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Herbicide resistance mechanisms: Why doesn't that weed die?

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Introduction

According to the International Survey of Herbicide Resistant Weeds (http://www.weedscience.org) there are currently 461 unique cases of herbicide-resistant (HR) weeds. The United States leads the way in number of HR weeds, with twice as many as any other country (Table 1). The prevalence of herbicide resistance is determined primarily by the acres under cultivation and type of production systems utilized, rather than how well individual farmers manage weeds. The USA leads the world in HR weeds because we have expansive acreage devoted to production systems heavily reliant on herbicides, not because our farmers are less adept at managing herbicide resistance than their foreign counterparts.

Table 1. Global leadership in number of herbicide-resistant weeds.

Country	Resistant weeds	Country	Resistant weeds
United States	153	China	41
Australia	76	Israel	36
Canada	61	Japan	36
France	45	Brazil	34

Most persons involved in production agriculture have a basic understanding of herbicide resistance – why it develops and how we can minimize its impacts. The purpose of this presentation is to provide a more in-depth discussion of the mechanisms that allow weeds to survive herbicides that once were effective against that particular species. Management practices that lead to the evolution of resistant biotypes or how to manage resistance once it is present will not be discussed.

Herbicide resistance mechanisms

A HR weed possesses an inherited trait that allows it to survive a herbicide that is effective against other biotypes of that species. Four resistance mechanisms are responsible for the majority of known cases of resistance. The known resistance mechanisms for herbicides commonly used in Iowa are shown in Table 2. Decreased absorption and/or translocation is another mechanism that may be involved in evolved resistance.

Table 1. Herbicide resistance mechanisms reported for herbicides commonly used in Iowa.

Resistance mechanism	HG 1 (ACC ase)	HG 2 (ALS)	HG 3 (DNA)	HG 4 (Auxin)	HG 5 (Triazine)	HG 9 (glyphosate)	HG 14 (PPO)	HG 27 (HPPD)
Target-site	X	X	X	X	X	X	X	
Metabolism	X	X			X			X
Sequestration						X		
Gene amplification						X		

Target-site based resistance

Herbicides kill plants by binding to a specific molecule in the plant. Most target-sites are proteins that function as enzymes. Binding of the herbicide to the protein prevents the protein from functioning, therefore stopping an essential metabolic process. For example, Group 2 herbicides bind to acetolactate synthase (ALS), an enzyme involved in the synthesis of the branch-chain amino acids. The loss of these amino acids prevents the formation of proteins ultimately resulting in plant death. Symptoms of Group 2 herbicides initially occur on new growth since protein synthesis is essential in cell division and development. Target-site based resistance is the most common resistance mechanism, and has been identified in resistant biotypes of Group 1, 2, 3, 5, 9 and 14 herbicides.

Binding of herbicides to a target-site is an intricate process and has been compared to a "lock and key" mechanism. Herbicides are relatively small molecules in comparison to the target-site. The herbicides are able to bind to the target-site because their molecular structure conforms to a particular location on the surface of the target-site. Target-site-based herbicide resistance occurs when there is a change in the target-site that alters the surface where the herbicide attaches. In most cases the change is due to a substitution of a single amino acid in the protein (proteins are long chains of amino acids). The change in the target-site must not interfere with the function of the protein, otherwise it would be lethal to the plant. Group 5 target-site resistance reduces the efficiency of photosynthesis, therefore making Group 5 resistant weeds less fit than their susceptible wild types. No or little fitness penalty has been found with target-site resistance to Group 2 herbicides.

Multiple herbicide families can attack some target-sites. For example, ALS is the target-site for both sulfonylurea and imidazolinone herbicides. There are multiple mutations to ALS that provide resistance to Group 2 herbicides. Some mutations provide cross resistance to herbicides from the two families, whereas others are specific to herbicides from one of the families. Group 5 (triazine), 6 (nitriles) and 7 (ureas) herbicides all bind to the same protein involved in electron transfer in Photosystem II. The target-site modification that provides resistance to Group 5 herbicides has little impact on binding of group 6 and 7 herbicides (Fuerst et al. 1986).

