

# Enhanced Metabolic Degradation: The Last Evolved Glyphosate Resistance Mechanism of Weeds?

For the past two decades, glyphosate has been the most used herbicide worldwide, resulting in prolonged, extreme selection pressure for glyphosate-resistant (GR) weeds. Glyphosate's only target as a herbicide is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme of the shikimate pathway. It forms a stable dead-end complex by binding EPSP to form a transition-state analog that is competitive with phosphoenolpyruvate (Sammons and Gaines, 2014). Its widespread use has been due both to its superior properties as a foliar-applied, nonselective herbicide and the widespread adoption of transgenic, GR crops (Duke and Powles, 2008). After glyphosate introduction as a commercial herbicide in 1974, the lag phase for evolution of GR weeds was more than 20 years. To date, both target site (TS) and non-target site (NTS) mechanisms of glyphosate resistance have evolved in 45 weed species (Table 1; Heap and Duke, 2018; Heap, 2019). This relatively long lag phase compared to that of some other herbicide classes has been partly due to the inability of a one-codon change (Pro-106 to Ser, Ala, Thr, or Leu and Thr-102Ser) in the gene for EPSPS to provide robust TS glyphosate resistance (Sammons and Gaines, 2014). Significantly higher levels of TS glyphosate resistance eventually evolved in some weed species via two- (Thr-102Ile and Pro-106Ser) or three-codon mutations (Thr-102Ile, Ala-103Val, and Pro-106Ser) in the *EPSPS* gene or by duplication of the *EPSPS* gene to produce higher concentrations of EPSPS in the plant than can be effectively inhibited by recommended doses of the herbicide (Gaines et al., 2019). The molecular mechanisms of gene duplication are understood in only two of the eight reported cases of gene duplication, and these two mechanisms differ (Table 1). No other cases of herbicide resistance involve multiple mutations in the same gene, and there is only one additional reported case of gene duplication-mediated TS herbicide resistance (Laforest et al., 2017), other than the eight species with this mechanism for glyphosate resistance (Gaines et al., 2019). The intense selection pressure by glyphosate over vast geographic areas annually for decades has resulted in TS mechanisms not seen or rarely seen in evolved resistance to other herbicides.

Considering the complications in evolving robust TS-based glyphosate resistance, NTS-based resistance might have been expected to predominate. Indeed, there are well-documented cases of glyphosate resistance in some species that are based upon reduced

movement of the herbicide into the plant (Heap and Duke, 2018), reduced translocation, and/or enhanced sequestration into vacuoles (Gaines et al., 2019). Vacuolar sequestration is one mechanism for reduced translocation. TS and NTS mechanisms of resistance have been found in the same GR biotypes of several weed species (Sammons and Gaines, 2014). For example, some populations of *Lolium rigidum* have evolved both the single codon Pro-106Ser EPSPS mutation and reduced translocation of glyphosate in the same weed biotype (Bostamam et al., 2012), resulting in more robust resistance than either change alone would afford. An extreme mechanism of NTS-based resistance is that of *Ambrosia trifida*, in which the weed has evolved to respond rapidly to this normally slow-acting herbicide (Van Horn et al., 2018). In this GR weed, rapid necrosis occurs in foliage directly contacted by the herbicide spray, preventing its translocation to meristems. The plant then regrows from these unaffected meristems (representing the so-called "Phoenix" phenomenon) after the tissues containing glyphosate die.

The NTS-based mechanism of resistance that is common to many weeds resistant to other classes of herbicides (e.g. diclofop resistance in *L. rigidum*) and for crops that are naturally tolerant to certain herbicides (e.g. propanil tolerance in rice [*Oryza sativa*]) is enhanced metabolic degradation of the herbicide to non-phytotoxic compounds. Metabolic degradation mechanisms of resistance or tolerance of crops can provide protection from even fast-acting herbicides, such as soybean (*Glycine max*) tolerance to the protoporphyrinogen oxidase inhibitor, acifluorfen. Until the work of Pan et al. (2019), none of the well-documented, evolved mechanisms of glyphosate resistance (Table 1) have represented enhanced metabolic degradation.

