CROP INSIGHTS

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Glyphosate Resistance in Weeds

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Summary

- Glyphosate was on the market for over 20 years before the first resistance case was confirmed in 1996. Resistance has currently been documented in 24 weed species.
- Three mechanisms of resistance to glyphosate have been identified: a target site mutation of the EPSP synthase gene, an altered pattern of glyphosate translocation, and amplification of the EPSP synthase gene.
- Resistance has most commonly occurred in areas where glyphosate was used every year, sometimes multiple times a season.
- Continuous glyphosate use may also lead to a shift in the weed community toward species that are naturally able to survive or avoid exposure to glyphosate.
- Practices that reduce glyphosate selection intensity such as combination or rotation of herbicides, crop rotation, or tillage can reduce the risk of resistance.
- In many cases, weed populations with resistance to glyphosate have already developed resistance to other herbicides as well, which can limit weed control options.

Introduction

Weed resistance to herbicides has been a management challenge for nearly as long as herbicides have been used for weed control. The first reported case of weed resistance to a herbicide occurred in 1957 with spreading dayflower resistance to 2,4-D in sugarcane in Hawaii. As herbicide use in crops increased in subsequent years, so did cases of resistance. Herbicide-resistant populations have now been documented in 203 weed species around the world (Heap 2012).

The first major outbreak of herbicide resistance to impact corn production in North America was triazine resistance, beginning in the early 1970's. Extensive use of atrazine in corn led to widespread triazine resistance in pigweeds, lambsquarters, kochia and many other species throughout North America and beyond. Triazine resistant weeds accounted for more cases of herbicide resistance than any other herbicide mode of action up through the mid-1990s.

The first case of glyphosate resistance in North America was discovered in 2000 in a population of horseweed (or marestail) in Delaware.

In 1982, acetolactate synthase (ALS)-inhibiting herbicides debuted in the marketplace. Many ALS herbicides were developed in the following years in the sulfonylurea, imidazolinone, pyrimidinylthiobenzoate, and triazolopyrimidine families and were used widely in the 1980s and 1990s. The extensive use of these herbicides across multiple crops, coupled with their strong selection pressure and multiple mutations capable of conferring resistance, led to a rapid increase in resistant weed populations during the 1990s. By 2000, ALS inhibitors had surpassed triazines as the herbicide mode of action with the most cases of resistance.

The introduction of crops with engineered resistance to glyphosate in 1996 appeared to be a possible solution to the problem of herbicide resistance. At the time, glyphosate had already been in use for over 20 years for preplant and preemergence (burndown) weed management, post-directed weed management, as well as for vegetation management in non-crop areas, with no known cases of resistance evolution. This led to doubt that resistance to glyphosate would ever develop. The relative scarcity of plant species expressing natural tolerance to glyphosate, coupled with the unlikelihood of the complex processes involved in creating glyphosate-resistant crops being duplicated under field conditions, lent support to this doubt. This outlook was

short-lived, however, as cases of evolved resistance to glyphosate began to appear. Glyphosate-resistant weeds are now a major challenge to weed management in many areas, and the continued widespread use of glyphosate makes it likely that the problem will continue to get worse.

Glyphosate Resistance

The first case of evolved resistance to glyphosate was confirmed in rigid ryegrass in Australia in 1996. A second occurrence in Australia was confirmed soon thereafter. Both of these cases involved rigid ryegrass populations in orchards where glyphosate had been applied 2-3 times a year for more than 15 years.

The first case of glyphosate resistance in the U.S. occurred in 2000 in a population of horseweed, or marestail, in Delaware. This discovery was significant because it was the first case of glyphosate resistance specifically associated with glyphosate-resistant crops. Additionally, the site where the resistant population was confirmed had been under repeated annual glyphosate use for only 3 years. This case demonstrated the risk of resistance evolution within a much shorter time-frame than previously observed. The subsequent discoveries of resistance to glyphosate in common ragweed, Palmer amaranth, and common waterhemp were also specifically associated with repeated annual glyphosate use in glyphosate-resistant crops.