Several weeds have evolved resistance to Group 4 herbicides. While target-site based resistance has not been conclusively confirmed, evidence suggests this is the mechanism involved with this site of action.

Enhanced metabolism

Differential metabolism is the most common herbicide selectivity mechanism. For example, corn's tolerance to atrazine, acetochlor (Harness) and mesotrione (Callisto) is due to corn's ability to metabolize the herbicide before it reaches the target-site at toxic concentrations. Susceptible plants are less efficient at metabolizing the herbicide, therefore the herbicide reaches the target-site at lethal concentrations.

Several enzyme systems are involved in metabolism of herbicides, probably the most important is a class of enzymes known as Cytochrome P450. Plants have many genes that code for different forms of this enzyme, each having a unique spectrum of molecules that they are able to metabolize. Metabolism-based herbicide resistance can provide cross resistance to herbicides from multiple herbicide groups. An Australian weed scientist, Dr. Stephen Powles, is fond of saying metabolism-based resistance can provide resistance to herbicides that have yet to be discovered (Powles and Yu 2010). The mechanics of enhanced metabolism-based herbicide resistance are poorly understood. Metabolism-based resistance in insects is due to overexpression of the enzymes involved in insecticide metabolism.

An Illinois waterhemp population is resistant to both HG 5 and 27 herbicides (Ma et al. 2013). The resistance to HG 5 is provided by enhanced metabolism of atrazine by glutathione conjugation, whereas resistance to HG 27 was due to enhanced metabolism by a Cyt P450 enzyme. It is interesting that the

initial cases of triazine resistance in *Amaranthus* spp. was due to an altered target-site, the finding of enhanced metabolism is a second resistance mechanism for this herbicide group in waterhemp.

Resistance to 2,4-D, glufosinate, and dicamba in Enlist, Liberty Link, and Roundup Ready Xtend HR crops, respectively is achieved by insertion of genes that code for enzymes that metabolize the target herbicides. In contrast, Roundup Ready corn and soybean gain resistance due to an altered target-site, EPSPS.

Sequestration

Animals eliminate toxins via their excretory system, this is why urine of cows fed hay treated with picloram or other persistent auxin herbicides may contain toxic herbicide concentrations. Plants don't have this system, thus they store toxins in areas where they won't interfere with cell functions. Herbicides are metabolized into less toxic forms that can be placed in the cell vacuole (often called the "cell garbage can") or be exported outside of the cell into the cell wall.

Glyphosate-resistant horseweed/marestail rapidly sends glyphosate that has entered the cell into the vacuole. Moving the glyphosate into the vacuole reduces the amount of the herbicide that is available to be transported to meristematic regions via the phloem (Ge et al. 2010). This sequestration is likely responsible for the decreased translocation reported in earlier research of glyphosate resistance in this species (Dinelli et al. 2006).

Gene amplification

An increase in the copies of a particular gene in a plant's DNA can result in an increase in the protein that gene is responsible for. Glyphosate resistance in both waterhemp and Palmer amaranth is due to additional copies of the gene for glyphosate's target-site. Resistant biotypes of Palmer amaranth had 5 to 160 times more copies of the EPSPS gene than susceptible biotypes (Gaines et al. 2010). Multiple copies of the gene result in excess EPSPS in resistant plants, therefore diluting the glyphosate that enters the plant. In resistant biotypes some of the EPSPS will be shut down by glyphosate, but a sufficient amount of the enzyme is left 'untouched' to sustain plant growth. Identification of this mechanism in the Amaranthus species is the first report of gene amplification as a herbicide resistance mechanism.

Mutations: the underlying cause of herbicide resistance

A HR weed biotype possesses a trait not found in the wild type of the weed. The source of this trait is a mutation in the genetic code of the plant. Mutations are relatively common and are the original source of variation within a species. For example, red hair is caused by a mutation on chromosome 16 of the human genome. Mutations play a role in both species evolution and cancer.