Most higher plant species can metabolize glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate (Duke, 2011). Although a comprehensive taxonomic survey of higher plant metabolism of glyphosate has not been conducted, degradation of glyphosate to AMPA appears to be more pronounced in dicotyledonous than monocotyledonous species. The enzyme(s) that produce(s) AMPA in plants has/have been unknown, but it has been presumed to be similar to the glyphosate oxidoreductase (GOX) of some microbes that degrade glyphosate to AMPA and glyoxylate (Duke, 2011). The first commercial transgenic GR canola (*Brassica napus*) varieties contained transgenes from microbes for both GOX and GR EPSPS, so that glyphosate was metabolically degraded, and the plant could maintain the shikimate pathway with the GR EPSPS.

Glyphosate is a slow-acting herbicide, which should provide the time needed to reduce the herbicide

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**Table 1.** Mechanisms of evolved resistance to glyphosate

Mechanism	GR Species	Reference
TS Mechanisms		
Mutated EPSPS		
One-codon change—Pro-106 to Ser, Ala, Thr, or Leu	Several—e.g. <i>Eleusine indica</i> and <i>Lolium</i> spp	Sammons and Gaines, 2014
Thr-102Ile	<i>Tridax procumbens</i>	Li et al., 2018
Two-codon changes (Thr-102Ile and Pro-106Ser)	<i>E. indica</i>	Yu et al., 2015
Three-codon change (Thr-102Ile, Ala-103Val, and Pro-106Ser)	<i>Amaranthus hybridus</i>	Perotti et al., 2019
EPSPS gene duplication		
On an extrachromosomal circular DNA	Eight species—e.g. <i>L. perrene</i> , <i>Bromus diandrus</i> , <i>Chloris truncata</i>	Patterson et al., 2018
Tandem duplication at a single locus	<i>Amaranthus palmeri</i> <i>Kochia scoparia</i> , <i>A. tuberculatis</i>	Koo et al., 2018 Gaines et al., 2019
NTS mechanisms		
Reduced movement of glyphosate into the plant	Several—e.g. <i>Sorghum halepense</i> , <i>Leptochloa virgata</i>	Heap and Duke, 2018
Reduced translocation of glyphosate	Several— <i>Chloris elata</i> , <i>Conyza canadensis</i>	Heap and Duke, 2018
Vacuolar sequestration of glyphosate	<i>C. canadensis</i> , <i>Lolium</i> spp	Gaines et al., 2019
Phoenix phenomenon (rapid necrosis, followed by regeneration)	<i>A. trifida</i>	Van Horn et al., 2018
Enhanced degradation to AMPA and glyoxylate by elevated AKR activity	<i>E. colona</i>	Pan et al., 2019
TS plus NTS mechanisms		
For example, one-codon change in EPSPS and reduced translocation	Several—e.g. <i>A. tuberculatis</i> , <i>L. rigidum</i>	Sammons and Gaines, 2014

concentration to nonlethal levels by metabolic degradation, provided there is sufficient enzymatic activity. Pan et al. (2019) clearly document enhanced degradation of glyphosate as the mechanism of resistance in a GR weed (*Echinochloa colona*). Perhaps more important, this work identifies the enzyme (an aldo-keto reductase; AKR) that oxidizes glyphosate to AMPA and glyoxylate in an evolved GR weed. This AKR protein has little similarity to GOX and Gly oxidase of microbes that generate AMPA and glyoxylate from glyphosate. Vemanna et al. (2017) had already shown that overexpression of transgenes of *Pseudomonas* and rice encoding AKR provides glyphosate resistance in tobacco (*Nicotiana tabacum*), and that silencing or mutating AKR genes in both plants and microbes increased sensitivity to glyphosate. These findings provided the clue that evolution of enhanced expression of an AKR gene in a weed could provide glyphosate resistance.

Now that enhanced expression of a plant degradation enzyme has been shown to provide evolved resistance to glyphosate, other questions might be more easily answered. Do plant species that naturally degrade significant amounts of glyphosate to AMPA and glyoxylate (e.g. soybean) do so with an AKR? Why does relatively rapid degradation of glyphosate by some plant species (e.g. soybean) not provide tolerance? Considering the fact that many plant species can degrade glyphosate, why has enhanced degradation been so rare as a mechanism of evolved glyphosate resistance? Is there a fitness penalty associated with high AKR activity? Is AKR the only plant enzyme involved in glyphosate degradation?

The panoply of mechanisms of resistance to glyphosate now clearly includes enhanced metabolic degradation (Table 1). This amazing range of evolved responses to the massive selection pressure of glyphosate is wider than for any other herbicide or herbicide class. Darwin would probably have been amazed at the diversity of resistance mechanisms that have evolved to a single selection pressure in such a short time period.

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