To date, glyphosate resistance has been confirmed in 24 weed species worldwide, including 14 in North America (Heap 2012). Glyphosate resistant weed populations have been confirmed in 29 states and two Canadian provinces (Figure 1). The number of weed species in which glyphosate resistance has evolved (24) is still relatively small compared to ALS inhibitor resistance (127) and triazine resistance (69), however the high level of dependence upon glyphosate as a primary weed management tool across multiple crops makes the development and spread of resistant populations of particular concern.

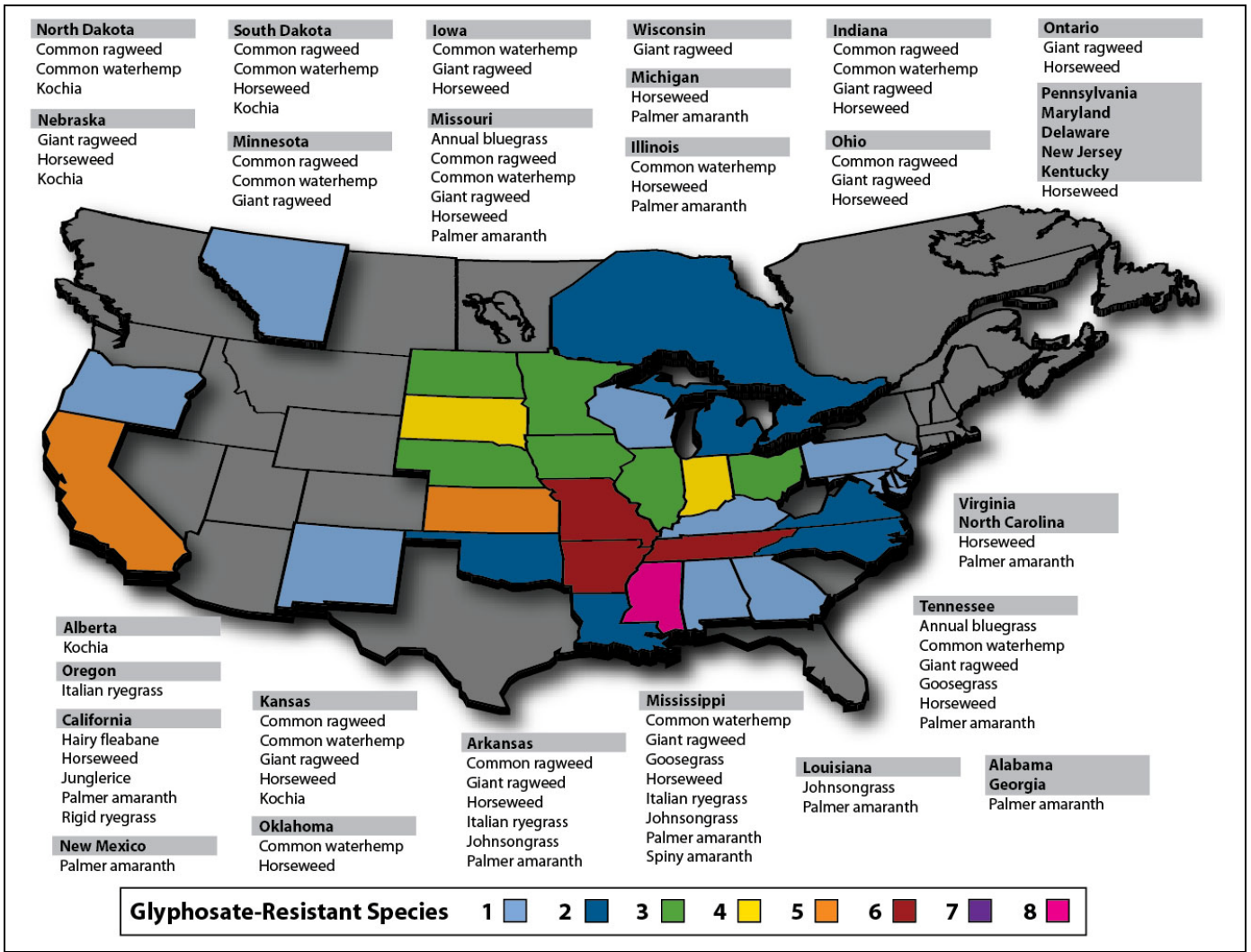


Figure 1. Confirmed glyphosate-resistant weed populations in North America (Fall 2012).

How do weeds become resistant?

Herbicide resistance is defined as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. Herbicides do not induce resistance in weed species, rather they simply select for resistant individuals that naturally occur within the weed population. The more a herbicide is used, the greater the likelihood of encountering a resistant individual in a field. Once a resistant plant has been selected, repeated use of a herbicide over multiple generations allows the resistant plants to proliferate as susceptible plants are eliminated.

Once a resistance gene has occurred within a population, failure of the herbicide can be rapid. Weed resistance to continuous use of the same herbicide occurs on a logarithmic rate of seed increase (Table 1). Resistant weed biotypes will produce seeds comprising an increasing proportion of the soil seed bank. The percentage of weeds in the population that are resistant to the herbicide gradually