Evolution of Glyphosate-Resistant Weeds

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Contents

Abstract Widespread adoption of glyphosate-resistant crops and concomitant reliance on glyphosate for weed control set an unprecedented stage for the evolution of herbicide-resistant weeds. There are now 48 weed species that have evolved glyphosate resistance. Diverse glyphosate-resistance mechanisms have evolved, including single, double, and triple amino acid substitutions in the target-site gene, duplication of the gene encoding the target site, and others that are rare or nonexistent for evolved resistance to other herbicides. This review summarizes these resistance

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mechanisms, discusses what is known about their evolution, and concludes with some of the impacts glyphosate-resistant weeds have had on weed management.

Keywords EPSPS · Evolution · Glyphosate · Herbicide resistance · Resistance mechanisms · Weed management

Abbreviations

AKR	Aldo-keto reductase
AMPA	Aminomethylphosphonic acid
C-P	Carbon-phosphorus
eccDNA	Extrachromosomal circular DNA
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
FISH	Fluorescent in situ hybridization
GOX	Glyphosate oxidoreductase

1 Introduction

Glyphosate competes with phosphoenolpyruvate to bind the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), preventing synthesis of the essential amino acids phenylalanine, tyrosine, and tryptophan (Steinrücken and Amrhein 1980; Schönbrunn et al. 2001). It was commercialized for use as a herbicide in 1974 for nonselective weed control (i.e., in non-crop areas or prior to planting) (Duke and Powles 2008). In this Volume of *Reviews of Environmental Contamination and Toxicology*, Duke (2021) provides a detailed review of the mode of action and use of glyphosate, and Green and Siehl (2021) discuss the development of glyphosate-resistant crops, which led to the unprecedented reliance on glyphosate for weed control.

Prior to the commercialization of these glyphosate-resistant crops, it was infamously argued in a 1997 paper (Bradshaw et al. 1997) that "the probability of evolution of glyphosate resistance seems low." To be fair, resistance to glyphosate does appear to arise spontaneously at a lower frequency than it does for other herbicides (Jander et al. 2003; Brotherton et al. 2007). Nevertheless, the probability that herbicide resistance occurs in weeds depends not only on the ease at which resistance to that herbicide evolves but also on the selection intensity imposed by that herbicide. In regards to glyphosate, perhaps not even Bradshaw et al. (1997) anticipated the unprecedented selection pressure that would be applied by this herbicide in the years following their publication.

After about a decade of very successful weed control, the beginning of the end of glyphosate as a stand-alone herbicide used in conjunction with glyphosate-resistant crops occurred around 2005, with the evolution of glyphosate-resistant populations

of *Amaranthus palmeri* and *Amaranthus tuberculatus* (Culpepper et al. 2006; Legleiter and Bradley 2008). Although these were not the first two weeds to evolve glyphosate resistance (Heap 2020), they are driver weeds in USA cotton and soybean fields – the two crops for which glyphosate-resistant varieties were first rapidly adopted.

Heap and Duke (2018) provided a relatively recent and comprehensive description of the occurrence and distribution of 38 glyphosate-resistant weed species known at the time. Since then, 10 additional glyphosate-resistant weeds have been reported, bringing the total to 48, equally split between grass and broadleaf species (Heap 2020). Glyphosate-resistant weeds now have been documented in 30 countries (Table 1). Of these 30 countries, however, most (24) have reports of less than five species, with Australia, the USA, and Argentina being notable exceptions, with 19, 17, and 15 glyphosate-resistant species, respectively. The earliest glyphosate-resistant weed species reported, including *Lolium* spp., *Conyza* spp., and *Eleusine indica*, are also now the most widely distributed glyphosate-resistant species among different countries. Early and widespread occurrence of glyphosate-resistant populations of these species likely reflects some combination of these species' widespread occurrence, their propensity for gene flow [e.g., *Conyza spp*. seeds are wind dispersed over broad geographies (Weaver 2001)], and their innate abilities to evolve glyphosate resistance.

When considering the timeline of the appearance of glyphosate-resistant weeds, it is important to keep in mind that glyphosate selection might have occurred prior to the adoption of glyphosate-resistant crops (through traditional use of glyphosate), only after the adoption of such crops, or both, depending on the species. For example, glyphosate-resistant *Lolium rigidum* was reported in Australia in 1996, representing a clear case of glyphosate resistance occurring due to the traditional use of glyphosate. In contrast, *A. tuberculatus*, for example, occurs primarily in crop fields and germinates relatively late in the growing season (Costea et al. 2005). Consequently, there likely was a relatively limited selection for glyphosate-resistant biotypes of this species prior to 1996. *Conyza canadensis*, first reported glyphosate-resistant in 2000, likely was selected by glyphosate applied both traditionally and in glyphosate-resistant crops (VanGessel 2001), contributing to this species evolving resistance sooner than, e.g., *A. tuberculatus*.

It is interesting that in the USA and Brazil, where glyphosate-resistant crops were first widely adopted, most of the glyphosate-resistant weed species were reported within a decade after the adoption of those crops, with no new species having been reported from these countries since 2015. The recent lack of new glyphosate-resistant species in these countries cannot be explained entirely by local curtailing of glyphosate use: glyphosate often is used even in areas where glyphosate-resistant weeds exist to provide control of other weed species. For example, in the USA as recently as 2017, glyphosate was still used on three-fourths of the soybean hectares (https://www.nass.usda.gov). Perhaps evolutionary rescue of glyphosate selection is not possible, or highly improbable, in several weed species. Alternatively, after the evolution of an initial glyphosate-resistant weed species in a given field, glyphosate was more likely to be applied at higher use rates and in combination with one or more other herbicides, limiting the subsequent evolution of glyphosate-resistant species.

Table 1 Distribution of glyphosate-resistant weeds (listed by year first reported) and their known resistance mechanisms

No.	Weed	Year	Australia	USA	Argentina	Brazil	Canada	Spain	Columbia	Greece	Italy	Japan	Paraguay	Portugal	South Africa	China	Costa Rica	France	Israel	Malaysia	Mexico	New Zealand	Bolivia	Chile	Czech Republic	Hungary	Indonesia	Poland	South Korea	Switzerland	Turkey	Venezuela	Resistance mechanisms
1	*Lolium rigidum*	1996	X	X				X		X	X				X			X	X														RTU, STM
2	*Eleusine indica*	1997		X	X	X			X			X				X	X			X			X				X						STM, DTM, GD
3	*Conyza canadensis*	2000		X		X	X	X		X	X	X		X		X									X	X		X	X				RTU
4	*Lolium perenne ssp. multiflorum*	2001	X	X	X		X			X	X										X		X						X				RTU, STM, GD
5	*Conyza bonariensis*	2003	X	X	X	X		X	X	X				X	X				X														RTU
6	*Plantago lanceolata*	2003													X																		–
7	*Ambrosia artemisiifolia*	2004		X			X																										–
8	*Ambrosia trifida*	2004		X			X																										RTU, RR
9	*Parthenium hysterophorus*	2004		X					X																								RTU, STM
10	*Amaranthus palmeri*	2005		X	X	X																											STM, GD
11	*Amaranthus tuberculatus*	2005		X			X																										RTU, STM, GD
12	*Digitaria insularis*	2005			X	X							X																				RTU, DTM
13	*Hedyotis verticillata*	2005																		X													–
14	*Sorghum halepense*	2005		X	X																												RTU
15	*Echinochloa colona*	2007	X	X	X																											X	STM, MB
16	*Bassia scoparia*	2007		X			X																										GD
17	*Cynodon hirsutus*	2008			X																												–

18	*Lolium perenne*	2008			X								X							X										GD
19	*Urochloa panicoides*	2008	X		X																									–
20	*Conyza sumatrensis*	2009	X			X		X		X			X					X											X		RTU, STM
21	*Chloris truncata*	2010	X																												GD
22	*Leptochloa virgata*	2010																		X											RTU, STM
23	*Paspalum paniculatum*	2010														X															–
24	*Poa annua*	2010	X	X																											STM, GD
25	*Raphanus raphanistrum*	2010	X																												–
26	*Bromus diandrus*	2011	X																												GD
27	*Amaranthus spinosus*	2012		X																											GD
28	*Brassica rapa*	2012			X		X																								TG
29	*Amaranthus hybridus*	2013			X	X																									TTM
30	*Bidens pilosa*	2014																		X											RTU, STM, DTM
31	*Brachiaria eruciformis*	2014	X																												–
32	*Bromus rubens*	2014	X																												–
33	*Chloris elata*	2014				X																									RTU
34	*Sonchus oleraceus*	2014	X																												–
35	*Chloris virgata*	2015	X																												STM
36	*Helianthus annuus*	2015		X																											–
37	*Lactuca serriola*	2015	X																												–
38	*Salsola tragus*	2015		X																											–
39	*Hordeum murinum*	2016	X																												GD
40	*Tridax procumbens*	2016	X																												STM
41	*Bromus catharticus*	2017			X																										–
42	*Lactuca saligna*	2017	X																												–
43	*Avena fatua*	2018	X																												–
44	*Avena sterilis*	2016	X																												RTU
45	*Bidens subalternans*	2018											X																		DTM

(continued)

Table 1 (continued)

No.	Weed	Year	Australia	USA	Argentina	Brazil	Canada	Spain	Columbia	Greece	Italy	Japan	Paraguay	Portugal	South Africa	China	Costa Rica	France	Israel	Malaysia	Mexico	New Zealand	Bolivia	Chile	Czech Republic	Hungary	Indonesia	Poland	South Korea	Switzerland	Turkey	Venezuela	Resistance mechanisms
46	*Carduus acanthoides*	2019			X																												–
47	*Chloris radiata*	2019							X																								–
48	*Echinochloa crus-galli*	2019			X																												–
	Total		19	17	15	9	6	5	4	4	3	3	3	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	

Abbreviations: *RTU* reduced translocation and/or uptake, *STM* single target site mutation, *DTM* double target site mutation, *TTM* triple target site mutation, *GD* target-site gene duplication (or increased expression), *MB* metabolism, *RR* rapid response, *TG* Transgene escape. Species, year, and country data are from Heap (2020). References for resistance mechanisms are listed by No. **1** Lorraine-Colwill et al. (2002), Wakelin et al. (2004), González-Torralva et al. (2012a). **2** Baerson et al. (2002b), Zhang et al. (2015), Yu et al. (2015), Chen et al. (2015), Franci et al. (2020). **3** Feng et al. (2004), Koger and Reddy (2005), Ge et al. (2010), González-Torralva et al. (2012b, 2017). **4** Salas et al. (2012), González-Torralva et al. (2012a), Liu et al. (2016), Karn and Jasieniuk (2017), Brunharo and Hanson (2018). **5** Dinelli et al. (2008), Shaner (2009), Moretti et al. (2013), Kleinman and Rubin (2017). **7** Brewer and Oliver (2009). **8** Lespérance (2015), Nandula et al. (2015), Van Horn (2016), Moretti et al. (2018). **9** Bracamonte et al. (2016). **10** Gaines et al. (2010), Dominguez-Valenzuela et al. (2017). **11** Bell et al. (2013), Nandula et al. (2013), Lorentz et al. (2014). **12** de Carvalho et al. (2012). **14** Vila-Aiub et al. (2012). **15** Han et al. (2016), Goh et al. (2018), Pan et al. (2019), McElroy and Hall (2020). **16** Jugulam et al. (2014). **18** Yanniccari et al. (2017). **20** González-Torralva et al. (2014), Amaro-Blanco et al. (2018). **21** Ngo et al. (2018b). **22** Alcántara-de la Cruz et al. (2016b). **24** Brunharo et al. (2019). **26** Malone et al. (2016). **27** Nandula et al. (2014). **28** Pandolfo et al. (2018). **29** García et al. (2019), Perotti et al. (2019). **30** Alcántara-de la Cruz et al. (2016a). **33** Brunharo et al. (2016). **35** Ngo et al. (2018a). **39** Adu-Yeboah et al. (2019). **40** Li et al. (2018). **44** Fernández-Moreno et al. (2016). **45** Takano et al. (2020)

The most recent reports of new glyphosate-resistant weed species have come from other South American countries and Australia. In fact, of the 10 species added to the list since Heap and Duke's review (2018), half were in Australia and the other half were in the South American countries of Argentina, Columbia, or Paraguay. Glyphosate selection from both traditional use and in glyphosate-resistant crops is continuing to increase the number of glyphosate-resistant weeds.

Just as humans have to think "outside the box" when confronted with new challenges, weeds had to evolve "outside the box" when confronted with glyphosate selection. Hence, the combination of the difficulty in evolving glyphosate resistance and the intense glyphosate selection pressure resulted in diverse and unusual resistance mechanisms (Gaines et al. 2019). In this review, we discuss these diverse resistance mechanisms and what is known about their evolution. We conclude with a discussion of how the vast, real-world glyphosate evolutionary experiment has impacted weed management.

As is the case for any other herbicide, resistance mechanisms for glyphosate can be broadly grouped into target-site and nontarget-site mechanisms (Gaines et al. 2020). Historically, target-site resistance was described as a mutation in the gene encoding the protein that directly interacts with the herbicide, leading to a reduced affinity between the herbicide and its target site. Nontarget-site resistance includes all other mechanisms, primarily including herbicide detoxification (metabolism), reduced herbicide uptake, and reduced herbicide translocation. In general, nontarget-site mechanisms confer resistance by essentially reducing the concentration of herbicide that reaches the target site. A relatively new resistance mechanism, associated primarily with glyphosate resistance, is increased expression of the target site via gene duplication (discussed in Sect. 3.2). Duplication of the target-site gene has been categorized as another form of target-site resistance (Gaines et al. 2020). From a physiological perspective, however, resistance due to increased expression of the target site is more like nontarget-site resistance in that the net result in both cases is reduced concentration of herbicide per unit of target site. Additionally, from a genetic perspective, gene duplication in some cases results in resistance being inherited from multiple loci – as often is the case with nontarget-site resistance (Délye 2013) – whereas traditional target-site resistance involves a single locus. Nevertheless, in this review we will include *EPSPS* duplication as a form of target-site resistance.

