HOW MANY WAYS CAN NATURE KILL THE GOOSE THAT LAID THE GOLDEN EGG? – THE MANY MECHANISMS OF EVOLVED GLYPHOSATE RESISTANCE

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Introduction

Weeding has been the bane of humanity since the dawn of agriculture. For about 70 years, synthetic herbicides have removed much of the drudgery of this onerous task. Glyphosate was introduced as a non-selective herbicide in 1974. Its ideal properties made it a very popular herbicide, and the introduction of glyphosate-resistant (GR) crops allowed its use as a selective herbicide, greatly expanding its use to become the most used herbicide on earth (Duke & Powles, 2004; Benbrook, 2016). For farmers who used glyphosate in GR crops, it was the golden age of weed management, as this technology significantly improved the efficacy and reduced the cost of weed management. Weed management was also simplified, an asset that was particularly valuable to part-time farmers. Furthermore, this technology provided the environmental benefits (reduced soil loss and fossil fuel use) of significantly reducing tillage. Farmers saved billions of US$, and weed management became more effective and simple. Indeed, to many farmers, glyphosate with GR crops became the goose that laid the golden egg.

After more than 20 years of use, the first cases of GR weeds were reported in the latter 1990s (Figure 1). After a lag period of less than 10 years after the first GR weed was reported, the number of species reported to have evolved glyphosate resistance began to increase in a linear fashion, reaching 53 species in 2021, third only to atrazine (66 species), a much older herbicide and to ALS inhibitors (168 species), which include several different herbicides used in numerous crops since the 1980s (Heap, 2021). The long lag phase before any resistance was detected led some to believe by the mid-1990s that evolution of resistance was improbable (Bradshaw *et al*., 1997). By this time, glyphosate use was greatly increasing, especially in GR crops, an ideal situation for the evolution of resistance.

After this, the number of glyphosate-resistance cases exploded, and the mechanisms of resistance to many of these cases of resistance were determined. A recent, short commentary detailed these mechanisms after a new mechanism of resistance was reported (Duke, 2019). The number of mechanisms for resistance to no other herbicide comes close to those of glyphosate. In the present paper, we briefly describe the many evolved mechanisms by which weeds have evolved resistance to glyphosate.

Mechanisms of Resistance

The known mechanisms of evolved glyphosate resistance are listed in Table 1. More detail on these mechanisms can be found in a recent review (Baek *et al*., 2021). They are broken down into target site (TS) and non-target site (NTS) mechanisms in Table 1. The most common TS mechanism is a change in the target site, so that the herbicide no longer binds to it. The other TS mechanism is caused by an increase in the production of the target site enzyme (protein), thus requiring more herbicide to block the function of the target site enzyme sufficiently. Nearly all NTS mechanisms reduce the amount of herbicide that reaches the target site. This can be through reduced movement of the herbicide into the plant or within the plant, sequestration of the herbicide away from the target site, or enhanced metabolic degradation of the herbicide before it reaches the target site. All of these mechanisms have now been reported for glyphosate resistance.

Target Site Mechanisms

The enzyme targeted by glyphosate is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is required for synthesis of the three essential aromatic amino acids. Proof that inhibition of EPSPS is the only mode of action of glyphosate is that GR crops that are made resistant with a bacterial GR EPSPS transgene are about 50-fold resistant to glyphosate (Nandula *et al*. 2007). The first cases of evolved glyphosate resistance in weeds were found to be single mutations (causing a change in one codon) in the gene for EPSPS, providing a low level