increases at an imperceptible rate and then makes a logarithmic jump to become more than half the weed population. This is why fields typically go from adequate control (>90% control) to failure (<50% control) in one year. The problem has actually been building for several years, but only becomes noticeable when it is too late to reverse. This is why weed resistance to herbicides must be managed before resistance occurs.

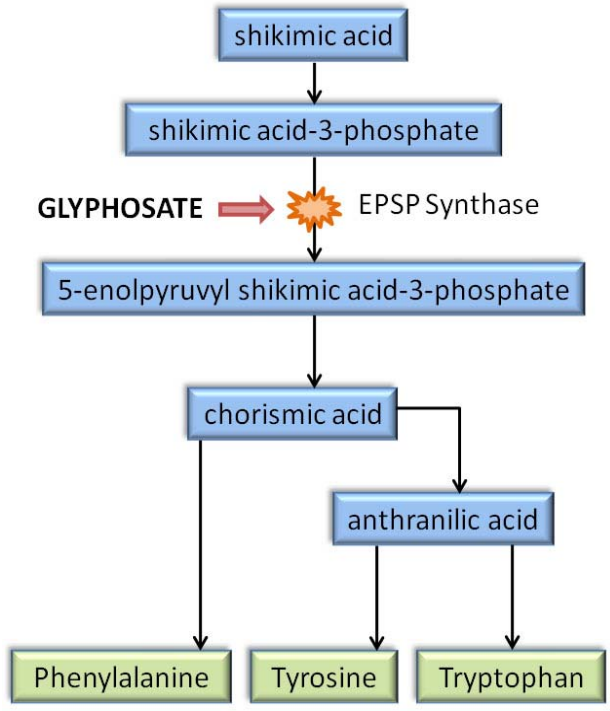
Table 1. Logarithmic progression of weed resistance to herbicides[1].

Treatment year	% Resistant weeds in total population	Weed control
0	.0001	Excellent
1st Application	.00143	Excellent
2nd Application	.0205	Excellent
3rd Application	.294	Excellent
4th Application	4.22	Excellent
5th Application	**60.5**	**Failure**

[1] Hypothetical model data. The actual time to occurrence depends on many factors. Martin, A. U. of Nebraska.

Mechanisms of Resistance

Target site mutations are by far the most common mechanism of herbicide resistance. These mutations alter the target site in such a way that it can still function but herbicide molecules can no longer bind to it. Other mechanisms, such as reduced translocation of the herbicide in the plant, have been reported but are far less common. Three mechanisms of resistance to glyphosate have been reported: a target site mutation of the EPSP synthase gene,



The shikimate pathway in plants produces the amino acids phenylalanine, tyrosine and tryptophan. Glyphosate binds to 5-enolpyruvyl shikimic acid-3-phosphate (EPSP) synthase which blocks the pathway and kills the plant.

an altered pattern of glyphosate translocation, and amplification of the EPSP synthase gene.