2 Nontarget-Site Resistance

2.1 Uptake, Translocation, and Sequestration

The effectiveness of any herbicide is highly dependent on the active ingredient reaching the target site. The delivery of the herbicide to the target site is defined by the uptake and translocation of the herbicide in the plant, which, in turn, are dependent on factors such as plant cuticle physiology, herbicide formulation,

environmental factors, and molecular properties of the herbicide (e.g., size and polarity) (Hess and Duke 1985).

The polar nature of glyphosate makes it poorly absorbed by leaves, but once absorbed, it can be rapidly translocated into plant meristems (Preston and Wakelin 2008). Glyphosate is mainly translocated via phloem following the source-to-sink pattern of photoassimilates. Translocation via xylem can also occur, but it rapidly goes back into the phloem and accumulates more in sink tissues (Bromilow et al. 1990).

Reduced translocation and absorption of glyphosate are known mechanisms of nontarget-site resistance documented in some weed species (Sprankle et al. 1975; Feng et al. 2004; Wakelin et al. 2004; Preston and Wakelin 2008; Shaner 2009; Vazquez-Garcia et al. 2020). Glyphosate uptake reduction occurs when chemical or morphological changes in the leaf cuticle or leaf shape reduce the amount of herbicide entering the plant. Most cases of reduced glyphosate uptake show a variation in leaf angle and cuticle properties and were observed in grass species (Michitte et al. 2007; Vila-Aiub et al. 2012; de Carvalho et al. 2012; Alcántara-de la Cruz et al. 2016b).

Reduced glyphosate translocation occurs when the herbicide molecules have limited or no movement to the plant meristem, a factor that can profoundly affect herbicide efficacy, and has evolved as a resistance mechanism. In some species, such as *Conyza* spp. and *Lolium* spp., the reduced translocation is attributed to a rapid-vacuolar sequestration mechanism (Ge et al. 2010, 2012). Such sequestration prevents translocation of the glyphosate molecules to meristematic tissue.

Vacuoles are degradative organelles, similar to lysosomes in animal cells, and are the largest organelles of plant cells, representing around 80% of the total cell space (Martinoia 1992). These large cell compartments serve as reservoirs for ions and metabolites and play fundamental roles in detoxification and maintaining cell homeostasis (Marty 1999). Studies have shown that active tonoplast transporters such as ABC transporters are possibly linked with the movement of glyphosate into the vacuoles, suggesting that ABC transporter genes regulate this resistance mechanism (Nol et al. 2012; Ge et al. 2014; Tani et al. 2015).

Environmental factors are also known to affect these key genes in the uptake and translocation of glyphosate. Studies suggest that glyphosate uptake may vary in different light regimes, showing greater uptake when conditions are optimum for high ATP levels (Kells and Rieck 1979; Devine et al. 1983; Ge et al. 2010). Temperature can also play a role in glyphosate uptake and translocation (Vila-Aiub et al. 2013; Palma-Bautista et al. 2019). Vacuole sequestration was shown to vary with temperature: with low temperature, glyphosate-resistant plants showed a reduction in the resistance level and herbicide retention in the vacuoles (Ge et al. 2011). Because vacuolar sequestration provides a relatively low level of resistance (Fig. 1), it potentially could be overcome by making applications when temperatures are low. Further studies of vacuolar sequestration are still required for a better understanding of this nontarget-site resistance mechanism at the molecular level.

Fig. 1 Comparison of resistance index conferred by different glyphosate-resistance mechanisms. Mean resistance indices (±1 standard error) were calculated from resistance ratios obtained from the literature. Data aggregated from: (Baerson et al. 2002b; Wakelin et al. 2004; Culpepper et al. 2006; Yu et al. 2007, 2015; Perez-Jones et al. 2007; Dinelli et al. 2008; Lamego and Vidal 2008; Jasieniuk et al. 2008; Kaundun et al. 2011; Chandi et al. 2012; Salas et al. 2012; Vila-Aiub et al. 2012; de Carvalho et al. 2012; Gaines et al. 2012; González-Torralva et al. 2012a; Bell et al. 2013; Moretti et al. 2013; Nandula et al. 2013, 2014; Mohseni-Moghadam et al. 2013; Lorentz et al. 2014; Wiersma et al. 2015; Alcántara-de la Cruz et al. 2016b, a; Brunharo et al. 2016, 2019; Kleinman and Rubin 2017; Yanniccari et al. 2017; Amaro-Blanco et al. 2018; Morran et al. 2018; Ngo et al. 2018b, a; Pandolfo et al. 2018; Beres et al. 2018; Li et al. 2018; Brunharo and Hanson 2018; Takano et al. 2019; Perotti et al. 2019)

2.2 Rapid Response (Phoenix Phenomenon)

Because glyphosate's herbicidal activity involves plants starving for aromatic amino acids, it generally takes several days for plants to die after application. First documented in 2008, some biotypes of *Ambrosia trifida* have evolved a rapid-response glyphosate-resistance mechanism in which leaves treated with the herbicide quickly wither and fall from the plant (Brabham et al. 2011; Moretti et al. 2018; Van Horn et al. 2018). This rapid cell death limits the ability of the herbicide to move throughout the plant and, therefore, can be considered a "reduced translocation" mechanism. After shedding tissue containing glyphosate, the plant begins new growth, seemingly from the ashes of a dead plant, and hence the name "Phoenix" phenomenon. This rapid cell death also affects the efficacy of other herbicides included in the spray mixture, because translocation is generally inhibited (Harre et al. 2018). Though this mechanism is still not well understood, it can be reversed with the application of exogenous phenylalanine and tyrosine, indicating that it is somehow involved with a deregulation of the shikimate pathway (Moretti et al. 2018). Increased accumulation of reactive oxygen species following glyphosate application in leaf discs displaying the rapid-response phenotype when compared

to sensitive leaf discs points to the possibility that an accumulation of reactive oxygen species plays a role in rapid cell death, though this remains to be elucidated. It is assumed that this resistance mechanism requires an actively metabolizing plant, given rapid-response plants do not display rapid cell death in the absence of light and sucrose (Moretti et al. 2018).

Recently, Queiroz et al. (2020) reported a similar resistance phenotype to the auxinic herbicide 2,4-D in *Conyza sumatrensis*, in which herbicide application results in hydrogen peroxide accumulation and rapid cell death. While this report means the rapid-response resistance mechanism no longer is unique to glyphosate, it is unknown how similar the two resistance mechanisms are at the molecular level.

2.3 Metabolism

Studies of metabolic-based herbicide-resistance mechanisms began occurring in earnest in the United Kingdom and Australia in the mid-1980s due to increasing cases of resistances particularly in *Alopecurus myosuroides* and *Lolium* spp. (Moss and Cussans 1985; Heap and Knight 1986). Documented cases have become more common in recent years with the innovation of biochemical and genetic tools that allow researchers to identify specific genes and metabolic pathways conveying such resistance. Given that glyphosate is a relatively slow-acting herbicide that causes depletion of aromatic amino acids, enhanced metabolism would be a highly effective mechanism of resistance; one in which plants would be able to detoxify the herbicide before the significant injury occurred.

Many known cases of metabolic herbicide resistance are due to mutated or overexpressed cytochrome P450, glucosyltransferase, or glutathione S-transferase enzymes (Yuan et al. 2007; Yu and Powles 2014). These enzymes belong to large protein families and have many roles in primary and secondary metabolism, with some having specificity to herbicide molecules. To date, there has been no report of a protein from either of these families that significantly interacts with glyphosate in plants. However, Van Etten et al. (2020) reported several genomic regions of *Ipomoea purpurea* that are associated with an increase in glyphosate tolerance and enriched for genes from these families. Further physiological validation to confirm the roles of these gene families in glyphosate metabolism may help elucidate previously reported variation of glyphosate tolerance within and among populations of this species (Baucom and Mauricio 2010; Kuester et al. 2015).

Two enzymes have been reported to metabolize glyphosate: glyphosate oxidoreductase (GOX), which cleaves a C-N bond within glyphosate, and carbon-phosphorus (C-P) lyase, which cleaves glyphosate's C-P bond (Liu et al. 1991; Van Eerd et al. 2003; also see Fig. 3 in Green and Siehl 2021). An unknown enzyme that acts similarly to GOX is suspected to be the primary catalyst for glyphosate detoxification in plants (Reddy et al. 2008). Along with the primary product of GOX-mediated detoxification of glyphosate, aminomethylphosphonic acid (AMPA), several other metabolites of glyphosate have been detected in higher

plants, including glycine, glyoxylate, sarcosine, formaldehyde, and inorganic phosphate (Marshall et al. 1987; Duke 2011; Rojano-Delgado et al. 2012; Gomes et al. 2014). Formaldehyde and hydrogen peroxide are compounds associated with C-P lyase-mediated metabolism of glyphosate, and their phytotoxicity in plants may explain why C-P lyase has not evolved to be the primary catalyst of glyphosate degradation in plants (Mutters et al. 1993; Goyer et al. 2004; Reddy et al. 2008). Although most metabolites of GOX-mediated glyphosate degradation are common compounds in plants and are unlikely to cause damage, AMPA has some evidence of phytotoxicity in plants. For example, AMPA was shown to accumulate as a result of glyphosate application and to cause injury in glyphosate-resistant soybean (Hoagland 1980; Duke 2011). Gomes et al. (2014) hypothesized that AMPA's phytotoxic effects are the result of competitive inhibition of glycine decarboxylase, thereby inhibiting chlorophyll biosynthesis. However, a microbial GOX has been used as a transgene to successfully confer glyphosate resistance to tobacco and rape, indicating that these plant species – and likely others – possess the molecular machinery sufficient for further metabolizing any products of GOX-mediated glyphosate metabolism (Duke 2011; Pollegioni et al. 2011).

Previously, de Carvalho et al. (2012) showed increased metabolism of glyphosate in resistant varieties of *Digitaria insularis* when compared to sensitive varieties, but failed to tease this effect from other possible mechanisms in the population. Additionally, Rojano-Delgado et al. (2012) proposed that glyphosate metabolism worked in conjunction with limited uptake and translocation to convey glyphosate tolerance in *Mucuna pruriens* but failed to quantify these effects. Recently, an aldo-keto reductase (AKR) enzyme was found to metabolize glyphosate to AMPA and glyoxylate in an Australian population of *Echinochloa colona*, just as GOX does in bacteria (Pan et al. 2019). While no variation in coding sequence of this AKR delimited resistant and sensitive populations, increased expression was shown to be associated with resistance to glyphosate. To further verify this AKR as the causative agent of glyphosate resistance, rice was transformed with AKR cDNA from *E. colona*. Calli and seedlings overexpressing the transcript and displaying increased AKR activity were resistant to glyphosate (Pan et al. 2019). McElroy and Hall (2020) later revisited this population of *E. colona*, however, and discovered the presence of the Pro-106-Thr substitution encoded within *EPSPS*, a target site mutation previously shown to reduce EPSPS affinity for glyphosate in this species (Alarcón-Reverte et al. 2015; Han et al. 2016). This discovery obscures, but does not eliminate, the effect of increased expression of AKR on the evolution of glyphosate resistance in *E. colona*. In any case, the discovery of AKR's role in glyphosate metabolism emphasizes the need for future metabolism research efforts to treat all candidate genes as true candidates in lieu of searching solely for common herbicide metabolism genes such as cytochrome P450s or glutathione S-transferases. In short, metabolism of glyphosate seems to have the potential to be a viable mechanism of resistance, and it is surprising that more cases of metabolism-based resistance have not been documented.

3 Target-site Resistance

3.1 Insensitive Target Site

Within the context of glyphosate resistance, an insensitive target site occurs through modifications to the primary amino acid sequence of EPSPS (Heap and Duke 2018). When considering the total length of the enzyme (520 amino acids – GenBank accession AT2G45300), relatively few amino acids are associated with resistance. That there are few target-site mutations for glyphosate resistance is attributed to the similarity in how glyphosate and phosphoenolpyruvate bind the EPSPS enzyme (Schönbrunn et al. 2001). Such similarity means that structural changes that reduce EPSPS affinity for glyphosate likely will also reduce its affinity for the phosphoenolpyruvate substrate. Indeed, only three amino acid positions have been implicated in evolved herbicide resistance in weed species: Thr-102, Ala-103, and Pro-106 (Murphy and Tranel 2019). Amino acid substitutions at the Pro-106 position, to Ser, Leu, Thr, or Ala, alone are sufficient for resistance to glyphosate (Heap and Duke 2018; Morran et al. 2018; Brunharo and Hanson 2018). Substitutions at Thr-102 and Ala-103 generally have only been observed coexisting with Pro-106 substitutions. Previously, Thr-102 substitutions observed in combination with Pro-106 substitutions contained Ile as the substitute amino acid, and it was suggested that this Thr-102-Ile mutation would not occur on its own because of its negative effect on EPSPS enzyme activity (Sammons and Gaines 2014). Recently, however, a Thr-102-Ser substitution was identified to confer glyphosate resistance in the tetraploid *Tridax procumbens* (Li et al. 2018). Effects of substitution at Ala-103 are not well known, and this substitution has been observed only in a triple substitution referred to as TAP-IVS (Thr-102, Ala-103, and Pro-106 are substituted with Ile, Val, and Ser, respectively) in Argentinian *Amaranthus hybridus*, (García et al. 2019; Perotti et al. 2019). Green and Siehl (2021) in this same Volume provide further discussion of the effects of different amino acid substitutions on EPSPS kinetics, and a database of EPSPS amino acid changes conferring glyphosate resistance in weeds is maintained by Gaines and Heap (2020).

