http://www.weeds.iastate.edu/mgmt/2007/glysurvival.pdf

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How plants survive glyphosate

Glyphosate is the most widely used herbicide in the world for several reasons, including: 1) high level of effectiveness, 2) flexibility in application, 3) large margin of crop safety in glyphosate resistant crops, and 4) safety to applicators and the environment. Glyphosate was used for more than 20 years before weeds developed resistance to the chemical. This relative low risk of glyphosate resistance compared to other herbicide classes resulted in considerable debate on how best to use the technology to minimize selection of resistant biotypes. This paper will briefly review the factors that make glyphosate such an effective herbicide, and then describe the mechanisms that provide resistance to glyphosate in glyphosate resistant crops (GRC) and weeds.

Glyphosate activity

Several factors contribute to glyphosate's unprecedented performance in controlling weeds, including disruption of an important metabolic process, inefficient metabolism of glyphosate by plants, and efficient translocation to growing points. The primary target for glyphosate is the enzyme EPSPS (5-enolpyruvoylshikimate 3-phosphate synthase). When glyphosate binds to EPSPS it forms a very stable complex that essentially permanently disables the enzyme. This enzyme is involved in the shikimic acid pathway, products of this system include aromatic amino acids, anthocyanins, phytoallexins, lignin, growth promoters and inhibitors, and many other compounds. It is estimated that up to 35% of the dry weight of plants is composed of aromatic compounds produced by the shikimic acid pathway.

The primary selectivity mechanism for most herbicides is the ability to metabolize a herbicide to non-toxic compounds. Tolerant plants are able to detoxify the herbicide before it reaches the target site. This is how corn survives atrazine and Steadfast, and how soybeans tolerate Pursuit and Valor. Sensitive plants are able to metabolize most herbicides, just not quickly enough to prevent the compound from accumulating at the target site. For example, residues of dicamba in soybean decreased from 4.8 ppm to 0.3 ppm in 12 days. In contrast, most studies with glyphosate indicate that glyphosate metabolism in plants is very slow.

The introduction of the phenoxy herbicides in the 1940's represented the first systemic herbicides, and provided new opportunities to control perennial weeds. While many systemic herbicides are on the market, none are moved as efficiently within the plant as glyphosate. Rapid movement and accumulation of glyphosate at plant growing points, combined with the disruption of a critical metabolic pathway, results in what has been called the herbicide of the century.

Glyphosate resistant crops

The development of GRC by Monsanto in 1995 changed not only the way Iowa farmers control weeds, but also their entire outlook on weed management. Since the initial release of Roundup Ready soybeans in 1996, GRC have dominated weed management systems in soybean and cotton, and are quickly becoming key players in corn and several other crops.

Numerous methods of creating GRC were evaluated, including increasing the expression of the EPSPS enzyme, introducing enzymes capable of metabolizing glyphosate, and introduction of an insensitive

EPSPS (Dill, 2005). Initially the search for an insensitive EPSPS focused on creating an altered target site through mutagenesis, similar methods as used in developing ALS resistant crops in the 1980's. Initial efforts to create an enzyme not inhibited by glyphosate but that would allow the plant to develop normally were unsuccessful.

The majority of glyphosate resistant crops on the market today use an insensitive EPSPS known as CP4. The gene for the CP4 enzyme was isolated from an *Agrobacterium* species. A second insensitive enzyme (GA21) isolated from corn and developed through mutagenesis is used in Agrisure GT corn hybrids. The mechanism for glyphosate resistance with the insensitive target site is shown in Figure 1. When glyphosate is applied to the plant, the native EPSPS is shut down, but the insensitive enzyme that has been inserted into the plant provides an alternative route for the shikimic acid pathway.