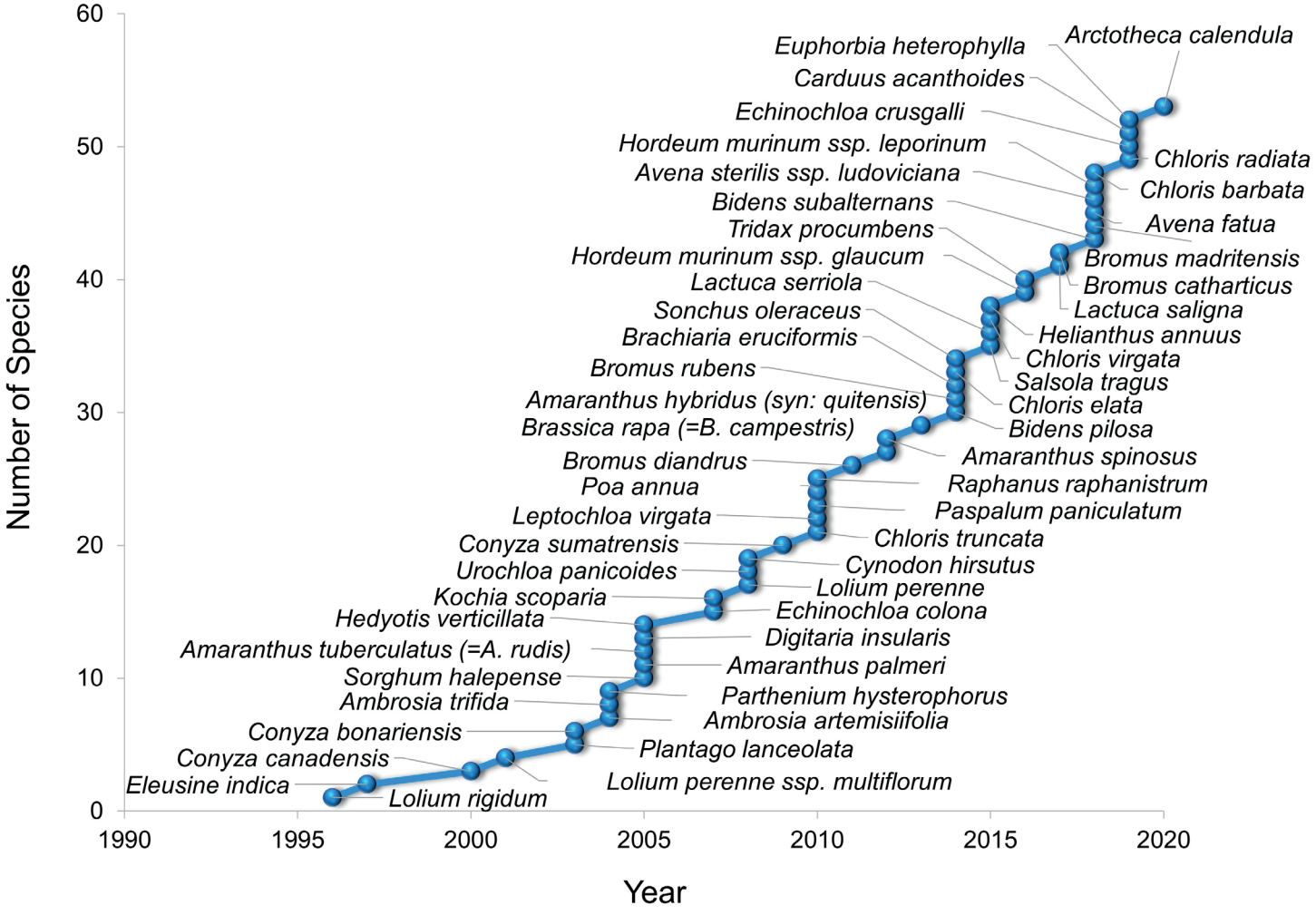

Figure 1. Time course for the appearance of glyphosate resistance in 53 weed species worldwide. From Heap (2021).