Some mutations occur during normal replication of DNA, some during DNA repair processes, whereas others may be induced by the environment (mutagens). Herbicides are not the cause of these mutations, although it has been speculated that non-lethal doses of a herbicide might increase the occurrence of mutations due to stress placed on the plant. In most cases the mutation that provides herbicide resistance occurred prior to discovery of the herbicide. In the absence of the herbicide the mutation remains at a low frequency in the population since there is no advantage for a plant to possess the resistance trait if the herbicide.

There are different types of mutations. The simplest mutations involve errors during the replication of the DNA, resulting in a change in the nucleotide sequence of a gene. A gene is essentially a template that determines the sequence of amino acids in a protein. A mutation changes the nucleotide sequence of the gene. The change in nucleotide sequence may result in the substitution of an amino acid in the protein, thereby altering the configuration of that protein.

A small section of the nucleotide sequence of the ALS gene is shown in Figure 1 (Yu et al. 2003). A codon is a series of three nucleotides, and a codon essentially tells the organism which amino acid to insert in a protein. In the third codon of the HG2-resistant biotype, a mutation replaced the C with an A. This change in a single nucleotide results in histidine being substituted for proline in the ALS enzyme, therefore preventing binding of Group 2 herbicides. Numerous mutations of this type have been identified on the ALS gene that provide resistance to Group 2 herbicides. The high frequency of these mutations is the reason for the rapid evolution of Group 2 resistance.

Wild type ALS gene					
Nucleotide codon	CAG	GTG	CCT	CGT	CGG
Amino acid	Glycine	Valine	Proline	Arginine	Arginine
			↓ *mutation*		
HG 2 resistant ALS gene					
Nucleotide codon	CAG	GTG	CAT	CGT	CGG
Amino acid	Glycine	Valine	Histidine	Arginine	Arginine

Figure 1. Nucleotide sequence and derived amino acids in the wild type and a HG 2 resistant biotype of wild radish (Adapted from Yu et al. 2003).

Another type of mutation is the duplication of large sections of DNA. This can result in a plant having multiple copies of genes. The only known example of this type of mutation providing herbicide resistance is found in glyphosate resistant *Amaranthus* species.

Summary

The intent of this presentation is to increase understanding and appreciation of the complexity of herbicide resistance. Mutations occur fairly often during DNA replication, and a very small percentage of these may occur on genes that play a role in herbicide activity. Herbicides place tremendous selection pressure on weed populations. Therefore, any weed biotype that possesses one of these mutations for herbicide resistance has a tremendous advantage over the wild type whenever any herbicide group is heavily relied on.

The frequency of herbicide resistance within weed populations varies among herbicide groups – this is why resistance appears rapidly following introduction of some herbicide groups (e.g. HG 1 and 2), whereas with other groups it takes many years of repeated use for resistance to evolve (e.g. HG 9 and 15). It was known that the initial frequency of resistance to glyphosate was low compared to most other herbicide groups, but heavy reliance on this product led to the selection of weeds having a range of herbicide resistance mechanisms. The numerous mechanisms that allow plants to survive herbicides reinforces the importance of developing resilient weed management strategies that attack weeds with not only multiple herbicide groups, but with additional selection pressures.

References

Dinelli et al. 2006. Physiological and molecular insight on the mechanisms of resistance to glyphosate in *Conyza canadensis* biotypes. Pest. Biochem. Physiol. 86:30-41.

Fuerst et al. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Sci. 34:344-353.

Gaines et al. 2010. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*). PNAS 107:1029-1034.

Ge, X. et al. 2010. Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag. Sci. 66:345-348.

Ma, R. et al. 2013. Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. Plant Phsiol. 163:363-377.

Powles and Yu. 2010. Evolution in Action: Plants Resistant to Herbicides. Ann. Rev. Pl. Biol. 61:317-347.

Yu et al. 2003. ALS gene proline (197) mutations confer ALS herbicide resistance in eight separated wild radish populations. Weed Sci. 51:831-838.