Experiences with resistance to other herbicide groups, particularly to inhibitors of photosystem II, acetolactate synthase, and acetyl-CoA-carboxylase, have indicated that single amino acid changes to herbicide target sites confer very robust levels of resistance relative to nontarget-site resistance mechanisms (Powles and Yu 2010). In the case of glyphosate, however, resistance derived from a single target-site substitution is often associated with weak resistance relative to other glyphosate-resistance mechanisms (Fig. 1). Consistently, single mutation events provide some of the lowest levels of resistance when compared to all other mechanisms in both grass and broadleaf weed species. In comparison, the documented double and triple substitutions to EPSPS confer resistance levels greater than those provided by nontarget-site mechanisms. This is consistent with attempts to develop glyphosate resistance traits in crops through site-directed mutagenesis. The pairing of Thr-102-Ile and Pro-106-Ser substitutions, which has evolved in weeds, also resulted in

commercial resistant germplasm developed through site-directed mutagenesis (Dill 2005). Indeed, the introduction of single point mutations through ethyl methanesulfonate was widely unsuccessful in the creation of an acceptable resistance phenotype for commercial use, consistent with modest levels of resistance conferred by single amino acid substitutions in EPSPS.

3.2 EPSPS Gene Duplication

Beginning with its discovery in *A. palmeri* in 2010 (Gaines et al. 2010), EPSPS gene duplication has become a relatively common mechanism of glyphosate resistance. Thus far, three other broadleaf species (*A. tuberculatus*, *Amaranthus spinosus*, and *Bassia scoparia*) and six grass species (*Lolium perenne*, *Bromus diandrus*, *E. indica*, *Chloris truncata*, *Poa annua*, and *Hordeum glaucum*) have evolved glyphosate resistance via this mechanism (Salas et al. 2012; Nandula et al. 2014; Lorentz et al. 2014; Jugulam et al. 2014; Chen et al. 2015; Malone et al. 2016; Ngo et al. 2018a; Adu-Yeboah et al. 2019; Brunharo et al. 2019). These species appear to require differing levels of genomic copies for resistance. Three of these species, *A. tuberculatus*, *B. scoparia*, and *H. glaucum*, show resistance with relatively low numbers of *EPSPS* genomic copies (Fig. 2), often between 3 and 14, with a minimum of three copies needed to confer glyphosate resistance (Lorentz et al. 2014; Wiersma et al. 2015; Chatham et al. 2015; Godar et al. 2015; Adu-Yeboah et al. 2019). One study has reported plants with >15 *EPSPS* copies in *A. tuberculatus* (Dillon et al. 2017), but this appears to be the exception to the norm. EPSPS expression mostly correlates with *EPSPS* genomic copy number for *B. scoparia* and *A. tuberculatus* but does not correlate well with resistance level, with most resistant accessions showing similar levels of glyphosate resistance despite varying levels of *EPSPS* copies (Fig. 2). Few studies examine all potential glyphosate resistance mechanisms, so some of this disconnect between *EPSPS* copy number and resistance may be due to the presence of alternative mechanisms of resistance. For *H. glaucum*, there was no correlation between copy number and expression, but some evidence of correlation between copy number and glyphosate resistance (Adu-Yeboah et al. 2019).

In contrast, for all other species with this resistance mechanism, at least 10 *EPSPS* gene copies have been shown to be necessary for resistance. Around 10–36 copies have been documented in *B. diandrus* (Malone et al. 2016), 32–48 copies in *C. truncata* (Ngo et al. 2018a), and 33–37 in *A. spinosus* (Nandula et al. 2014). These mid-range levels of EPSPS copy numbers confer approximately the same level of resistance to glyphosate (compared to a sensitive control) as observed in *B. scoparia* and *A. tuberculatus*, with resistance between about 3- and 7-fold. Much higher *EPSPS* copy numbers have been observed in *A. palmeri* (35–160); (Gaines et al. 2010), *L. perenne* (11–151); (Salas et al. 2012), and *E. indica* (89); (Chen et al. 2015), with both *A. palmeri* and *L. perenne* demonstrating increasing levels of glyphosate resistance with increasing numbers of *EPSPS* gene copies (Fig. 2).

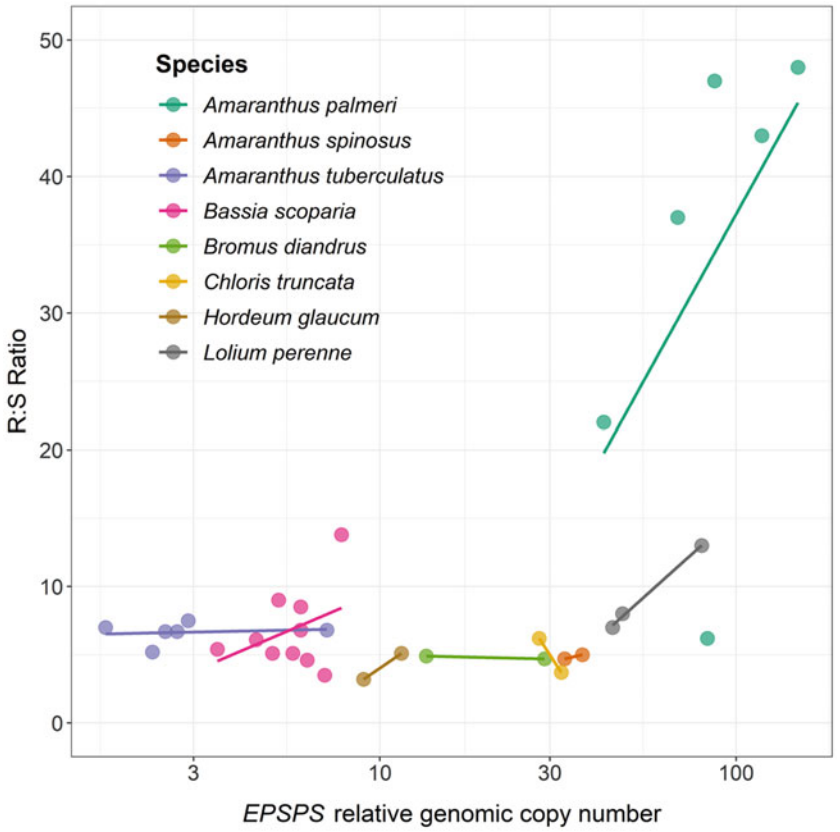

Fig. 2 Average *EPSPS* genomic copy number plotted against the resistant:susceptible (R:S) ratio for glyphosate-resistant populations. Each dot is a single population and each color indicates a different weed species, with linear regression lines plotted separately for each species. Data aggregated from (Gaines et al. 2010; Chandi et al. 2012; Salas et al. 2012; Nandula et al. 2014; Lorentz et al. 2014; Wiersma et al. 2015; Godar et al. 2015; Malone et al. 2016; Chahal et al. 2017; Ngo et al. 2018b; Singh et al. 2018; Adu-Yeboah et al. 2019)

Eleusine indica has shown a positive correlation between *EPSPS* gene copy number and expression, but whether this translates to higher levels of glyphosate resistance is not yet known (Chen et al. 2015). A population of *P. annua* was reported with 18-fold resistance to glyphosate but with only seven *EPSPS* copies (Brunharo et al. 2019). This was a novel case, however, in which, for the first time, it was reported that the duplicated *EPSPS* gene also encoded a glyphosate-resistant enzyme (Pro-106-Leu). The relative contribution of each (duplication and mutation) to glyphosate resistance is unknown, but they both likely contributed, because the magnitude of resistance was greater than that typically conferred by either mechanism alone (Fig. 1).

The correlation of *EPSPS* genomic copy number and the resistance phenotype has been investigated in multiple species, as indicated with some examples just discussed. At a population level, a positive correlation between genomic copy number and resistance has been reported within *B. scoparia*; however, this relationship does not appear to be linear (Godar et al. 2015; Gaines et al. 2016). In some cases, relationships may be population-specific, suggesting that each evolved event may follow a distinct relationship (Gaines et al. 2016). In fact, this is well supported by the meta-analysis shown in Fig. 2, because a diversity of relationships are observable among species. For instance, while a strong linear correlation is observed within *A. palmeri* data points, such a correlation is not consistent in other species. The relationship between genomic copy number and resistance should be established for each species, if not for each evolved instance of this mechanism. Breakdowns in the relationship between genomic copy number and resistance are not wholly unexpected. For example, an increase in genomic copy number is several steps removed from an increase in protein abundance. Consequently, demonstration of an elevated protein abundance is necessary to attribute increases in genomic copy with the resistance phenotype. And, as previously mentioned, the coexistence of one or more other resistance mechanisms within individual plants can be a confounding factor and typically can be ruled out only by further genetic analyses.

4 Distribution of Resistance Mechanisms Among Species

Of the 48 glyphosate-resistant weed species, there is strong evidence for the existence of a particular resistance mechanism in 29 of them (Table 1). Although only one glyphosate-resistance mechanism has been documented in 16 weed species, there are 9 species for which two mechanisms have been reported and 4 species in which three different mechanisms have been reported. Reduced uptake/translocation and single EPSPS amino acid substitutions are the most common mechanisms, with each having been reported in 14 different weed species. As mentioned above, gene duplication, although not known as a herbicide-resistance mechanism prior to glyphosate resistance, is now also a quite common glyphosate-resistance mechanism, being reported in 10 different weed species.

In general, there do not appear to be significant differences in the distributions of glyphosate-resistance mechanisms between grass and broadleaf weed species. In fact, the three most common categories of mechanisms shown in Table 1 (reduced uptake/translocation, single EPSPS substitution, and *EPSPS* duplication) are surprisingly evenly distributed, with *EPSPS* duplication showing the greatest deviation from 1:1 (4 broadleaf species:6 grass species). However, as noted above (Sect. 2.1), reduced glyphosate uptake tends to be more common in grass than in broadleaf species.

There are 19 reported glyphosate-resistant weeds for which resistance mechanisms have not yet been confirmed. It will be interesting to see what new glyphosate-resistant mechanisms might be found in these weeds. To be sure, there very well

might be additional resistance mechanisms, which simply have not been identified yet, in the 29 species for which mechanisms have already been reported. And, of course, new glyphosate-resistant species certainly will be added to the list shown in Table 1. It should also be noted that the categories of resistance mechanisms listed in Table 1 underreport the variety of mechanisms at the molecular level. For example, as discussed in Sect. 3.1, a variety of single amino acid substitutions can confer glyphosate resistance, but they are all grouped together under the category of "single target site mutation" in Table 1. Additionally, quite a variety of molecular mechanisms associated with a variety of different genes could contribute to altered glyphosate uptake/translocation. Clearly, weeds have evolved diverse mechanisms to survive glyphosate, and more mechanisms likely await future discovery.

5 Evolutionary Origins of Resistance

As described in Sects. 2 and 3, glyphosate resistance can be mediated by a variety of mechanisms. These resistance mechanisms arise as a result of changes to one or more locations in the genome, resulting in structural or regulatory changes to gene products. Genetic changes that are beneficial (e.g., confer reduced sensitivity to glyphosate) are selected and increase in frequency in the selected populations. The source of the genetic differences that can be selected include standing genetic variation (i.e., they already exist in the population before the onset of selection), immigration from a different population or species, or new mutations. As is the case with resistance to other herbicides, the relative contribution of these sources for glyphosate-resistance evolution are largely unknown (Casale et al. 2019). Ultimately, a better understanding of the evolution of herbicide resistance could lead to novel strategies to mitigate it (Neve et al. 2009).

Naturally occurring plant tolerance cases to a given chemistry may provide insight into what mechanisms may evolve in the future. Several plant species have exhibited a natural tolerance to glyphosate, although the underlying mechanisms have been investigated in few cases. In both *Convolvulus arvensis* and lilyturf species, gene copy number has been attributed to at least part of the observed tolerance phenotype (Mao et al. 2016; Huang et al. 2019). However, these tolerance cases are frequently due to a combination of mechanisms. In lilyturf species, for example, EPSPS structural differences were also noted, relative to other plant EPSPS enzymes, due to multiple amino acid substitutions and deletions Both modeling and in vitro enzyme assays indicated that these structural differences resulted in reduced glyphosate sensitivity (Mao et al. 2016). In *C. arvensis*, a promoter-mediated overexpression, associated with glyphosate application, was also observed in addition to increased *EPSPS* copy number (Huang et al. 2019). Reduced glyphosate translocation was associated with increased tolerance in *Ipomoea lacunosa* (Ribeiro et al. 2015), whereas increased glyphosate metabolism is hypothesized to confer tolerance in *I. purpurea* (Van Etten et al. 2020). While there does not appear to be an overarching trend in tolerance mechanisms among species, similar mechanisms are

observed across plant tolerance and resistance. The structure-based tolerance of lilyturf could be considered analogous to target-site resistance. Promoter-mediated overexpression, resulting in an increase in EPSPS protein abundance, also has been occasionally associated with evolved resistance (Baerson et al. 2002a). Gene copy number increase and reduced translocation both have been implicated in both tolerance and evolved resistance. The investigation of tolerance mechanisms to a given chemistry, even beyond the scope of glyphosate, can provide insight into what mechanisms might evolve in response to selection.