Table 1. Mechanisms of evolved resistance to glyphosate.[1]

Mechanism	Example of GR weed species
	– Target site (TS) resistance –
Mutated EPSPS	
Single mutation	Several – e.g., *Eleusine indica* and *Lolium* spp.
Double mutation	*E. indica*
Triple mutation	Amaranthus hybridus
EPSPS gene duplication	Several species – e.g., *Kochia scoparia*, *Amaranthus palmerii*
EPSPS gene overexpression	*E. indica*
	Non-target site (NTS) resistance –
Reduced movement into the plant	Several – e.g., *Sorghum halepense*, *Leptochloa virgate*
Reduced translocation	Several – e.g., *Chloris elata*, *Conyza canadensis*
Enhanced degradation	*Echinochloa colona*
Phoenix phenomenon	*Amaranthus trifida*
	– TS plus NTS –
One codon change, plus reduced translocation	Several – e.g., *Amaranthus tuberculatus*, *Lolium rigidum*
Enhanced degradation and one codon change in EPSPS	*E. colona*

[1]Adapted and updated from Duke (2019.

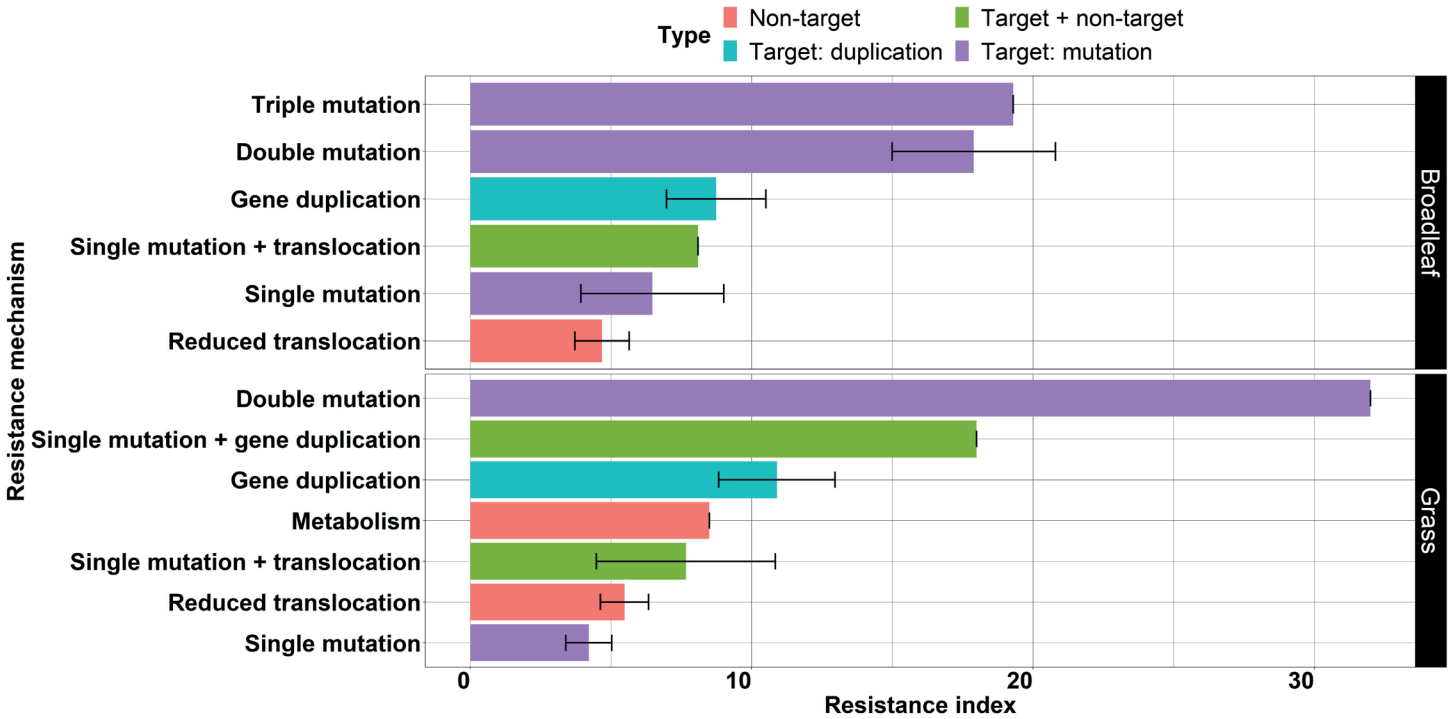

Figure 2. Comparison of the resistance index (fold resistance level in the field compared to the susceptible weed biotype) imparted by different glyphosate resistance mechanisms. The papers from which the data originated are provided in Bael *et al.* (2021). Reprinted by permission of Springer, *Reviews of Environmental Contamination and Toxicology*, Evolution of glyphosate-resistant weeds, Baek *et al.*, 2021, https://doi.org/10.1007/398_2020_55

of glyphosate resistance. Later, cases of a two codon and a three-codon changes were found, providing an even more resistant form of EPSPS, resulting in a higher level of resistance for the weed (Figure 2).

Early research on selection of glyphosate resistance in plant tissue cultures in the laboratory found duplication of the EPSPS gene (also called gene amplification), resulting in more EPSPS enzyme in the plant cell and, therefore, less sensitivity to glyphosate. Glyphosate resistance due to gene amplification was first found in an agricultural field in *Amaranthus palmeri* in the southeast USA, providing a higher level of resistance than a single-codon change in the EPSPS gene (Figure 2). *A. palmeri* had been a minor weed problem before this, but this GR mutant rapidly spread and became a major problem in GR crops, especially where populations of the weed occurred with evolved resistance to additional herbicides. There are now at least ten weed species (both grass and broadleaf) with gene duplication as the mechanism of glyphosate resistance. Other than for glyphosate, the