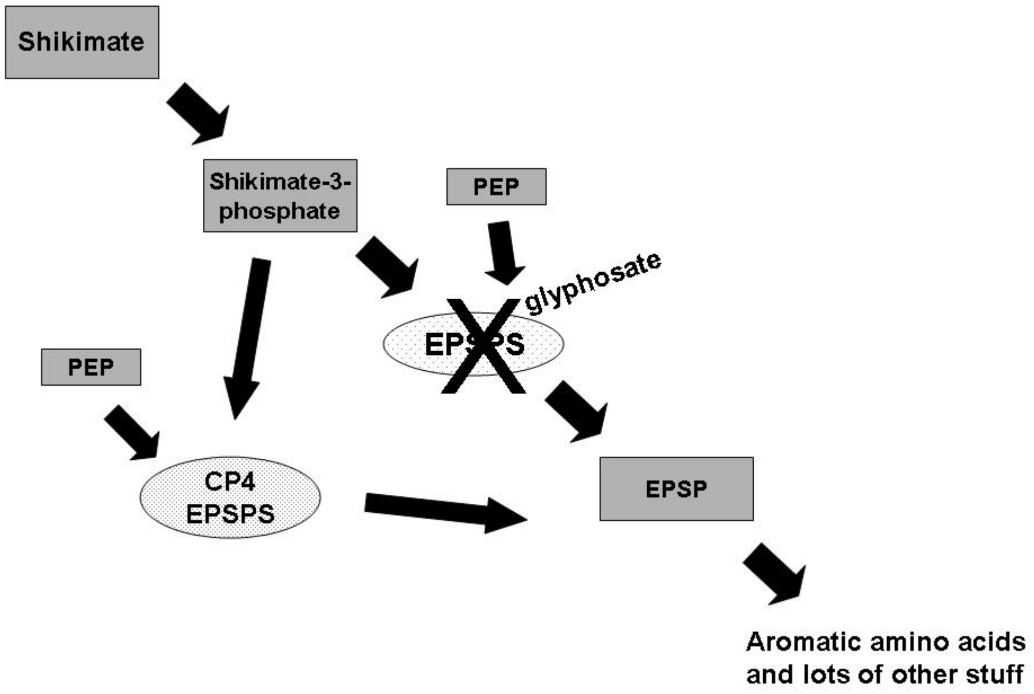

Figure 1. Resistance due to insensitive CP4 EPSPS.

Glyphosate oxidase (GOX), a bacterial enzyme that metabolizes glyphosate, is used in combination with an the CP4 EPSPS in GR canola. The GOX enzyme failed to provide a sufficient level of glyphosate resistance by itself. DuPont has developed a new metabolism based gene that is used in the Optimum GAT trait, a technology that combines two resistance mechanisms, one for glyphosate and one for ALS inhibiting herbicides.

Glyphosate Resistant Weeds

Shortly after the introduction of Roundup Ready soybeans, the first GR weed was reported (rigid ryegrass in Australia). The first resistant weed selected in a cropping system using GRC was horseweed, identified in Delaware in 2000. Currently, twelve weed species have been identified with GR biotypes. Several mechanisms providing resistance to glyphosate have been identified, and many of the GR biotypes possess more than one mechanism.

Horseweed (Conyza canadensis) The R:S Index (effective dose for resistant biotype / effective dose for susceptible biotype) is commonly use to measure the level of resistance. Resistance in several horseweed (also called marestail) biotypes varied depending upon application timing (Table 1). When glyphosate was applied at the two-leaf stage, the resistant biotypes responded similarly to susceptible biotypes (R:S = 1.0). However, the R:S Index for the four resistant biotypes treated at the rosette stage was 4 or greater, indicating these biotypes could survive glyphosate rates four times greater than needed to kill a susceptible biotype. While this is sufficient resistance to allow a plant to survive herbicide applications, R:I Indices for other herbicide classes typically have been 100 or greater.

Table 1. Glyphosate resistance characteristics of three horseweed populations[1].

Growth stage	Delaware	Ohio	Virginia	Arkansas
	-------------- R:S Index --------------			
Two-leaf	0.8	1	0.9	1.1
Rosette	4.2	4.7	4	4.2

[1] Dinelli et al. 2006.