There have been a couple of cases in which a weed evolved glyphosate resistance via gene flow from a related species. In one case, the weed *Brassica rapa* acquired the transgene (*CP4 EPSPS*) conferring glyphosate resistance from cultivated rape (Warwick et al. 2003). This evolutionary path to glyphosate resistance in *B. rapa* subsequently has been shown to be a common event and has occurred in multiple countries (Simard et al. 2006; Pandolfo et al. 2018). Another case involves weed-to-weed gene flow, in which *A. spinosus* acquired *EPSPS* gene duplication that had evolved in *A. palmeri* (Nandula et al. 2014). These cases are the exception to the norm, however, and most weed species have evolved glyphosate resistance from either standing genetic variation or new mutations.

5.1 Nontarget-Site Mechanisms

In general, nontarget-site glyphosate resistance mechanisms are still poorly understood, and even less is known about their evolutionary origins. In regard to enhanced detoxification, because glyphosate is metabolized readily through multiple pathways in bacteria, horizontal gene transfer could certainly be a source of resistance, though no evidence exists for this having occurred. As discussed above, AKR likely plays a role in glyphosate resistance in *E. colona*, perhaps via enhanced expression, and remains the only plant protein proven to directly metabolize glyphosate (Duke 2019). The evolutionary origin of enhanced expression of AKR in *E. colona*, or of any other herbicide-metabolizing enzyme selected in weed populations, remains unknown. Now that AKR has been identified to metabolize glyphosate, evaluation of homologous genes in other weed species likely will follow and should reveal the potential of AKR to confer glyphosate resistance in other species.

Because inheritance studies have not yet been published regarding the rapid-response glyphosate-resistance mechanism, its genetic complexity is not known. Additionally, though similarities exist with a recently identified resistance mechanism to 2,4-D (Queiroz et al. 2020), it is unclear if these rapid response mechanisms have any evolutionary relatedness. The similarities with plant pathogen response (e.g., hypersensitivity and rapid cell death) suggest this mechanism evolved by somehow co-opting a pathway for plant defense against abiotic attack (Roden and Ingle 2009).

As discussed above, glyphosate resistance due to vacuolar sequestration might be mediated by an ABC transporter and has been most studied in *C. canadensis*. Just as enhanced herbicide metabolism can evolve through increased expression of a herbicide-metabolizing enzyme, sequestration could evolve through increased expression of an ABC transporter. A previous study found glyphosate resistance in *C. canadensis* to be mediated by a single gene (Zelaya et al. 2004), although the identity of that gene is unknown. Increased expression of both *EPSPS* and ABC transporters in a glyphosate-resistant *C. canadensis* biotype prompted Margaritopoulou et al. (2018) to investigate methylation of the *EPSPS* gene. Their finding of differential *EPSPS* methylation between resistant and sensitive biotypes suggests epigenetic changes could be playing an evolutionary role. The contribution of epigenetic changes to herbicide-resistance evolution in general, not just specifically to glyphosate, remains an unanswered question (Markus et al. 2018).

Nontarget-site herbicide resistance offers the field of weed science many novel research questions to be answered through a variety of omics-based approaches (Maroli et al. 2018; Patterson et al. 2019a). The recent establishment of an International Weed Genomics Consortium promises the development of reference genome assemblies for many of the world's most problematic weeds (Ravet et al. 2018). This effort will supplement other recent but less coordinated efforts to produce genomic resources for driver weed species, including *L. multiflorum* (Copetti et al. 2019), *A. tuberculatus* (Kreiner et al. 2019), *B. scoparia* (Patterson et al. 2019b), and *C. canadensis* (Laforest et al. 2020). The availability of these genomic resources enables genetic mapping of traits such as glyphosate resistance (Korte and Farlow 2013; Van Etten et al. 2020) and will complement previous transcriptomic studies designed to identify candidate genes that may be involved in herbicide resistance (Piasecki et al. 2019). The identification of genomic regions associated with the trait of interest, via a genetic mapping experiment, allows for the filtering of candidate genes identified via expression- or variant-based transcriptomic analyses and hedges against the possibility that the trait is ultimately controlled by some regulatory element located far from the genes that would be identified through expression-based transcriptomic approaches. These filtered candidates should be judged, based on physiological characteristics of the trait, and functionally validated via loss- or gain-of-function experiments (Sauka-Spengler and Barembaum 2008; Housden et al. 2017). Pan et al. (2019) provide a good model for functional validation of a glyphosate-resistance gene (AKR), but additional genetic study may have identified the second locus (*EPSPS*, see Sect. 2.3) contributing to glyphosate resistance. With the identification of the genes involved in nontarget-site glyphosate resistance, researchers will be able to better understand the evolutionary origins of such resistance and predict how likely it is that other species will evolve similar resistance mechanisms in the future.

5.2 *EPSPS Gene Duplication*

Because of the novelty and importance of *EPSPS* gene duplication as a resistance mechanism, its evolutionary origin is of great interest and has been addressed in several studies (Patterson et al. 2018). Except for the case of *A. spinosus*, wherein the *EPSPS* amplicon from *A. palmeri* introgressed into the *A. spinosus* population after a hybridization event (Nandula et al. 2014), *EPSPS* gene duplication evolved independently in each of these species. Accordingly, the mechanism of duplication and the length and content of the *EPSPS* amplicon varies across the different species. For two species with relatively low *EPSPS* copy numbers, *A. tuberculatus* and *B. scoparia*, cytogenomic analysis using fluorescent in situ hybridization (FISH) has shown the duplicated *EPSPS* genes are arranged as tandem repeats along one chromosome pair. In *B. scoparia*, these tandem repeats of *EPSPS* occurred at the distal end of one pair of homologous chromosomes, with approximately 40–70 kb between *EPSPS* genes and one copy inverted compared to the rest (Jugulam et al. 2014). The tandem arrangement of the *EPSPS* genes and their location in the telomeres suggests an unequal recombination-based mechanism of gene duplication since unequal crossing over occurs most frequently in telomeric regions of the chromosome and leads to tandem duplications. Similarly, in *A. tuberculatus*, the *EPSPS* repeats were found to occur at a single locus in one set of homologous chromosomes, but unlike in *B. scoparia*, these repeats were in the pericentromeric region of the chromosome, where recombination is less likely to occur (Dillon et al. 2017). Whether the mechanism of gene duplication in this species is also unequal recombination or some other form of chromosomal rearrangement or segmental duplication is unknown.

To further complicate the story, some *A. tuberculatus* individuals with higher *EPSPS* copy numbers (>15 copies) showed multiple *EPSPS* signals on an additional small chromosome (Dillon et al. 2017). Further cytogenomic work found this extra chromosome to be a ring chromosome that was derived from the pericentromeric region of the chromosome with multiple *EPSPS* gene duplications (Koo et al. 2018a). FISH assays of F_1 progeny showed variation in the size and *EPSPS* copy number of these ring chromosomes across different individuals and, surprisingly, additional *EPSPS* gene copies on other pairs of chromosomes, indicating reintegration of the ring chromosomes into the linear chromosomes through ectopic recombination (Koo et al. 2018a). The hypothesized model of ring chromosome formation includes breakage of the linear chromosome at two spots flanking the original *EPSPS* gene duplicates (perhaps via aneuploidy-triggered destabilization), followed by fusion of the broken chromosome ends into a shortened linear chromosome. The excised middle region containing one or more *EPSPS* genes then undergoes fusion of its proximal ends to form a ring chromosome, that may then form varying sizes of ring chromosomes via a breakage-fusion-bridge cycle model (Koo et al. 2018a). Work looking into the *EPSPS* gene duplication mechanism in *A. palmeri* has found similar results, with the additional *EPSPS* gene copies occurring on extrachromosomal DNA. In the initial report of gene duplication in this species, a FISH image

showed *EPSPS* gene signals distributed across all 34 chromosomes of *A. palmeri* (Gaines et al. 2010), but a later study (Koo et al. 2018b) showed these gene signals were not actually on the linear chromosomes but were located on extrachromosomal circular DNA (eccDNA) tethered to the main chromosomes. Inheritance of these eccDNA molecules was highly variable and displayed unequal mitotic segregation, illustrating the need for glyphosate selection for retention of glyphosate-resistant plants with high numbers of *EPSPS* copies. Further work has highlighted that these eccDNA molecules are highly structured with 59 genes, 41 of which are expressed under glyphosate application, and a complex array of mobile genetic elements, repeat sequences, and clustered palindromes (Molin et al. 2017, 2020). The contribution of these additional genes/sequences to the overall resistance phenotype is unknown. Syntenic analysis using genomic assembly of closely related species (*Amaranthus hypochondriacus* and *A. tuberculutus*) suggested that the eccDNA was built from several regions across the genome, rather than derived from a single locus (Molin et al. 2020). Consequently, some of the genes (in addition to *EPSPS*) within the eccDNA may have been selected by glyphosate. An alternative hypothesis is that one or more genes in the eccDNA were selected in the evolutionary past by some other plant stress, and *EPSPS* happened to get captured within the amplicon, priming the species for the later evolution of glyphosate resistance.

In grass species with the *EPSPS* gene duplication mechanism, some recent publications have begun to shed light on the arrangement and origin of the *EPSPS* gene copies. In *L. perenne* ssp. *multiflorum*, FISH mapping of the *EPSPS* gene on somatic metaphase chromosomes revealed a similar pattern as that observed in *A. palmeri*, with *EPSPS* signals distributed across all chromosomes in plants with high *EPSPS* gene copy number (Putta 2017). As with *A. palmeri*, the signals appeared to be on the outer edges of the chromosomes, perhaps indicating a similar mechanism of gene duplication involving circular extrachromosomal DNA tethered to the main chromosomes, but conclusive evidence of this does not yet exist. Conversely, in *E. indica*, *EPSPS* gene copies in a resistant individual appeared to be restricted to two pairs of homologous chromosomes, as indicated by FISH work in this species (Chen et al. 2019). In *B. diandrus*, no FISH assays have yet been published, but inheritance work has shown F_2 offspring to have a range (3–30) of *EPSPS* gene copies, with all F_2 offspring showing an increase in the baseline copy number (Malone et al. 2016). If the *EPSPS* gene copies were inherited as a single locus, as would be expected in a tandem repeat model, 25% of the F_2s should have a single *EPSPS* copy, and the fact that this is not observed indicates these *EPSPS* gene copies likely occur on multiple chromosomes. For the other three grass species (*C. truncate*, *H. glaucum*, and *P. annua*), no cytogenetic or inheritance work has yet been completed and the mechanism of *EPSPS* gene duplication is unknown.

Gene duplication as a herbicide-resistance mechanism thus far has been reported in only one other case, resistance to acetyl-CoA-carboxylase inhibitors (Laforest et al. 2017). Why, then, has it repeatedly evolved for glyphosate resistance? As can be seen in Fig. 1, besides multiple amino acid substitutions in EPSPS, gene duplication confers the highest magnitude of resistance among the known resistance mechanisms evolved to date. Perhaps *EPSPS* duplication is the evolutionary "path of least resistance" for robust glyphosate resistance (Tranel 2017).

Recent population genetics analysis of glyphosate-resistance evolution in *A. tuberculatus* indicated that *EPSPS* duplication in this species – which appears to be due primarily to tandem duplications – independently occurred multiple times (Kreiner et al. 2019). In contrast, the *EPSPS*-containing eccDNA in *A. palmeri* was nearly identical among geographically dispersed populations, suggesting a single evolutionary origin (Molin et al. 2018). Conservation of the eccDNA among these populations suggests a relatively recent evolutionary event, arguing against the hypothesis mentioned above, that the amplicon was selected by some plant stress prior to glyphosate selection. Kreiner et al. (2019) presented evidence suggesting *EPSPS* duplication preexisted as standing genetic variation in *A. tuberculatus*, in contrast to the eccDNA in *A. palmeri* being a relatively recent event. Certainly, more work is needed, but comparison of these two species suggests that tandem duplication is a higher probability event than the eccDNA-based duplication. Why these two related species used different evolutionary paths to *EPSPS* duplication is unknown. One possibility is that tandem duplication may not have evolved as a glyphosate-resistance mechanism in *A. palmeri* because this species is inherently more sensitive than *A. tuberculatus* to glyphosate. Therefore, *A. palmeri* needed tens of copies of *EPSPS* for resistance, which was enabled only after evolution of the *EPSPS*-containing eccDNA. In fact, if the linear correlation between *EPSPS* copy number in *A. palmeri* and resistance magnitude shown in Fig. 2 is extrapolated, resistance would not be observed below 10 copies. As mentioned above, it is also possible that other genes within the eccDNA augment the glyphosate resistance conferred by *EPSPS* duplication.

5.3 Target-Site Mutations

The relative contributions of standing genetic variation versus new mutations for target-site resistance likely vary among herbicides. In the case of target-site resistance to glyphosate, repeated occurrence of double mutations and the occurrence of a triple mutation (discussed in Sect. 3.1) present additional evolutionary questions. These multiple-mutation alleles could preexist in a population as part of the standing genetic variation, or the multiple mutations could arise sequentially during the course of herbicide selection. In addition, the spontaneous occurrence of a double or triple-mutation allele (e.g., both or all three of the mutations occurring in a single generation) is formally possible, but the probability is so low that this route probably can be considered inconsequential (Ossowski et al. 2010). Sequential evolution could occur by a second mutation occurring in an allele that already has one mutation, or via recombination between two alleles each carrying one of the two mutations. Given the close proximity of the double and triple mutation sites in the gene, however, recombination between them will be exceedingly rare. Therefore, the two most likely evolutionary paths to the multiple-mutation alleles are either they existed prior to selection or a single-mutation allele increased in frequency as a result of herbicide selection, and then acquired one or more additional mutations.