only other case of evolved gene duplication providing herbicide resistance is that of resistance of *Digitaria sanguinalis* to acetyl-CoA carboxylase-inhibiting herbicides (Laforest et al., 2017). In addition to gene duplication, an evolved mechanism for higher amounts of EPSPS is overexpression of existing *EPSPS* genes when the plant is treated with glyphosate (Zhang *et al.*, 2021).

Non-target Site Mechanisms

Glyphosate is a foliar-applied herbicide that does not easily move from the sprayed leaf surface to its site of action (EPSPS) in living cells. Glyphosate uptake is even further reduced in some GR weed species, apparently by changes in properties of the leaf cuticle. One of the reasons that glyphosate is such an effective herbicide is that once it is taken up, it translocates readily to growing tissues. Translocation of glyphosate in some GR weeds is greatly reduced, apparently due to rapid sequestration of the herbicide into the vacuole (Ge *et al.* 2010), where it is not in contact with EPSPS and cannot reach vascular tissues for translocation. More recently, a GR biotype of the grass weed *Echinocloa colona* was found that prevents glyphosate from accumulating in plant cells by increased activity of a transport protein that moves glyphosate across the cell membrane to outside the living cytoplasm (Pan *et al.* 2021). This prevents downward translocation from sprayed leaves to growing tissues.

A common NTS resistance mechanism to other herbicides is enhanced metabolic degradation of the herbicide, but this mechanism has rarely been found in GR weeds, as none of the enzymes found to degrade and inactivate other herbicides are active on glyphosate. However, microbes have enzymes to readily break glyphosate down, the main reason for its relatively short half-life in soil and water. For years, whether glyphosate was metabolized in plants was controversial, but now it is clear that it is significantly metabolized to aminomethylphosponic acid (AMPA) and glyoxylate in many plant species, especially some legumes (Duke, 2011, 2019, 2021). Even though some plants apparently have an enzyme to break down glyphosate, there were no rigorously proven examples of GR weeds using enhanced enzymatic degradation as a resistance mechanism. A GR *E. colona* biotype was recently found in Australia with greater levels of an aldo-keto reductase that converts glyphosate to AMPA and glyoxylate, providing a level of resistance higher than some TS mechanisms (Figure 2).