A **target site mutation** has been associated with resistance of goosegrass, rigid ryegrass, and Italian ryegrass to glyphosate. An amino acid substitution at Pro_{106} in the EPSP synthase gene causes it to produce enzyme molecules that glyphosate is less likely to bind to. Pro_{106} is not actually at the glyphosate binding site, but an amino acid substitution at this position is believed to reorient the binding site in such a way that it has a reduced affinity for glyphosate. Mutations at the binding site typically confer a much higher level of herbicide resistance, which explains the relatively low level of resistance associated with this mutation (2-4 fold).

An **altered pattern of herbicide translocation** has been identified as the mechanism of resistance in populations of rigid ryegrass, Italian ryegrass, and horseweed. In resistant plants, glyphosate accumulates in the leaves rather than being translocated throughout the plant. Extensive translocation throughout the plant is a key factor that makes glyphosate such an effective herbicide, which may explain why a reduction in translocation can produce such a dramatic reduction in efficacy. Altered translocation has been shown to produce a higher level of glyphosate resistance (8-12 fold) than the EPSP synthase target site mutation (2-4 fold).

Gene amplification has been identified as the mechanism of glyphosate resistance in palmer amaranth. Genomes of resistant plants have between 5x and 160x more copies of the EPSP synthase gene than susceptible plants. In resistant plants, EPSP synthase activity is still inhibited by glyphosate, but the high levels of EPSP synthase expression allows the plants to survive the application.

Factors Influencing Resistance Risk

Several factors influence the evolution of weed resistance to herbicides, including mutation rate for resistance traits, number of genes required to confer resistance, dominance of the resistance allele, inheritance of resistance traits, fitness of resistant plants, and herbicide selection intensity.

Mutation rates for resistance traits have not been determined empirically in weed species, and are commonly estimated based on expected rates of spontaneous mutation. Estimates of mutation rates for resistance traits typically range from 1×10^{-5} to 1×10^{-12}. The frequency of mutations conferring resistance to glyphosate has not been determined, but has been assumed to be much lower than that for triazines and ALS inhibitors due to the relative scarcity of weed species that have evolved resistance to glyphosate.

Nearly all cases of resistance to herbicides are conferred by a single gene. Under conditions of high selection intensity, resistance conferred by a single gene tends to evolve much more rapidly in a weed population than multigenic resistance, which requires the convergence of multiple alleles to yield a resistant phenotype. Studies of glyphosate resistant weeds have found that resistance is usually a single gene trait, but not always. Inheritance patterns of glyphosate-resistant rigid ryegrass in California suggest that two genes are involved. Variability in glyphosate response in common waterhemp may also be a function of more than one gene.

In most cases, resistance to herbicides is conferred by a dominant or partially dominant allele. A resistance allele that is at least partially dominant is much more likely to establish and spread within a weed population due to expression of the dominant phenotype by heterozygous plants. Similarly, most mutations conferring herbicide resistance are nuclear-encoded, which allows resistance traits to be spread through pollen as well as seeds. Nuclear-encoded resistance is associated with many commonly used classes of herbicides including acetolactate synthase (ALS) inhibitors. A major exception is resistance to the triazine class of herbicides, of which most cases are conferred by a target-site mutation in the chloroplast genome and are maternally-inherited. Maternally-inherited traits can only be spread by seed. So far, all known mechanisms of glyphosate resistance are associated with a dominant or incompletely-dominant, nuclear-encoded gene.