If a multiple-mutation allele preexisted in the population, then one would expect it to have a limited fitness cost, because a large fitness cost would result in it having been purged from the population. From limited studies to date on fitness costs of multiple-mutation *EPSPS* alleles, however, at least some seem to have significant fitness costs (see Sect. 5.4). Additionally, if a multiple-mutation allele preexisted, one would expect to find this allele in essentially all resistant plants, i.e., occurrence of alleles containing only one of the mutations would be rare (since they would only come about via recombination or a mutation back to wild type). In an *E. indica* population with the Thr-102-Ile + Pro-106-Ser double mutation, both the double mutant and the single mutant Pro-106-Ser, but not the single mutant Thr-102-Ile, allele were found at high frequencies, leading the authors to conclude that the two mutations evolved sequentially (Yu et al. 2015).

In the cases of multiple-mutation *EPSPS* alleles in *Bidens subalternans* (double mutant) and *A. hybridus* (triple mutant), however, only the multiple-mutant alleles were observed (Perotti et al. 2019; Takano et al. 2020), which is consistent with the alleles preexisting in the population. Furthermore, because *B. subalternans* is tetraploid, it was suggested that fitness cost of the double-mutation allele could be masked by the second, wild type *EPSPS* gene (Takano et al. 2020), which could explain how such an allele persisted in the population prior to glyphosate selection. Because the multiple-mutation alleles confer higher resistance than the single-mutation alleles, there are caveats with the expectation that lack of finding the single-mutation alleles is evidence of the multiple-mutation alleles preexisting in the population. For example, with repeated selection of glyphosate, especially with high doses, the multiple-mutation alleles will be favored over the single-mutation alleles and, therefore, the single-mutation alleles will be purged over time. Thus, one must consider the glyphosate selection timeframe. In addition, if the multiple-mutation allele arose sequentially in one population, but then migrated to a second population, analysis of the second population would incorrectly lead to support of the hypothesis that the multi-mutation allele preexisted.

In summary, there is good evidence that multiple-mutation *EPSPS* alleles evolved from sequential events in at least some cases. More evidence is needed, however, to conclude that glyphosate resistance also has evolved via selection of multiple-mutation *EPSPS* alleles that preexisted as part of the standing genetic variation of a population.

5.4 Fitness Costs

In many organisms, the evolutionary adaptation to a new environment or to a new selection pressure is often accompanied by tradeoffs that can affect the general fitness of the organism, commonly referred to as fitness cost (Purrington 2000; Strauss et al. 2002; Vila-Aiub 2019). The presence of resistance alleles in a biotype can cause pleiotropic effects that will enhance some negative phenotypes, such as lower number and viability of seeds, less biomass, and less attraction to pollinators.

All of these effects can prevent the fixation of resistance alleles, making the adaptation process occur slower (Tian et al. 2003; Vila-Aiub 2019). On the other hand, studies have also shown that, in some cases, no fitness cost was observed due to the presence of herbicide-resistance alleles (Vila-Aiub 2019). Understanding fitness costs related to the presence of herbicide resistance traits is important to understand the evolution patterns that these traits will follow (Cousens and Fournier-Level 2018).

Studies to investigate fitness cost due to glyphosate resistance have shown different results according to the mechanism of resistance involved. In the case of target-site glyphosate resistance, there is generally a correlation between higher levels of resistance and greater fitness costs (Vila-Aiub et al. 2019). For example, substitution of two amino acids in EPSPS in *E. indica* was accompanied by a high fitness cost, whereas a single mutation in the same species—which provided lower resistance—conferred a negligible fitness cost (Yu et al. 2015; Han et al. 2017). Fitness studies of *EPSPS* gene duplication generally have identified little if any fitness costs, although costs may be higher in certain genetic backgrounds (Giacomini et al. 2014; Vila-Aiub et al. 2014; Martin et al. 2017; Osipitan and Dille 2019). That *EPSPS* duplication does not confer a large fitness penalty is particularly surprising in *A. palmeri*, given both the large number of copies in resistant plants and the size of the amplicon (Vila-Aiub et al. 2019). The *EPSPS* amplicon in *A. tuberculatus* also appears quite large (Kreiner et al. 2019) but, nevertheless, only modestly decreased in frequency in a multi-generational fitness study (Wu et al. 2017). Vila-Aiub (2019) provides a recent and more comprehensive review of fitness costs associated with glyphosate resistance. When considering fitness costs of herbicide-resistance mechanisms, it is important to keep in mind that those mechanisms that confer extremely high fitness penalties are unlikely to be selected. Consequently, our vantage point is skewed by studying only those mechanisms that have evolved in weed populations.

6 Impacts on Weed Management

Widespread adoption of glyphosate-resistant crops resulted in reliance on glyphosate for weed control in those crops and a dramatic drop in the use of alternative herbicides (Young 2006). A primary impact of glyphosate-resistant weeds has been a reversal of that trend. Initially, farmers typically responded to glyphosate-resistant weeds by increasing the glyphosate use rate (Weller et al. 2010). However, because glyphosate-resistant weeds often can withstand maximum labeled use rates, such an approach was largely futile. The second approach often was to use a tank mix, spraying a second herbicide with glyphosate. For example, in the case of glyphosate-resistant *A. tuberculatus* and *A. palmeri*, a herbicide that inhibits protoporphyrinogen oxidase often was added. This is reflected in the use of these herbicides in the USA declining precipitously, beginning in 1996, but then beginning to increase in 2013, coinciding with increasing occurrence of glyphosate-

resistant *Amaranthus* populations (Dayan et al. 2018). Similar management responses, i.e., initially increasing the glyphosate rate, and then adding an appropriate tank-mix partner, were not restricted to USA farmers (Valverde 2010). Other chemical strategies such as returning to the use of soil-residual herbicides and rotating herbicides (e.g., not using glyphosate ever year) also were implemented in response to glyphosate-resistant weeds.

From the broader weed science industry perspective, a major impact of glyphosate-resistant crops was a decrease in herbicide discovery efforts (Duke 2012). Consequently, there are essentially no new herbicide options for farmers to turn to for combatting glyphosate-resistant weeds. This is particularly problematic for those weed populations that possess multiple resistance to other herbicides. Therefore, some farmers reluctantly responded to glyphosate-resistant weeds by implementing nonchemical strategies, including hand-weeding, tillage, and growing cover crops (Sosnoskie and Culpepper 2014; Duzy et al. 2016). Ironically, farmers are having to use diverse tactics to control glyphosate-resistant weeds, which are the same tactics that would have mitigated the evolution of these biotypes in the first place (Powles 2008).

Widespread adoption of glyphosate plus glyphosate-resistant crops also may have contributed to the range expansion of some of the weed species that evolved glyphosate resistance. *Conyza canadensis*, for example, was one of the first weeds to evolve glyphosate resistance, occurring originally in Delaware, USA (VanGessel 2001). Although at that time it was already a widespread weed in the USA (and elsewhere), long-distance wind dispersal of seeds with glyphosate resistance across a landscape heavily dominated by glyphosate-based weed management undoubtedly contributed to its invasiveness as a weed (Weaver 2001; Shah et al. 2014). Glyphosate resistance in both *A. tuberculatus* and *A. palmeri* also likely fostered their expansions. For example, glyphosate-resistant *A. tuberculatus* was identified in Canada, and at least one such population likely arrived via seed movement from the USA Midwest (Kreiner et al. 2019). Perhaps even more widespread dissemination of *A. palmeri* has occurred over the past few years, both within and beyond the USA, as a seed contaminant in, e.g., harvest equipment, livestock feed, and conservation-planting mixtures (Kistner and Hatfield 2018) and by migratory waterfowl (Farmer et al. 2017). To be sure, glyphosate resistance is not a prerequisite for the expansion of weed species, and maybe these weeds would have similarly expanded in a non-glyphosate scenario. However, it cannot be discounted that these weeds evolved glyphosate resistance in an era in which glyphosate was the sole means of chemical weed control in many fields, allowing populations of these species to explode in size. The increased population sizes increased the likelihood that seeds of these species would be disseminated.

Over the past few years, dicamba, coupled with dicamba-resistant crops, has been rapidly adopted in USA soybean and cotton production, largely to provide a solution for managing glyphosate-resistant weeds (Byker et al. 2013; Cahoon et al. 2015). It is unfortunate that glyphosate-resistant weeds have created such a demand for this technology, given the off-target concerns with dicamba, which are only exacerbated by wider adoption (Soltani et al. 2020).

The emergence of glyphosate resistance was the motivation behind an epidemiology approach to understand the spread of this resistance in *A. tuberculatus* (Evans et al. 2016). As expected, frequent use of glyphosate was identified as a key driver. However, this study also identified that, at least for glyphosate resistance in *A. tuberculatus*, the use of annual herbicide rotation was ineffective, whereas the use of herbicide mixtures was effective, at mitigating resistance evolution. A follow-up modeling study predicted that glyphosate-resistant *A. tuberculatus* evolution could have been even more effectively mitigated if practices such as herbicide mixing were coordinated at regional scales (Evans et al. 2018). Subsequently to these *A. tuberculatus* studies, a somewhat similar epidemiological approach was taken to proactively predict glyphosate-resistance evolution in *A. myosuroides* (Comont et al. 2019). Although glyphosate resistance has not yet been reported in this species, the study identified heritable variation for glyphosate sensitivity and that directional selection towards glyphosate resistance was occurring. Recently, there has been a call to increase the use of these types of epidemiological approaches to better predict, understand, and ultimately mitigate herbicide-resistance evolution in weeds (Comont and Neve 2020).

Currently, there is substantial interest in the development of novel, nonchemical, weed management technologies; much of this interest is largely (although not solely) attributable to glyphosate-resistant weeds. Examples of such new technologies include gene drives and robots (Neve 2018; McAllister et al. 2019). In retrospect, perhaps a positive outcome of the occurrence of glyphosate-resistant weeds will be spurred development of novel, nonchemical weed management strategies, which are particularly needed because the glyphosate-resistant crop era stifled the development of new herbicides.

Herbicide resistance is not a new phenomenon. In the 1990s, the widespread and rapid occurrence of resistance to inhibitors of acetolactate synthase taught us the importance of not relying on a single weed-control tactic (Tranel and Wright 2002). Apparently, that lesson was largely forgotten and then relearned through glyphosate-resistant weeds. Hopefully, this lesson will not be forgotten again.

7 Conclusion

Investigation of glyphosate-resistant weeds has revealed new mechanisms that weeds can evolve in response to intense herbicide selection. Although some of these mechanisms have been thus far associated exclusively or nearly exclusively with glyphosate resistance, now that they have been identified, it will be interesting to see if corresponding mechanisms for other herbicides are indeed rare, or simply have been overlooked. The source, i.e., new mutations vs. standing genetic variation, of adaptive glyphosate-resistance mechanisms remains largely unknown. Beyond herbicide resistance, the source of adaptive alleles is a fundamental and unresolved question in evolutionary biology. We suggest that glyphosate resistance, given its recent and rapid evolution, and the evolution of multiple adaptive mechanisms,

provides an appropriate model system for this broad evolutionary question. There is a need to increase the use of genomics approaches to better understand resistance mechanisms to glyphosate, as well as to other herbicides. In particular, genetic mapping, which is just beginning to become a viable strategy with the availability of assembled weed genomes, offers great promise for elucidating previously intractable herbicide-resistance mechanisms. Such studies, together with epidemiological and population genetics approaches, should shed much light on glyphosate-resistance evolution. Lessons learned from studying the evolution of glyphosate-resistant weeds likely could be broadly translated to inform mitigation strategies for future herbicides. However, a major challenge posed by glyphosate-resistant weeds is that they evolved in an era coinciding with reduced research and development for alternative herbicides, ironically owing to the success of glyphosate/glyphosate-resistant crops. Consequently, there is a dearth of new herbicides to manage glyphosate-resistant weeds. Although only time will tell, glyphosate-resistant weeds should serve as a lasting example of the perils of relying on a single pest-management strategy.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Adu-Yeboah P, Malone JM, Fleet B et al (2019) *EPSPS* gene amplification confers resistance to glyphosate resistant populations of *Hordeum glaucum* Stued (northern barley grass) in South Australia. Pest Manag Sci n/a. https://doi.org/10.1002/ps.5671

Alarcón-Reverte R, García A, Watson SB et al (2015) Concerted action of target-site mutations and high EPSPS activity in glyphosate-resistant junglerice (*Echinochloa colona*) from California. Pest Manag Sci 71:996–1007. https://doi.org/10.1002/ps.3878

Alcántara-de la Cruz R, Fernández-Moreno PT, Ozuna CV et al (2016a) Target and non-target site mechanisms developed by glyphosate-resistant hairy beggarticks (*Bidens pilosa* l.) populations from mexico. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01492

Alcántara-de la Cruz R, Rojano-Delgado AM, Giménez MJ et al (2016b) First resistance mechanisms characterization in glyphosate-resistant *Leptochloa virgata*. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01742

Amaro-Blanco I, Fernández-Moreno PT, Osuna-Ruiz MD et al (2018) Mechanisms of glyphosate resistance and response to alternative herbicide-based management in populations of the three *Conyza* species introduced in southern Spain. Pest Manag Sci 74:1925–1937. https://doi.org/10.1002/ps.4896

Baerson SR, Rodriguez DJ, Biest NA et al (2002a) Investigating the mechanism of glyphosate resistance in rigid ryegrass (*Lolium ridigum*). Weed Sci 50:721–730. https://doi.org/10.1614/0043-1745(2002)050[0721:ITMOGR]2.0.CO;2

Baerson SR, Rodriguez DJ, Tran M et al (2002b) Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiol 129:1265–1275. https://doi.org/10.1104/pp.001560

Baucom RS, Mauricio R (2010) Defence against the herbicide RoundUp® predates its widespread use. Evol Ecol Res 12:131–141

Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (Amaranthus tuberculatus). Weed Sci 61:460–468

Beres ZT, Ernst EE, Ackley BA et al (2018) High levels of glyphosate resistance in *Conyza canadensis* from agricultural and non-agricultural sites in Ohio and Iowa. Sci Rep 8:1–8. https://doi.org/10.1038/s41598-018-28163-w

Brabham CB, Gerber CK, Johnson WG (2011) Fate of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in the presence and absence of glyphosate. Weed Sci 59:506–511. https://doi.org/10.1614/WS-D-11-00050.1

Bracamonte E, Fernández-Moreno PT, Barro F, De Prado R (2016) Glyphosate-resistant *Parthenium hysterophorus* in the Caribbean islands: non target site resistance and target site resistance in relation to resistance levels. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01845

Bradshaw LD, Padgette SR, Kimball SL, Wells BH (1997) Perspectives on glyphosate resistance. Weed Technol 11:189–198

Brewer CE, Oliver LR (2009) Confirmation and resistance mechanisms in glyphosate-resistant common ragweed (*Ambrosia artemisiifolia*) in Arkansas. Weed Sci 57:567–573

Bromilow RH, Chamberlain K, Evans AA (1990) Physicochemical aspects of phloem translocation of herbicides. Weed Sci 38:305–314

Brotherton JE, Jeschke MR, Tranel PJ, Widholm JM (2007) Identification of *Arabidopsis thaliana* variants with differential glyphosate responses. J Plant Physiol 164:1337–1345. https://doi.org/10.1016/j.jplph.2006.08.008

Brunharo CA, Patterson EL, Carrijo DR et al (2016) Confirmation and mechanism of glyphosate resistance in tall windmill grass (*Chloris elata*) from Brazil. Pest Manag Sci 72:1758–1764. https://doi.org/10.1002/ps.4205

Brunharo CACG, Hanson BD (2018) Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: Characterization, mechanism of resistance, and chemical management. Weed Sci 66:696–701. https://doi.org/10.1017/wsc.2018.50

Brunharo CADCG, Morran S, Martin K et al (2019) EPSPS duplication and mutation involved in glyphosate resistance in the allotetraploid weed species *Poa annua* L. Pest Manag Sci 75:1663–1670. https://doi.org/10.1002/ps.5284

Byker HP, Soltani N, Robinson DE et al (2013) Control of glyphosate-resistant horseweed (*Conyza canadensis*) with dicamba applied preplant and postemergence in dicamba-resistant soybean. Weed Technol 27:492–496. https://doi.org/10.1614/WT-D-13-00023.1

Cahoon CW, York AC, Jordan DL et al (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758–770. https://doi.org/10.1614/WT-D-15-00041.1

Casale FA, Giacomini DA, Tranel PJ (2019) Empirical investigation of mutation rate for herbicide resistance. Weed Sci 67:361–368. https://doi.org/10.1017/wsc.2019.19

Chahal PS, Varanasi VK, Jugulam M, Jhala AJ (2017) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska: confirmation, *EPSPS* gene amplification, and response to post corn and soybean herbicides. Weed Technol 31:80–93. https://doi.org/10.1614/WT-D-16-00109.1

Chandi A, Milla-Lewis SR, Giacomini D et al (2012) Inheritance of evolved glyphosate resistance in a north carolina palmer amaranth (*Amaranthus palmeri*) biotype. Int J Agron. https://www.hindawi.com/journals/ija/2012/176108/. Accessed 5 Dec 2019

Chatham LA, Bradley KW, Kruger GR et al (2015) A multistate study of the association between glyphosate resistance and EPSPS gene amplification in waterhemp (*Amaranthus tuberculatus*). Weed Sci 63:569–577. https://doi.org/10.1614/WS-D-14-00149.1

Chen J, Huang H, Zhang C et al (2015) Mutations and amplification of *EPSPS* gene confer resistance to glyphosate in goosegrass (*Eleusine indica*). Planta 242:859–868. https://doi.org/10.1007/s00425-015-2324-2

Chen J, Cui H, Ma X et al (2019) Distribution differences in the *EPSPS* gene in chromosomes between glyphosate-resistant and -susceptible goosegrass (*Eleusine indica*). Weed Sci:1–28. https://doi.org/10.1017/wsc.2019.72

Comont D, Neve P (2020) Adopting epidemiological approaches for herbicide resistance monitoring and management. Weed Res n/a: https://doi.org/10.1111/wre.12420
Comont D, Hicks H, Crook L et al (2019) Evolutionary epidemiology predicts the emergence of glyphosate resistance in a major agricultural weed. New Phytol 223:1584–1594. https://doi.org/10.1111/nph.15800
Copetti D, Kuon J, Yates S et al (2019) A diploid phased genome assembly of the Italian ryegrass (Lolium multiflorum Lam.) cv. Rabiosa. 1 p. https://doi.org/10.3929/ETHZ-B-000353845
Costea M, Weaver SE, Tardif FJ (2005) The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. Can J Plant Sci 85:507–522. https://doi.org/10.4141/P04-101
Cousens RD, Fournier-Level A (2018) Herbicide resistance costs: what are we actually measuring and why? Pest Manag Sci 74:1539–1546
Culpepper AS, Grey TL, Vencill WK et al (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620–626. https://doi.org/10.1614/WS-06-001R.1
Dayan FE, Barker A, Tranel PJ (2018) Origins and structure of chloroplastic and mitochondrial plant protoporphyrinogen oxidases: implications for the evolution of herbicide resistance. Pest Manag Sci 74:2226–2234. https://doi.org/10.1002/ps.4744
de Carvalho LB, Alves PL d CA, González-Torralva F et al (2012) Pool of resistance mechanisms to glyphosate in *Digitaria insularis*. J Agric Food Chem 60:615–622. https://doi.org/10.1021/jf204089d
Délye C (2013) Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Manag Sci 69:176–187. https://doi.org/10.1002/ps.3318
Devine MD, Bandeen JD, McKERSIE BD (1983) Temperature effects on glyphosate absorption, translocation, and distribution in quackgrass (*Agropyron repens*). Weed Sci 31:461–464
Dill GM (2005) Glyphosate-resistant crops: history, status and future. Pest Manag Sci 61:219–224. https://doi.org/10.1002/ps.1008
Dillon A, Varanasi VK, Danilova TV et al (2017) Physical mapping of amplified copies of the 5-enolpyruvylshikimate-3-phosphate synthase gene in glyphosate-resistant *Amaranthus tuberculatus*. Plant Physiol 173:1226–1234. https://doi.org/10.1104/pp.16.01427
Dinelli G, Marotti I, Bonetti A et al (2008) Physiological and molecular bases of glyphosate resistance in *Conyza bonariensis* biotypes from Spain. Weed Res 48:257–265. https://doi.org/10.1111/j.1365-3180.2008.00623.x
Dominguez-Valenzuela JA, Gherekhloo J, Fernández-Moreno PT et al (2017) First confirmation and characterization of target and non-target site resistance to glyphosate in Palmer amaranth (*Amaranthus palmeri*) from Mexico. Plant Physiol Biochem 115:212–218. https://doi.org/10.1016/j.plaphy.2017.03.022
Duke SO (2011) Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. J Agric Food Chem 59:5835–5841. https://doi.org/10.1021/jf102704x
Duke SO (2012) Why have no new herbicide modes of action appeared in recent years? Pest Manag Sci 68:505–512. https://doi.org/10.1002/ps.2333
Duke SO (2019) Enhanced metabolic degradation: the last evolved glyphosate resistance mechanism of weeds? Plant Physiol 181:1401–1403. https://doi.org/10.1104/pp.19.01245
Duke SO (2021) Glyphosate: uses other than in glyphosate-resistant crops, mode of action, degradation in plants, and effects on non-target plants and agricultural microbes. Rev Environ Contam Toxicol (this volume). https://doi.org/10.1007/398_2020_53
Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325. https://doi.org/10.1002/ps.1518
Duzy LM, Price AJ, Balkcom KS, Aulakh JS (2016) Assessing the economic impact of inversion tillage, cover crops, and herbicide regimes in Palmer amaranth *(Amaranthus palmeri)* infested cotton. Int J Agron 2016:1–9. https://doi.org/10.1155/2016/1524389

Evans JA, Tranel PJ, Hager AG et al (2016) Managing the evolution of herbicide resistance. Pest Manag Sci 72:74–80. https://doi.org/10.1002/ps.4009

Evans JA, Williams A, Hager AG et al (2018) Confronting herbicide resistance with cooperative management. Pest Manag Sci 74:2424–2431. https://doi.org/10.1002/ps.5105

Farmer JA, Webb EB, Pierce RA, Bradley KW (2017) Evaluating the potential for weed seed dispersal based on waterfowl consumption and seed viability. Pest Manag Sci 73:2592–2603. https://doi.org/10.1002/ps.4710

Feng PC, Tran M, Chiu T et al (2004) Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation, and metabolism. Weed Sci 52:498–505

Fernández-Moreno PT, Alcantara-de la Cruz R, Cruz-Hipólito HE et al (2016) Non-target site tolerance mechanisms describe tolerance to glyphosate in *Avena sterilis*. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01220

Franci J, Lam KW, Chuah TS, San Cha T (2020) Genetic diversity and in silico evidence of target-site mutation in the EPSPS gene in endowing glyphosate resistance in *Eleusine indica* (L.) from Malaysia. Pestic Biochem Physiol 104556

Gaines TA, Heap IM (2020) Mutations in Herbicide-Resistant Weeds to EPSPS Synthase Inhibitors. http://www.weedscience.com. Accessed 11 June 2020

Gaines TA, Zhang W, Wang D et al (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107:1029–1034. https://doi.org/10.1073/pnas.0906649107

Gaines TA, Cripps A, Powles SB (2012) Evolved resistance to glyphosate in junglerice (*Echinochloa colona*) from the tropical Ord River region in Australia. Weed Technol 26:480–484. https://doi.org/10.1614/WT-D-12-00029.1

Gaines TA, Barker AL, Patterson EL et al (2016) *EPSPS* gene copy number and whole-plant glyphosate resistance level in *Kochia scoparia*. PLOS ONE 11:e0168295. https://doi.org/10.1371/journal.pone.0168295

Gaines TA, Patterson EL, Neve P (2019) Molecular mechanisms of adaptive evolution revealed by global selection for glyphosate resistance. New Phytol 223:1770–1775. https://doi.org/10.1111/nph.15858

Gaines TA, Duke SO, Morran S et al (2020) Mechanisms of evolved herbicide resistance. J Biol Chem 295(30):10307–10330. https://doi.org/10.1074/jbc.REV120.013572

García MJ, Palma-Bautista C, Rojano-Delgado AM et al (2019) The triple amino acid substitution TAP-IVS in the *EPSPS* gene confers high glyphosate resistance to the superweed *Amaranthus hybridus*. Int J Mol Sci 20:2396. https://doi.org/10.3390/ijms20102396

Ge X, d'Avignon DA, Ackerman JJ, Sammons RD (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag Sci 66:345–348. https://doi.org/10.1002/ps.1911

Ge X, d'Avignon DA, Ackerman JJ et al (2011) Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by 31P NMR. Pest Manag Sci 67:1215–1221

Ge X, d'Avignon DA, Ackerman JJH et al (2012) Vacuolar glyphosate-sequestration correlates with glyphosate resistance in ryegrass (*Lolium* spp.) from Australia, South America, and Europe: a 31p nmr investigation. J Agric Food Chem 60:1243–1250. https://doi.org/10.1021/jf203472s

Ge X, d'Avignon DA, Ackerman JJ, Sammons RD (2014) In vivo 31P-nuclear magnetic resonance studies of glyphosate uptake, vacuolar sequestration, and tonoplast pump activity in glyphosate-resistant horseweed. Plant Physiol 166:1255–1268

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci 62:29–37

Godar AS, Stahlman PW, Jugulam M, Dille JA (2015) Glyphosate-resistant kochia (*Kochia scoparia*) in Kansas: *EPSPS* gene copy number in relation to resistance levels. Weed Sci 63:587–595. https://doi.org/10.1614/WS-D-14-00145.1

Goh SS, Yu Q, Han H et al (2018) Non-target-site glyphosate resistance in *Echinochloa colona* from Western Australia. Crop Prot 112:257–263. https://doi.org/10.1016/j.cropro.2018.06.013

Gomes MP, Smedbol E, Chalifour A et al (2014) Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. J Exp Bot 65:4691–4703. https://doi.org/10.1093/jxb/eru269

González-Torralva F, Gil-Humanes J, Barro F et al (2012a) Target site mutation and reduced translocation are present in a glyphosate-resistant *Lolium multiflorum* Lam. biotype from Spain. Plant Physiol Biochem 58:16–22. https://doi.org/10.1016/j.plaphy.2012.06.001

González-Torralva F, Rojano-Delgado AM, de Castro MDL et al (2012b) Two non-target mechanisms are involved in glyphosate-resistant horseweed (*Conyza canadensis* L. Cronq.) biotypes. J Plant Physiol 169:1673–1679

González-Torralva F, Gil-Humanes J, Barro F et al (2014) First evidence for a target site mutation in the EPSPS2 gene in glyphosate-resistant Sumatran fleabane from citrus orchards. Agron Sustain Dev 34:553–560. https://doi.org/10.1007/s13593-013-0163-8

González-Torralva F, Brown AP, Chivasa S (2017) Comparative proteomic analysis of horseweed (*Conyza canadensis*) biotypes identifies candidate proteins for glyphosate resistance. Sci Rep 7:42565. https://doi.org/10.1038/srep42565

Goyer A, Johnson TL, Olsen LJ et al (2004) Characterization and metabolic function of a peroxisomal sarcosine and pipecolate oxidase from Arabidopsis. J Biol Chem 279:16947–16953. https://doi.org/10.1074/jbc.M400071200