The "Phoenix" mechanism of resistance results in this normally slow-acting herbicide to cause rapid cell death to

Figure 3. The Phoenix mechanism of glyphosate resistance. Glyphosate-susceptible (top) and -resistant (bottom) *Ambrosia trifida* at 2 (left) and 21 (right) days after glyphosate treatment. From the work of Van Horn *et al.* (2018) at Colorado State University. Courtesy of Christopher Van Horn.

the tissues on which it is sprayed directly, thereby preventing its translocation to tissues from which the plant can regrow (from the ashes of the dead, sprayed leaves comes growth from unsprayed tissues, hence a Phoenix phenomenon) (Figure 3). This mechanism of glyphosate resistance has only been reported in *Ambrosia trifida*, and the reasons for its very fast action in this GR biotype are still unknown. The Phoenix mechanism of evolved herbicide resistance has only been reported for one other herbicide (2,4-D) in only one species, (*Conyza sumatrensis*) (De Queiroz *et al.*, 2020).

Stacked Mechanisms and Creeping Resistance

No other herbicide or herbicide class with a particular mode of action has as many mechanisms of evolved resistance associated with it. This is probably due to two factors. First, glyphosate has been used on a greater area continuously and thereby has imposed a higher selection pressure than any other herbicide. However, a more important explanation may be that one or two mechanisms of resistance to most other herbicides provide a very high level of resistance that is relatively easy to evolve.

For example, one-codon changes in genes for the target sites of several classes of herbicides (e.g., inhibitors of acetolactate synthase such as sulfonylurea herbicides) confer higher levels (*e.g.*, 100-fold or more) of resistance than any of those of GR weeds (Figure 2), removing the evolutionary pressure for selection for any further mechanisms. As mentioned earlier, plants have an arsenal of enzymes that can degrade and inactivate most herbicides if present in sufficient amounts. High levels of NTS resistance to herbicides other than glyphosate have often evolved by elevation in levels of these enzymes. Thus, evolved glyphosate resistance has often been found to be a form of "creeping" resistance, a phenomenon in which a weak resistance is augmented by the addition of another mutation that strengthens it. Thus, a single codon alteration in the EPSPS gene allowed a weakly resistant weed population to persist to gain a second EPSPS mutation to generate an even more resistant form of EPSPS. In other situations, a TS mechanism was followed by the addition of a NTS resistance trait, providing more robust resistance (Figure 2). In *Eleusine indica*, there are GR populations containing almost every combination of TS resistance mechanisms, with the double mutation of the *EPSPS* gene providing the highest resistance level (ca. 25X) when homozygous (Zhang *et al*., 2021).

With so many mechanisms of resistance to glyphosate, can more be expected to evolve? It is hard to imagine any more. However, in at least some species, glyphosate is exuded from the roots of sprayed plants into soil, where it is virtually inactive. Increased root exudation of glyphosate has been speculated to be a potential mechanism of glyphosate resistance (Ghanizadeh & Harrington, 2020). To our knowledge, this mechanism has not been searched for, but in those species in which root exudation has been described, this process probably reduces susceptibility to glyphosate to some extent.

The ever-increasing number of GR weed species and the astounding number of evolved resistance mechanisms has been a boon to academia, as research on this dire problem for some farmers has been the research focus of hundreds of graduate students and the basis for thousands of published studies. The rapidity with which these many resistance mechanisms have evolved after introduction of GR crops provides strong evidence of the power of Darwinian evolution.

