also in the top five countries that planted more than 90% of genetically modified (GM) crops worldwide. In 2018, the top five GM crop-growing countries, namely USA, Brazil, Argentina, Canada and India, produced 75.0, 51.3, 23.9, 12.7, and 11.6 million ha of GM crops, respectively [17].

The number of GR cases in USA was 4.6 times that in Australia. However, Australia was on the top in the number of weed species evolved resistance to glyphosate with 19 weed species (Table 1). There were 125.3 million kg of glyphosate active ingredient used in USA in 2014 [16], while annual glyphosate use in Australia was 24.1 million kg of active ingredient [18]. In Southeast Asia, glyphosate resistance has been reported in Malaysia and Indonesia in goosegrass (*Eleusine indica* (L.) Gaertn.) and woody borerria (*Hedyotis verticillata* (L.) Lam.). There were no cases of GR weed reported in Vietnam (Table 1).

**Table 1. GR weeds: number of cases and species by country.**

No	Country	No. of cases	No. of species
1	United States	169	17
2	Australia	36	19
3	Argentina	19	15
4	Brazil	17	10
5	Canada	14	6
6	Spain	6	5
7	Colombia	5	4
8	South Africa	5	2
9	Chile	4	1
10	Greece	4	4
11	Italy	4	3
12	New Zealand	4	2
13	France	3	2
14	Japan	3	3
15	Malaysia	3	2
16	Paraguay	3	3
17	Portugal	3	3
18	China	2	2
19	Costa	2	2
20	Mexico	2	2
21	Israel	2	2
22	Bolivia	1	1
23	Czech Republic	1	1
24	Hungary	1	1
25	Indonesia	1	1
26	Poland	1	1
27	Switzerland	1	1
28	Turkey	1	1
29	Venezuela	1	1

Adapted from [14] (accessed 30 March 2020) and [15]

Among the 49 different weed species that have evolved resistance to glyphosate, the top five weed species (in terms of number of cases) contributed to 160 cases (50.3%). They were horseweed (*Conyza canadensis* (L.) Cronq.), palmer amaranth (*Amaranthus palmeri* S. Watson), tall waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer), Italian ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot), and common ragweed (*Ambrosia artemisiifolia* L.) with 42, 42, 29, 26, and 21 reported cases, respectively. *Conyza canadensis* was the most commonly found GR weed species with 42 cases in 13 countries (Table 2). *Lolium rigidum* was the first weed species to evolve resistance to glyphosate in Australia in 1996 [12] followed by 48 others weed species. In 2020, *Chloris distichophylla* (Lag.) from areas of soybean

cultivation in Brazil was the 49<sup>th</sup> weed species that evolved resistance to glyphosate [15].

Unfortunately, 19 out of 49 GR weed species had evolved multiple herbicide resistance (Table 2), leaving growers with fewer herbicidal options for weed control. For example, *A. tuberculatus* in Ontario, Canada evolved multiple resistance (4 herbicide sites of action) to (1) EPSP synthase inhibitors (phosphanoglycine: glyphosate), (2) acetolactate synthase (ALS) inhibitors (imidazolinones), (3) Photosystem II inhibitors (triazines), and (4) protoporphyrinogen oxidase (PPO) inhibitors (diphenylether). *Amaranthus palmeri* in Arkansas, USA also evolved multiple resistance (5 herbicide sites of action) to (1) EPSP synthase inhibitors, (2) ALS inhibitors, (3) PPO inhibitors, (4) microtubule inhibitors (initroaniline), and (5) long chain fatty acid inhibitors (chloroacetamides).

In Vietnam, herbicides and mechanical and manual weeding are used for weed control. There were 104 glyphosate-based herbicides registered in Vietnam [19]. The annual glyphosate use in Vietnam was 3.22 million kg of active ingredient [18]. Glyphosate accounted for 36% of total herbicide use (in terms of active ingredient) and 35% of total spray area in Vietnam. Glyphosate use in rubber, coffee, rice, and sugarcane accounted for 57, 12, 6, and 5%, respectively, of total usage [18]. Fourteen genetically modified (GM) crop events for herbicide tolerance have been approved in Vietnam, include eight events (three events for glyphosate tolerance) of maize (*Zea mays* L.) and six events (three events for glyphosate tolerance) of soybean (*Glycine max* L.) [20]. Glyphosate-tolerant maize was grown on 55,000 ha in Vietnam [18]. GR weed has not been scientifically reported in Vietnam yet. This may be because there have been insufficient studies in weed science and herbicide resistance in Vietnam, and particularly, research in glyphosate resistance. Heap and Duke (2018) [3] also stated that herbicide resistant weeds were underreported in developing countries. Additional research needs to be conducted in weed science in order to improve weed management practices in these countries. However, populations of barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) collected at several locations in the Mekong delta, Vietnam were confirmed to be resistant to two herbicide sites of action, namely synthetic auxins inhibitors (quinclorac) and ALS inhibitors (bispyribac, penoxsulam) [21].