Resistance mutations can reduce the competitiveness and reproductive success of resistant plants rendering them at a disadvantage to susceptible populations in the absence of herbicide selection. Relative fitness of resistant plants has been extensively studied in triazine-resistant weed populations, which are typically less competitive than susceptible plants. In contrast, resistance to ALS inhibitors and ACCase inhibitors has not been associated with reduced weed fitness. There is evidence that glyphosate resistance may be associated with a reduction in weed fitness. Studies of rigid ryegrass have shown that the frequency of resistant plants in a population declined over time when glyphosate was not applied. This could have important implications for long-term resistance management. If resistant plants decline in frequency over time when glyphosate is not used, it may be possible to eventually restore the effectiveness of glyphosate.

Changes in Weed Communities

Resistance and reduced sensitivity of weeds to glyphosate can be a problem in glyphosate-resistant crops; however resistance cases are still relatively rare. A more likely problem that growers may encounter with continuous glyphosate use is a shift in the weed community toward species that are naturally able to survive or avoid exposure to glyphosate. Despite glyphosate's broad spectrum of weed control, there are some species that are naturally less susceptible to it. Morningglory species have increased in prevalence in some areas due to their natural tolerance to glyphosate. Common lambsquarters, giant ragweed, and velvetleaf also tend to vary in their tolerance to glyphosate.

Some weed species, such as morningglory, naturally have a higher tolerance to glyphosate. Tolerant species can increase in prevalence under continuous glyphosate use.

Weeds can also survive glyphosate application by avoiding exposure. Non-residually active herbicides such as glyphosate allow weeds that emerge after application to survive. Common lambsquarters and eastern black nightshade are two species that may escape control by emerging after a glyphosate application. A long term study of continuous glyphosate use showed increases in giant ragweed and shattercane over time due to their capacity for later emergence (Jeschke 2007).

Weed species with a wide window of emergence timing such as common lambsquarters (top) and giant ragweed (above) may be able to emerge after a glyphosate application and survive to produce seed.

Resistance Prevention and Management

Most factors that determine the risk of glyphosate resistance are inherent plant characteristics that cannot be influenced by a grower. The one risk factor a grower can control is herbicide selection intensity. Herbicide selection intensity is determined by herbicide efficacy, persistence, and frequency of application. The more weeds that are exposed to glyphosate, the greater the selection intensity upon that population. Decreased herbicide selection intensity will reduce the probability of resistance evolution and prolong the usefulness of the herbicide chemistry. Agronomic practices such as crop rotation and tillage can decrease herbicide selection intensity by reducing weed populations; however, effectiveness can vary among weed species (Jeschke 2007). Combination or rotation of herbicide modes of action is likely the most effective means to delay resistance. Using a pre-emergence herbicide followed by glyphosate is an example of a practice that can reduce glyphosate selection intensity and may improve weed control. However, it is essential to balance responsible herbicide stewardship with the need to maintain satisfactory levels of weed management.

The appearance of a herbicide-resistant weed species in the field does not necessarily mean that the herbicide can no longer be an effective weed management tool for that field. For example, atrazine is still widely used despite prevalent weed resistance; however it is typically used in combination with herbicides with other modes of action. The situation with glyphosate is complicated due to the fact that many of the weed species in which glyphosate resistance has occurred may already be resistant to other herbicides. Populations of several species have developed multiple resistance to glyphosate and other herbicides in North America. Multiple resistance limits viable alternative herbicides, and in some cases no good alternatives may be available. Development of new herbicide resistance technologies in crops may provide new options for dealing with herbicide resistant weeds. However, overreliance on any new technology is likely to yield the same result as overreliance on glyphosate.

References

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. October 10, 2012. Available www.weedscience.com

Jeschke, M. R. 2007. Weed Community Responses to Cropping System Factors in Glyphosate-Resistant Corn and Soybean. Ph.D. Thesis. Univ. of Wisconsin-Madison.

Nandula, V. K. (ed.) 2010. Glyphosate Resistance in Crops and Weeds. John Wiley and Sons, Inc., Hoboken, NJ.