Green JM, Siehl DS (2021) History and outlook for glyphosate-resistant crops. Rev Environ Contam Toxicol (this volume). https://doi.org/10.1007/398_2020_54

Han H, Yu Q, Widderick MJ, Powles SB (2016) Target-site EPSPS Pro-106 mutations: sufficient to endow glyphosate resistance in polyploid *Echinochloa colona*? Pest Manag Sci 72:264–271. https://doi.org/10.1002/ps.4038

Han H, Vila-Aiub MM, Jalaludin A et al (2017) A double *EPSPS* gene mutation endowing glyphosate resistance shows a remarkably high resistance cost. Plant Cell Environ 40:3031–3042. https://doi.org/10.1111/pce.13067

Harre NT, Young JM, Young BG (2018) Glyphosate-induced antagonism in rapid response giant ragweed (*Ambrosia trifida*). Weed Technol 32:52–59. https://doi.org/10.1017/wet.2017.92

Heap I, Duke SO (2018) Overview of glyphosate-resistant weeds worldwide. Pest Manag Sci 74:1040–1049. https://doi.org/10.1002/ps.4760

Heap I, Knight R (1986) The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. Aust J Agric Res 37:149–156. https://doi.org/10.1071/ar9860149

Heap IM (2020) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed 13 Feb 2020

Hess FD, Duke SO (1985) Herbicide absorption and translocation and their relationship to plant tolerances and susceptibility. In: Weed physiology, vol 2, herbicide physiology. CRC Press, Boca Raton, pp 191–214

Hoagland RE (1980) Effects of glyphosate on metabolism of phenolic compounds: vi. effects of glyphosine and glyphosate metabolites on phenylalanine ammonia-lyase activity, growth, and protein, chlorophyll, and anthocyanin levels in soybean (*glycine max*) seedlings. Weed Sci 28:393–400. https://doi.org/10.1017/S0043174500055545

Housden BE, Muhar M, Gemberling M et al (2017) Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. Nat Rev Genet 18:24–40. https://doi.org/10.1038/nrg.2016.118

Huang Z, Liu Y, Zhang C et al (2019) Molecular basis of natural tolerance to glyphosate in *Convolvulus arvensis*. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-44583-8

Jander G, Baerson SR, Hudak JA et al (2003) Ethylmethanesulfonate saturation mutagenesis in *Arabidopsis* to determine frequency of herbicide resistance. Plant Physiol 131:139–146. https://doi.org/10.1104/pp.102.010397

Jasieniuk M, Ahmad R, Sherwood AM et al (2008) Glyphosate-resistant italian ryegrass (*Lolium multiflorum*) in California: Distribution, response to glyphosate, and molecular evidence for an altered target enzyme. Weed Sci 56:496–502. https://doi.org/10.1614/WS-08-020.1

Jugulam M, Niehues K, Godar AS et al (2014) Tandem amplification of a chromosomal segment harboring *5-enolpyruvylshikimate-3-phosphate synthase* locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiol 166:1200–1207. https://doi.org/10.1104/pp.114.242826

Karn E, Jasieniuk M (2017) Nucleotide diversity at site 106 of *EPSPS* in *Lolium perenne* L. ssp. *multiflorum* from California indicates multiple evolutionary origins of herbicide resistance. Front Plant Sci 8:777

Kaundun SS, Dale RP, Zelaya IA et al (2011) A novel p106l mutation in epsps and an unknown mechanism(s) act additively to confer resistance to glyphosate in a South African *lolium rigidum* population. J Agric Food Chem 59:3227–3233. https://doi.org/10.1021/jf104934j

Kells JJ, Rieck CE (1979) Effects of illuminance and time on accumulation of glyphosate in johnsongrass (*Sorghum halepense*). Weed Sci 27:235–237

Kistner EJ, Hatfield JL (2018) Potential geographic distribution of Palmer amaranth under current and future climates. Agric Environ Lett 3:170044. https://doi.org/10.2134/ael2017.12.0044

Kleinman Z, Rubin B (2017) Non-target-site glyphosate resistance in *Conyza bonariensis* is based on modified subcellular distribution of the herbicide. Pest Manag Sci 73:246–253. https://doi.org/10.1002/ps.4293

Koger CH, Reddy KN (2005) Role of absorption and translocation in the mechanism of glyphosate resistance in horseweed (*Conyza canadensis*). Weed Sci 53:84–89

Koo D-H, Jugulam M, Putta K et al (2018a) Gene duplication and aneuploidy trigger rapid evolution of herbicide resistance in common waterhemp. Plant Physiol 176:1932–1938. https://doi.org/10.1104/pp.17.01668

Koo D-H, Molin WT, Saski CA et al (2018b) Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in crop weed *Amaranthus palmeri*. Proc Natl Acad Sci 115:3332–3337. https://doi.org/10.1073/pnas.1719354115

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29. https://doi.org/10.1186/1746-4811-9-29

Kreiner JM, Giacomini DA, Bemm F et al (2019) Multiple modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*. Proc Natl Acad Sci 116:21076–21084. https://doi.org/10.1073/pnas.1900870116

Kuester A, Chang S-M, Baucom RS (2015) The geographic mosaic of herbicide resistance evolution in the common morning glory, *Ipomoea purpurea*: Evidence for resistance hotspots and low genetic differentiation across the landscape. Evol Appl 8:821–833. https://doi.org/10.1111/eva.12290

Laforest M, Soufiane B, Simard M-J et al (2017) Acetyl-CoA carboxylase overexpression in herbicide-resistant large crabgrass (*Digitaria sanguinalis*). Pest Manag Sci 73:2227–2235. https://doi.org/10.1002/ps.4675

Laforest M, Martin SL, Bisaillon K et al (2020) A chromosome-scale draft sequence of the Canada fleabane genome. Pest Manag Sci 76:2158–2169. https://doi.org/10.1002/ps.5753

Lamego FP, Vidal RA (2008) Resistance to glyphosate in *Conyza bonariensis* and *Conyza canadensis* biotypes in Rio Grande do Sul, Brazil. Planta Daninha 26:467–471. https://doi.org/10.1590/S0100-83582008000200024

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587. https://doi.org/10.1614/WS-07-204.1

Lespérance MA (2015) Programmed cell death and altered translocation cause glyphosate resistance in giant ragweed (*Ambrosia trifida* L.). PhD thesis

Li J, Peng Q, Han H et al (2018) Glyphosate resistance in *Tridax procumbens* via a novel EPSPS Thr-102-Ser substitution. J Agric Food Chem 66:7880–7888. https://doi.org/10.1021/acs.jafc.8b01651

Liu C-M, McLean PA, Sookdeo CC, Cannon FC (1991) Degradation of the herbicide glyphosate by members of the family rhizobiaceae. Appl Environ Microbiol 57:1799–1804

Liu M, Hulting AG, Mallory-Smith C (2016) Characterization of multiple herbicide-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) populations from winter wheat fields in Oregon. Weed Sci 64:331–338

Lorentz L, Gaines TA, Nissen SJ et al (2014) Characterization of glyphosate resistance in *Amaranthus tuberculatus* populations. J Agric Food Chem 62:8134–8142. https://doi.org/10.1021/jf501040x

Lorraine-Colwill DF, Powles SB, Hawkes TR et al (2002) Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. Pestic Biochem Physiol 74:62–72

Malone JM, Morran S, Shirley N et al (2016) *EPSPS* gene amplification in glyphosate-resistant *Bromus diandrus*. Pest Manag Sci 72:81–88. https://doi.org/10.1002/ps.4019

Mao C, Xie H, Chen S et al (2016) Multiple mechanism confers natural tolerance of three lilyturf species to glyphosate. Planta 243:321–335. https://doi.org/10.1007/s00425-015-2408-z

Margaritopoulou T, Tani E, Chachalis D, Travlos I (2018) Involvement of epigenetic mechanisms in herbicide resistance: the case of *Conyza canadensis*. Agriculture 8:17. https://doi.org/10.3390/agriculture8010017

Markus C, Pecinka A, Karan R et al (2018) Epigenetic regulation – contribution to herbicide resistance in weeds? Pest Manag Sci 74:275–281. https://doi.org/10.1002/ps.4727

Maroli AS, Gaines TA, Foley ME et al (2018) Omics in weed science: a perspective from genomics, transcriptomics, and metabolomics approaches. Weed Sci 66:681–695. https://doi.org/10.1017/wsc.2018.33

Marshall G, Kirkwood RC, Martin DJ (1987) Studies on the mode of action of asulam, aminotriazole and glyphosate in Equisetum arvense L. (field horsetail). II: The metabolism of [14C]asulam, [14C]aminotriazole and [14C]glyphosate. Pestic Sci 18:65–77. https://doi.org/10.1002/ps.2780180107

Martin SL, Benedict L, Sauder CA et al (2017) Glyphosate resistance reduces kochia fitness: Comparison of segregating resistant and susceptible F2 populations. Plant Sci 261:69–79. https://doi.org/10.1016/j.plantsci.2017.04.010

Martinoia E (1992) Transport processes in vacuoles of higher plants. Bot Acta 105:232–245. https://doi.org/10.1111/j.1438-8677.1992.tb00293.x

Marty F (1999) Plant vacuoles. Plant Cell 11:587–599

McAllister W, Osipychev D, Davis A, Chowdhary G (2019) Agbots: Weeding a field with a team of autonomous robots. Comput Electron Agric 163:104827. https://doi.org/10.1016/j.compag.2019.05.036

McElroy JS, Hall ND (2020) *Echinochloa colona* with reported resistance to glyphosate conferred by aldo-keto reductase also contains a Pro-106-Thr EPSPS target-site mutation. Plant Physiol. https://doi.org/10.1104/pp.20.00064

Michitte P, De Prado R, Espinoza N et al (2007) Mechanisms of resistance to glyphosate in a ryegrass (*Lolium multiflorum*) biotype from Chile. Weed Sci 55:435–440

Mohseni-Moghadam M, Schroeder J, Ashigh J (2013) Mechanism of Resistance and Inheritance in Glyphosate Resistant Palmer amaranth (<i>Amaranthus palmeri<i>) Populations from New Mexico, USA. Weed Sci 61:517–525. https://doi.org/10.1614/WS-D-13-00028.1

Molin WT, Wright AA, Lawton-Rauh A, Saski CA (2017) The unique genomic landscape surrounding the *EPSPS* gene in glyphosate resistant *Amaranthus palmeri*: a repetitive path to resistance. BMC Genomics 18. https://doi.org/10.1186/s12864-016-3336-4

Molin WT, Wright AA, VanGessel MJ et al (2018) Survey of the genomic landscape surrounding the 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene in glyphosate-resistant *Amaranthus palmeri* from geographically distant populations in the USA. Pest Manag Sci 74:1109–1117. https://doi.org/10.1002/ps.4659

Molin WT, Yaguchi A, Blenner MA, Saski CA (2020) The eccDNA replicon: a heritable, extranuclear vehicle that enables gene amplification and glyphosate resistance in *Amaranthus palmeri*. Plant Cell. https://doi.org/10.1105/tpc.20.00099

Moretti ML, Hanson BD, Hembree KJ, Shrestha A (2013) Glyphosate resistance is more variable than paraquat resistance in a multiple-resistant hairy fleabane (*Conyza bonariensis*) population. Weed Sci 61:396–402. https://doi.org/10.1614/WS-D-12-00201.1

Moretti ML, Van Horn CR, Robertson R et al (2018) Glyphosate resistance in *Ambrosia trifida:* Part 2. Rapid response physiology and non-target-site resistance. Pest Manag Sci 74:1079–1088. https://doi.org/10.1002/ps.4569

Morran S, Moretti ML, Brunharo CA et al (2018) Multiple target site resistance to glyphosate in junglerice (*Echinochloa colona*) lines from California orchards. Pest Manag Sci 74:2747–2753. https://doi.org/10.1002/ps.5061

Moss SR, Cussans GW (1985) Variability in the susceptibility of *Alopecurus myosuroides* (black-grass) to chlortoluron and isoproturon. Asp Appl Biol 9:91–98

Murphy BP, Tranel PJ (2019) Target-site mutations conferring herbicide resistance. Plants 8:382. https://doi.org/10.3390/plants8100382

Mutters RG, Madore M, Bytnerowicz A (1993) Formaldehyde exposure affects growth and metabolism of common bean. Air Waste 43:113–116. https://doi.org/10.1080/1073161X.1993.10467112

Nandula VK, Ray JD, Ribeiro DN et al (2013) Glyphosate resistance in tall waterhemp (*Amaranthus tuberculatus*) from Mississippi is due to both altered target-site and nontarget-site mechanisms. Weed Sci 61:374–383. https://doi.org/10.1614/WS-D-12-00155.1

Nandula VK, Wright AA, Bond JA et al (2014) EPSPS amplification in glyphosate-resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer via interspecific hybridization from glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci 70:1902–1909. https://doi.org/10.1002/ps.3754

Nandula VK, Wright AA, Van Horn CR et al (2015) Glyphosate resistance in giant ragweed (*Ambrosia trifida* L.) from Mississippi is partly due to reduced translocation. Am J Plant Sci 6:2104

Neve P (2018) Gene drive systems: do they have a place in agricultural weed management? Pest Manag Sci 74:2671–2679. https://doi.org/10.1002/ps.5137

Neve P, Vila-Aiub M, Roux F (2009) Evolutionary-thinking in agricultural weed management. New Phytol 184:783–793. https://doi.org/10.1111/j.1469-8137.2009.03034.x