Table 2. GR weeds: number of cases and countries by species.

No	Species	No. of cases	No. of countries	Multiple resistance
1	<i>Conyza canadensis</i>	42	13	Yes
2	<i>Amaranthus palmeri</i>	42	3	Yes
3	<i>Amaranthus tuberculatus</i>	29	2	Yes
4	<i>Lolium perenne</i> ssp. <i>multiflorum</i>	26	9	Yes
5	<i>Ambrosia artemisiifolia</i>	21	2	Yes
6	<i>Lolium rigidum</i>	17	8	Yes
7	<i>Ambrosia trifida</i>	17	2	
8	<i>Kochia scoparia</i>	17	2	Yes
9	<i>Eleusine indica</i>	14	10	Yes
10	<i>Conyza bonariensis</i>	13	10	Yes
11	<i>Conyza sumatrensis</i>	11	7	Yes
12	<i>Poa annua</i>	7	2	Yes
13	<i>Echinochloa colona</i>	6	4	Yes
14	<i>Sorghum halepense</i>	5	2	Yes
15	<i>Lolium perenne</i>	4	3	Yes
16	<i>Amaranthus hybridus</i>	4	2	Yes
17	<i>Digitaria insularis</i>	3	3	
18	<i>Chloris virgata</i>	3	1	
19	<i>Salsola tragus</i>	3	1	
20	<i>Brassica rapa</i>	2	2	Yes
21	<i>Parthenium hysterophorus</i>	2	2	
22	<i>Urochloa panicoides</i>	2	2	
23	<i>Chloris truncata</i>	2	1	
24	<i>Amaranthus spinosus</i>	1	1	
25	<i>Avena fatua</i>	1	1	
26	<i>Avena sterilis</i> ssp. <i>ludoviciana</i>	1	1	
27	<i>Bidens pilosa</i>	1	1	
28	<i>Bidens subalternans</i>	1	1	
29	<i>Brachiaria eruciformis</i>	1	1	
30	<i>Bromus catharticus</i>	1	1	
31	<i>Bromus diandrus</i>	1	1	
32	<i>Bromus rubens</i>	1	1	
33	<i>Carduus acanthoides</i>	1	1	Yes
34	<i>Chloris elata</i>	1	1	
35	<i>Chloris radiata</i>	1	1	
36	<i>Cynodon hirsutus</i>	1	1	
37	<i>Echinochloa crus-galli</i> var. <i>crus-galli</i>	1	1	
38	<i>Hedyotis verticillata</i>	1	1	Yes
39	<i>Helianthus annuus</i>	1	1	
40	<i>Hordeum murinum</i> ssp. <i>glaucum</i>	1	1	
41	<i>Lactuca saligna</i>	1	1	
42	<i>Lactuca serriola</i>	1	1	
43	<i>Leptochloa virgata</i>	1	1	
44	<i>Paspalum paniculatum</i>	1	1	
45	<i>Plantago lanceolata</i>	1	1	
46	<i>Raphanus raphanistrum</i>	1	1	Yes
47	<i>Sonchus oleraceus</i>	1	1	
48	<i>Tridax procumbens</i>	1	1	
49	<i>Chloris distichophylla</i>	1	1	

Adapted from [14] (accessed 30 March 2020) and [15]

## Glyphosate resistance mechanisms

Resistance mechanisms to glyphosate in weeds include (1) target-site alterations and (2) non-target-site mechanisms.

### Target-site mechanisms

Target-site alterations include (1a) target-site mutation [22-24], represented by amino acid substitutions that affect herbicide interactions at the target enzyme; and (1b) target-site gene amplification [25-27], where sufficient EPSPS protein is produced so that the shikimate pathway can continue to operate despite the fact that glyphosate inhibits some of the enzyme.

Target-site mutations that change Pro106 of EPSPS to Ala, Leu, Ser, or Thr have been reported in GR populations of 6 different weed species [28, 29]. It was concluded that the Pro101 (position Pro106 in plant mature EPSPS consensus corresponds to position Pro101 in *E. coli*) is not directly involved in molecular interactions with either glyphosate or the substrate PEP, but any mutation at this site would shift other amino acids (Thr97 and Gly96) towards the inhibitor molecule resulting in a structural change to the glyphosate-binding site [30]. More recently, a double amino acid substitution in a single EPSPS allele (Thr102Ile+Pro106Ser) was found in glyphosate-resistant *E. indica* populations from Malaysia and China [23, 24]. This double amino acid substitution conferred high-level glyphosate resistance (more than 180-fold) [23] whereas the single Pro-106 mutations of the 6 weed species provided only moderate resistance (less than 10-fold) [29]. Another double TIPT mutation (Thr102Ile and Pro106Thr) was reported in a population of glyphosate-resistant greater beggarticks (*Bidens subalternans* DC.) from Paraguay [31]. Especially, a triple amino acid substitution from TAP to IVS was found in the EPSPS gene of a GR smooth pigweed population (*Amaranthus hybridus* L.) from Argentina. The nucleotide substitutions were Ile102 (ATA), Val103 (GTC), and Ser106 (TCA) in place of Thr102 (ACA), Ala103 (GCG), and Pro106 (CCA), respectively [32].