The Future of Glyphosate and GR Crops

Glyphosate is still extensively used because there are still hundreds of weed species that have not evolved resistance to it, and there are populations of species for which resistance has been reported in some places that are still susceptible in other locations. Therefore, even where there are problems with one or more GR weed species in a field, many farmers and vegetation management personnel continue to use glyphosate to control the still susceptible weeds, combined with other herbicides or non-herbicide methods to kill GR weeds. Thus, we can expect the evolution of more GR weeds as long as glyphosate is heavily used. The golden egg-laying goose is not as valuable as before, but farmers will abandon it only when the combination of larger numbers of GR weeds and better weed management options converge to reduce its value sufficiently.

References

Baek, Y., Bobadilla, L.K., Giacomini, D.A., Montgomery, J.S., Murphy, B.P. & Tranel, P.J. (2021) Evolution of glyphosate-resistant weeds. *Reviews of Environmental Contamination and Toxicology* **255**, https://doi.org/10.1007/398_2020_55

Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe* **28**:1–15

Bradshaw, L.D., Padgette, S.R., Kimball, S.L. & Wells B.H. (1997) Perspectives on glyphosate resistance. *Weed Technology* **11**, 189–198

Duke. S.O. (2011) Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. *Journal of Agricultural and Food Chemistry* **59**, 5835–5841

Duke, S.O. (2019) Enhanced metabolic degradation of glyphosate: The last evolved resistance mechanism? *Plant Physiology* **181**:1401–1403

Duke, S.O. (2021) Glyphosate: Uses other than in glyphosate-resistant crops, mode of action, degradation in plants, and effects on non-target plants and agricultural microbes. *Reviews of Environmental Contamination and Toxicology* **255**: 1–65, doi.org/10.1007/398_2020_53

Duke, S.O. & Powles, S.B., (2008) Glyphosate: A once in a century herbicide. *Pest Management Science* **64**, 319–325

Ge, X., d'Avignon, D.A., Ackermann, J.J. & Sammons, R.D. (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. *Pest Management Science* **66**, 345–348.

Ghanizadeh, H. & Harrington, K.C. (2020) Perspective: Root exudation of herbicides as a novel mode of herbicide resistance in weeds. *Pest Management Science* **76**: 2543–2547

Laforest, M., Soufiane, B., Simard, M.-J., Obeid, K, Page, E. & Nurse, R.E. (2017) Acetyl-CoA carboxylase overexpression in herbicide-resistant large crabgrass (*Digitaria sanguinalis*). *Pest Management Science* **73**, 2227–2235.

Heap, I. (2021) The International Herbicide-Resistant Weed Database. http://www.weedscience.org/ [Accessed, August 27, 2021]

Nandula, V.K., Reddy, K.N., Rimando, A.M., Duke, S.O. & Poston, D.H. (2007) Glyphosate-resistant and -susceptible soybean (*Glycine fmax*) and canola (*Brassica napus*) dose response and metabolism relationships with glyphosate. *Journal of Agricultural and Food Chemistry* **55**, 3540–354.

Pan, L, Yu, Q., Wang, J., Han, H., Mao L., Nyproko, A. *et al.* (2021) An ABCC-type transporter endowing glyphosate resistance in plants. *Proceedings of the National Academy of Science. USA* **118**, https://doi.org/10.1073/pnas.2100136118

De Queiroz, A.R.S., Delatorre, C.A., Lucio, F.R., Rossi, C.V., Zobiole, L.H.S. & Merrotto, A. (2020) Rapid necrosis: a novel plant resistance mechanism to 2,4-D. *Weed Science* **68**, 6–18

Van Horn, C.R., Moretti, M.L., Robertson, R.R., Segobye, K., Weller, S.C., Young, B.G. *et al.* (2018) Glyphosate resistance in *Ambrosia trifida*. Part 1. Novel rapid death response to glyphosate. *Pest Management Science* **74**, 1071–1078

Zhang, C., Yu, C.-J., Yu, Q., Guo, W.-L., Zhang, T.-J. & Tian X.-S. (2021) Evolution of multiple target-site resistance mechanisms in individual plants of glyphosate-resistant *Eleusine indica*. *Pest Management Science* **77**, 4810–4817

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