The authors attributed the resistance to three mechanisms: 1) altered translocation of glyphosate, increased levels of EPSPS, and increased branching. In the GR horseweed biotypes less glyphosate was translocated out of the leaves to the root and apical meristems than in susceptible biotypes. Retaining glyphosate within the leaves of treated GR horseweed resulted in the leaves being sacrificed, therefore protecting the roots and growing points from the effects of glyphosate. Horseweed seedlings treated at the 2-leaf stage would not have sufficient energy reserves to replace killed leaves, thus allowing glyphosate to kill small seedlings (R:S = 1.0). The specific mechanism responsible for the altered translocation pattern was not identified, but it was speculated that glyphosate was sequestered in the apoplast, therefore enhancing xylem transport to margins of leaves in which the glyphosate was absorbed.

Target site resistance can be achieved either via an insensitive target site or by increasing the amount of enzyme produced, therefore 'diluting' the effect of the herbicide. GR horseweed accumulated 2 to 3X higher levels of EPSPS mRNA than susceptible biotypes, and changes in concentrations of shikimate following glyphosate application was indicative of EPSPS overexpression. This trait could protect meristems from the toxic effects of glyphosate since the altered translocation patterns did not completely prevent movement of glyphosate to these tissues.

Italian ryegrass (Lolium multiflorum) Alterations in glyphosate translocation patterns similar to that observed in horseweed were observed in one GR Italian ryegrass biotype, but a second GR biotype did not demonstrate this response (Perez-Jones et al. 2007). In the GR1 biotype, nearly 80% of the glyphosate remained in the treated leaf, compared to only 51% in the susceptible biotype (Table 2). Translocation patterns in the GR2 biotype were similar to the susceptible biotype, suggesting that a different resistant mechanism was responsible in this biotype.

Table 2. Translocation patterns of glyphosate applied to a single leaf 24 hours after application.

Bitoype	% of absorbed glyphosate				
	Treated section	Tip of treated leaf	Untreated leaves	Stem	Roots
Susceptible	22	29	20	17	11
GR1	26	51	3	10	9
GR2	33	38	10	9	9

Perez-Jones et al. 2007. Planta.

The EPSPS enzyme is a protein consisting of approximately 425 amino acids. Analysis of the gene sequence for EPSPS in the GR2 biotype indentified a single amino acid substitution (proline at the 106 position changed to a serine). This same substitution has been identified in other plants, and is known to provide a moderate level of resistance to glyphosate. Thus, this research found that different mechanisms were responsible for GR in the two ryegrass populations that were studied.

Other GR weeds In the past two years GR has been reported in several important weed species that infest agronomic fields of Iowa and surrounding states, including waterhemp, giant and common ragweed and common lambsquarters. At this time the specific mechanisms responsible for GR resistance have not been identified, but altered translocation is suspected to be involved in many of these biotypes based on symptomology following application. The same EPSPS modification as described in Italian ryegrass has been identified in a GR goosegrass biotype, but a second GR goosegrass biotype with a different, but unidentified, resistance mechanism has been documented.

Conclusion

Our understanding of the mechanisms that provide GR in weeds is still in its infancy, but the identification of several different resistance mechanisms suggests that new reports of GR weeds should be expected. Glyphosate resistance is different from previous herbicide resistance issues faced in the Midwest due to both the multiple resistance mechanisms and the relative level of resistance. The use of correct glyphosate rates has been promoted as the critical factor in managing glyphosate resistance. Since most GR biotypes possess a relatively low resistance level, use of rates that allow significant numbers of weed escapes undoubtedly would enhance the rate that resistance evolves within a weed population. However, it should be noted that the majority of species in which GR has evolved have a relatively high natural tolerance to glyphosate. Simply using labeled rates is unlikely to be an effective resistance management strategy since escapes with these species are likely to occur whenever the environment places them under stress or other factors limit glyphosate efficacy. Effective management of glyphosate resistance will require the use of not only appropriate rate selection, but also inclusion of alternative weed management strategies and limiting reliance on glyphosate.

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