Glyphosate resistance due to extensive amplification of the EPSPS gene was first revealed in a population of *A. palmeri* in Georgia, USA in 2010 [27] and in Arkansas, USA in 2018 [33]. This mechanism has since been identified in 8 other weed species: *L. multiflorum* [26], spiny amaranth (*Amaranthus spinosus* L.) [34], *A. tuberculatus* [35, 36], *E. indica* [24], kochia (*Kochia scoparia* (L.) Schrad.) [37], rigpgut brome (*Bromus diandrus* Roth) [25], windmillgrass (*Chloris truncata* R. Br) [38], and smooth barley (*Hordeum*

*murinum* ssp. *glaucum* (Steud.) [39]. Individuals of the GR populations contained few to many more copies of the EPSPS gene than did the susceptible plants, with the number of EPSPS copies found to be variable both between and within populations. For example, *A. palmeri* from Georgia, the Carolinas, and New Mexico had, respectively, 5 to 160-fold [27], 22 to 36-fold [40], and 2 to 8-fold [41] more copies of the EPSPS gene and a 10 to 36-fold increase in *B. diandrus* [25] and a 10 to 25-fold increase in *L. multiflorum* [26]. It has been suggested that the effect of additional copies of the EPSPS is additive and additional copies confer higher levels of resistance to glyphosate [27]. This amplification of the EPSPS gene produces sufficient EPSPS protein to enable the shikimate pathway to continue to operate despite glyphosate inhibition of some of the enzymes [42].

#### *Non-target-site mechanisms*

Glyphosate is taken up through plant surfaces and leaf uptake rates vary considerably between species. Diffusion is the most likely mode of transport across the plant cuticle [1], which varies in composition and thickness among different plant species. Uptake is also dependent on several interdependent factors: droplet size and droplet spread, surfactant type and concentration, ionic strength and salt concentration, humidity, and, most importantly, glyphosate concentration [43]. After it is absorbed, the physicochemical properties of glyphosate enable it to be translocated from the leaf via phloem transport to meristematic growing points in the roots and shoots [1, 43]. The phloem movement of glyphosate and the efficiency of translocation are affected by the health and developmental stage of the plant, which is often related to environmental conditions [43] such as temperature.

Non-target-site mechanisms include (2a) reduced glyphosate uptake [44, 45], where less glyphosate is absorbed by resistant plants than susceptible plants; (2b) reduced glyphosate translocation [44, 46-48], where amounts of glyphosate absorbed by both resistant and susceptible plants are similar after glyphosate treatment; however, the absorbed herbicide mostly remains in the treated leaf in resistant biotypes and only a smaller amount of glyphosate was transported to the meristems of the treated plant; and (2c) enhanced glyphosate metabolism [49, 50].

Reduced glyphosate uptake has been reported in GR populations of sourgrass (*Digitaria insularis* (L.) Mez ex Ekman) and johnsongrass (*Sorghum halepense*

(L.) Pers.) [44, 45]. Carvalho, et al. (2012) [45] stated that reduced glyphosate uptake is one of the glyphosate resistance mechanisms in *D. insularis* in Brazil. The susceptible plants absorbed at least 12% more glyphosate than resistant sourgrass plants up to 48 h after treatment. Reduced glyphosate translocation has been found in GR plants of *S. halepense*, *L. rigidum*, and perennial ryegrass (*Lolium perenne* L.) [44, 46-48]. It was reported that reduced glyphosate translocation to meristems is the main mechanism conferring glyphosate resistance in *S. halepense* in Argentina. The amount that translocated to the meristems of the resistant plants was threefold less than in the susceptible plants of johnsongrass [44]. Enhanced glyphosate metabolism has been reported in some weed species such as an Australian population of *E. colona* [49] and in the populations of 3 *Conyza* species from Greece [50]. It was revealed that glyphosate was metabolized to produce aminomethylphosphonic acid and glyoxylate [49, 50].

#### Conclusions

In conclusion, weeds that are resistant to glyphosate will continue to increase in terms of both number of species and cases. However, this increase can be slowed down by improved resistance management. For example, it may not be sustainable to use a single herbicide to manage weeds. Herbicides need to be exploited in a more sustainable way and additional methods must be used to control GR weeds. To have sustainable weed management practices, herbicides should not only involve smart herbicide mixtures and rotations, but also be part of a much more intensive integrated weed management program [51] that includes mechanical, cultural, and crop-based weed management strategies.

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#### COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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