Ngo TD, Krishnan M, Boutsalis P et al (2018a) Target-site mutations conferring resistance to glyphosate in feathertop Rhodes grass (*Chloris virgata*) populations in Australia. Pest Manag Sci 74:1094–1100. https://doi.org/10.1002/ps.4512

Ngo TD, Malone JM, Boutsalis P et al (2018b) *EPSPS* gene amplification conferring resistance to glyphosate in windmill grass (*Chloris truncata*) in Australia. Pest Manag Sci 74:1101–1108. https://doi.org/10.1002/ps.4573

Nol N, Tsikou D, Eid M et al (2012) Shikimate leaf disc assay for early detection of glyphosate resistance in *Conyza canadensis* and relative transcript levels of EPSPS and ABC transporter genes. Weed Res 52:233–241

Osipitan OA, Dille JA (2019) No impact of increased *EPSPS* gene copy number on growth and fecundity of glyphosate-resistant kochia (*Bassia scoparia*). Weed Sci 67:22–28. https://doi.org/10.1017/wsc.2018.82

Ossowski S, Schneeberger K, Lucas-Lledo JI et al (2010) The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. Science 327:92–94. https://doi.org/10.1126/science.1180677

Palma-Bautista C, Alcántara-de la Cruz R, Rojano-Delgado AM et al (2019) Low temperatures enhance the absorption and translocation of 14C-glyphosate in glyphosate-resistant *Conyza sumatrensis*. J Plant Physiol 240:153009. https://doi.org/10.1016/j.jplph.2019.153009

Pan L, Yu Q, Han H et al (2019) Aldo-keto reductase metabolizes glyphosate and confers glyphosate resistance in *Echinochloa colona*. Plant Physiol 181:1519–1534. https://doi.org/10.1104/pp.19.00979

Pandolfo CE, Presotto A, Carbonell FT et al (2018) Transgene escape and persistence in an agroecosystem: the case of glyphosate-resistant *Brassica rapa* L. in central Argentina. Environ Sci Pollut Res 25:6251–6264. https://doi.org/10.1007/s11356-017-0726-3

Patterson EL, Pettinga DJ, Ravet K et al (2018) Glyphosate resistance and *EPSPS* gene duplication: convergent evolution in multiple plant species. J Hered 109:117–125. https://doi.org/10.1093/jhered/esx087

Patterson EL, Saski C, Küpper A et al (2019a) Omics potential in herbicide-resistant weed management. Plants 8:607. https://doi.org/10.3390/plants8120607

Patterson EL, Saski CA, Sloan DB et al (2019b) The draft genome of *Kochia scoparia* and the mechanism of glyphosate resistance via transposon-mediated *EPSPS* tandem gene duplication. Genome Biol Evol 11:2927–2940. https://doi.org/10.1093/gbe/evz198

Perez-Jones A, Park K-W, Polge N et al (2007) Investigating the mechanisms of glyphosate resistance in *Lolium multiflorum*. Planta 226:395–404. https://doi.org/10.1007/s00425-007-0490-6

Perotti VE, Larran AS, Palmieri VE et al (2019) A novel triple amino acid substitution in the *EPSPS* found in a high-level glyphosate-resistant *Amaranthus hybridus* population from Argentina. Pest Manag Sci 75:1242–1251. https://doi.org/10.1002/ps.5303

Piasecki C, Yang Y, Benemann DP et al (2019) Transcriptomic analysis identifies new non-target site glyphosate-resistance genes in *Conyza bonariensis*. Plants 8:157. https://doi.org/10.3390/plants8060157

Pollegioni L, Schonbrunn E, Siehl D (2011) Molecular basis of glyphosate resistance: Different approaches through protein engineering. FEBS J 278:2753–2766. https://doi.org/10.1111/j.1742-4658.2011.08214.x

Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Manag Sci 64:360–365. https://doi.org/10.1002/ps.1525

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347. https://doi.org/10.1146/annurev-arplant-042809-112119

Preston C, Wakelin AM (2008) Resistance to glyphosate from altered herbicide translocation patterns. Pest Manag Sci Former Pestic Sci 64:372–376

Purrington CB (2000) Costs of resistance. Curr Opin Plant Biol 3:305–308

Putta K (2017) Physical mapping of *EPSPS* gene copies in glyphosate resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*). Thesis, Kansas State University

Queiroz ARS, de Delatorre CA, Lucio FR et al (2020) Rapid necrosis: a novel plant resistance mechanism to 2,4-D. Weed Sci 68:6–18. https://doi.org/10.1017/wsc.2019.65

Ravet K, Patterson EL, Krähmer H et al (2018) The power and potential of genomics in weed biology and management. Pest Manag Sci 74:2216–2225. https://doi.org/10.1002/ps.5048

Reddy KN, Rimando AM, Duke SO, Nandula VK (2008) Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J Agric Food Chem 56:2125–2130. https://doi.org/10.1021/jf072954f

Ribeiro DN, Nandula VK, Dayan FE et al (2015) Possible glyphosate tolerance mechanism in pitted morningglory (*Ipomoea lacunosa* L.). J Agric Food Chem 63:1689–1697. https://doi.org/10.1021/jf5055722

Roden LC, Ingle RA (2009) Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant–pathogen interactions. Plant Cell 21:2546–2552. https://doi.org/10.1105/tpc.109.069922

Rojano-Delgado AM, Cruz-Hipolito H, De Prado R et al (2012) Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in *Mucuna pruriens* var. *utilis* plants. Phytochemistry 73:34–41. https://doi.org/10.1016/j.phytochem.2011.09.007

Salas RA, Dayan FE, Pan Z et al (2012) EPSPS gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) from Arkansas. Pest Manag Sci 68:1223–1230. https://doi.org/10.1002/ps.3342

Sammons RD, Gaines TA (2014) Glyphosate resistance: state of knowledge. Pest Manag Sci 70:1367–1377. https://doi.org/10.1002/ps.3743

Sauka-Spengler T, Barembaum M (2008) Chapter 12 Gain- and loss-of-function approaches in the chick embryo. In: Methods in cell biology. Academic Press, pp 237–256

Schönbrunn E, Eschenburg S, Shuttleworth WA et al (2001) Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci 98:1376–1380. https://doi.org/10.1073/pnas.98.4.1376

Shah MA, Callaway RM, Shah T et al (2014) *Conyza canadensis* suppresses plant diversity in its nonnative ranges but not at home: a transcontinental comparison. New Phytol 202:1286–1296. https://doi.org/10.1111/nph.12733

Shaner DL (2009) Role of translocation as a mechanism of resistance to glyphosate. Weed Sci 57:118–123

Simard M-J, Légère A, Warwick SI (2006) Transgenic *Brassica napus* fields and *Brassica rapa* weeds in Quebec: sympatry and weed-crop in situ hybridization. Can J Bot 84:1842–1851. https://doi.org/10.1139/b06-135

Singh S, Singh V, Lawton-Rauh A et al (2018) *EPSPS* gene amplification primarily confers glyphosate resistance among Arkansas Palmer amaranth (*Amaranthus palmeri*) populations. Weed Sci 66:293–300. https://doi.org/10.1017/wsc.2017.83

Soltani N, Oliveira MC, Alves GS et al (2020) Off-target movement assessment of dicamba in North America. Weed Technol 34:318–330. https://doi.org/10.1017/wet.2020.17

Sosnoskie LM, Culpepper AS (2014) Glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) increases herbicide use, tillage, and hand-weeding in georgia cotton. Weed Sci 62:393–402. https://doi.org/10.1614/WS-D-13-00077.1

Sprankle P, Meggitt WF, Penner D (1975) Absorption, action, and translocation of glyphosate. Weed Sci 23:235–240

Steinrücken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94:1207–1212. https://doi.org/10.1016/0006-291X(80)90547-1

Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002) Direct and ecological costs of resistance to herbivory. Trends Ecol Evol 17:278–285

Takano HK, Mendes RR, Scoz LB et al (2019) Proline-106 *EPSPS* mutation imparting glyphosate resistance in goosegrass (*Eleusine indica*) Emerges in South America. Weed Sci 67:48–56. https://doi.org/10.1017/wsc.2018.71

Takano HK, Fernandes VN, Adegas FS et al (2020) A novel TIPT double mutation in EPSPS conferring glyphosate resistance in tetraploid *Bidens subalternans*. Pest Manag Sci 76:95–102. https://doi.org/10.1002/ps.5535

Tani E, Chachalis D, Travlos IS (2015) A glyphosate resistance mechanism in *Conyza canadensis* involves synchronization of *EPSPS* and ABC-transporter genes. Plant Mol Biol Report 33:1721–1730

Tian D, Traw MB, Chen JQ et al (2003) Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. Nature 423:74

Tranel PJ (2017) Herbicide-resistance mechanisms: gene amplification is not just for glyphosate. Pest Manag Sci 73:2225–2226. https://doi.org/10.1002/ps.4679

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712. https://doi.org/10.1614/0043-1745(2002)050[0700:RROWTA]2.0.CO;2

Valverde BE (2010) Glyphosate resistance in Latin America. In: Nandula VK (ed) Glyphosate resistance in crops and weeds. Wiley, Hoboken, pp 249–280

Van Eerd LL, Hoagland RE, Zablotowicz RM, Hall JC (2003) Pesticide metabolism in plants and microorganisms. Weed Sci 51:472–495. https://doi.org/10.1614/0043-1745(2003)051[0472:PMIPAM]2.0.CO;2

Van Etten M, Lee KM, Chang S-M, Baucom RS (2020) Parallel and nonparallel genomic responses contribute to herbicide resistance in *Ipomoea purpurea*, a common agricultural weed. PLOS Genet 16:e1008593. https://doi.org/10.1371/journal.pgen.1008593

Van Horn CR (2016) Investigating resistance and the rapid response to glyphosate in giant ragweed (*Ambrosia trifida* L.). Ph.D., Colorado State University

Van Horn CR, Moretti ML, Robertson RR et al (2018) Glyphosate resistance in *Ambrosia trifida*: Part 1. Novel rapid cell death response to glyphosate. Pest Manag Sci 74:1071–1078. https://doi.org/10.1002/ps.4567

VanGessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Sci 49:703–705
Vazquez-Garcia JG, Palma-Bautista C, Rojano-Delgado AM et al (2020) The first case of glyphosate resistance in Johnsongrass (*Sorghum halepense* (l.) pers.) in Europe. Plants 9:313. https://doi.org/10.3390/plants9030313
Vila-Aiub MM (2019) Fitness of herbicide-resistant weeds: current knowledge and implications for management. Plants 8:469
Vila-Aiub MM, Balbi MC, Distéfano AJ et al (2012) Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Manag Sci 68:430–436
Vila-Aiub MM, Gundel PE, Yu Q, Powles SB (2013) Glyphosate resistance in *Sorghum halepense* and *Lolium rigidum* is reduced at suboptimal growing temperatures. Pest Manag Sci 69:228–232
Vila-Aiub MM, Goh SS, Gaines TA et al (2014) No fitness cost of glyphosate resistance endowed by massive *EPSPS* gene amplification in *Amaranthus palmeri*. Planta 239:793–801. https://doi.org/10.1007/s00425-013-2022-x
Vila-Aiub MM, Yu Q, Powles SB (2019) Do plants pay a fitness cost to be resistant to glyphosate? New Phytol. https://doi.org/10.1111/nph.15733
Wakelin AM, Lorraine-Colwill DF, Preston C (2004) Glyphosate resistance in four different populations of *Lolium rigidum* is associated with reduced translocation of glyphosate to meristematic zones. Weed Res 44:453–459
Warwick SI, Simard M-J, Légère A et al (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. Theor Appl Genet 107:528–539. https://doi.org/10.1007/s00122-003-1278-0
Weaver SE (2001) The biology of Canadian weeds. 115. *Conyza canadensis*. Can J Plant Sci 81:867–875. https://doi.org/10.4141/P00-196
Weller SC, Owen MDK, Johnson WG (2010) Managing glyphosate-resistant weeds and population shifts in midwestern U.S. ropping systems. In: Nandula VK (ed) Glyphosate resistance in crops and weeds. Wiley, Hoboken, pp 213–232
Wiersma AT, Gaines TA, Preston C et al (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463–474. https://doi.org/10.1007/s00425-014-2197-9
Wu C, Davis AS, Tranel PJ (2017) Limited fitness costs of herbicide-resistance traits in *Amaranthus tuberculatus* facilitate resistance evolution. Pest Manag Sci:9
Yanniccari M, Gómez-Lobato ME, Istilart C et al (2017) Mechanism of resistance to glyphosate in *Lolium perenne* from argentina. Front Ecol Evol 5. https://doi.org/10.3389/fevo.2017.00123
Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301–307. https://doi.org/10.1614/WT-04-189.1
Yu Q, Powles S (2014) Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. Plant Physiol 166:1106–1118. https://doi.org/10.1104/pp.114.242750
Yu Q, Cairns A, Powles S (2007) Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 225:499–513. https://doi.org/10.1007/s00425-006-0364-3
Yu Q, Jalaludin A, Han H et al (2015) Evolution of a double amino acid substitution in the 5-enolpyruvylshikimate-3-phosphate synthase in *Eleusine indica* conferring high-level glyphosate resistance. Plant Physiol 167:1440–1447. https://doi.org/10.1104/pp.15.00146
Yuan JS, Tranel PJ, Stewart CN (2007) Non-target-site herbicide resistance: a family business. Trends Plant Sci 12:6–13. https://doi.org/10.1016/j.tplants.2006.11.001
Zelaya IA, Owen MDK, VanGessel MJ (2004) Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L.) Cronq. Theor Appl Genet 110:58–70
Zhang C, Li F, HE T et al (2015) Investigating the mechanisms of glyphosate resistance in goosegrass (*Eleusine indica*) population from South China. J Integr Agric 14:909–918