OCCURRENCE AND MECHANISMS OF WEED RESISTANCE TO GLYPHOSATE

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Introduction

Glyphosate is a nonselective foliar-applied herbicide that has been used for over 20 years for the management of annual, perennial, and biennial herbaceous species of grasses, sedges, and broadleaf weeds, as well as woody brush and tree species (Bradshaw et al. 1997). Commercialization of engineered glyphosate resistance in several crop species has further expanded the in-crop use of the herbicide (Padgette et al. 1996). In addition to being highly effective on a broad spectrum of annual and perennial weed species common to many cropping systems, glyphosate has other favorable environmental characteristics such as strong sorption to soil and very low toxicity to mammals, birds, and fish (Padgette et al. 1996). These factors have contributed to glyphosate being the most widely used herbicide in the world (Powles et al. 1997).

Due to the widespread reliance on herbicides as a primary method of weed management, herbicide-resistant weed species have become an increasing problem in many cropping systems. Herbicide resistance can be defined as the inherited ability of a plant to survive and reproduce following exposure to a herbicide dose normally lethal to the wild type; resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis (Weed Science Society of America 1998). Weed resistance to herbicides has been confirmed in 156 species (94 dicots and 62 monocots) worldwide, most commonly to acetolactate synthase (ALS) inhibitors, triazines, or acetyl-coenzyme A carboxylase (ACCase) inhibitors (Heap 2003). In Wisconsin, intensive use of a single herbicide chemistry has been associated with the development of resistance of several weed species, including eastern black nightshade (*Solanum ptycanthum*) (Volenberg et al. 2000), giant foxtail (*Setaria faberi*) (Volenberg et al. 2001), and green foxtail (*Setaria viridis*) (Volenberg et al. 2002b) resistance to ALS inhibitors, as well as giant foxtail (Stoltenberg and Wiederholt 1995) and large crabgrass (*Digitaria sanguinalis*) (Wiederholt and Stoltenberg 1995) resistance to ACCase inhibitors.

Although strong arguments have been proposed against the likelihood of weeds developing resistance to glyphosate (Bradshaw et al. 1997), resistance to glyphosate has occurred. The first confirmed case of weed resistance to glyphosate was rigid ryegrass (*Lolium rigidum*) in Australia (Pratley et al. 1996). A second occurrence of glyphosate-resistant rigid ryegrass in Australia was confirmed soon thereafter (Powles et al. 1998). Additional cases of rigid ryegrass resistance to glyphosate were confirmed in California in 1998 and South Africa in 2001 (Heap 2003). However, resistance to glyphosate has not been limited to rigid ryegrass. Goosegrass (*Elucine indica*) in Malaysia (Lee and Ngim 2000) and Italian ryegrass (*Lolium multiflorum*) in Chile (Heap 2003) have developed resistance to glyphosate. Of much greater concern is the occurrence of glyphosate-resistant horseweed (*Conyza canadensis*) in soybean cropping systems in Delaware (VanGessel 2001) and the rapid increase in its occurrence across the eastern corn belt (Heap 2003). Furthermore, inconsistent control of common waterhemp (*Amaranthus tuberculatus*) populations in Iowa (Owen 2002) and in Illinois and Missouri (Smeda and Schuster 2002) with glyphosate, have raised concerns about resistance or tolerance to glyphosate in these weed populations.

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A considerable amount of research has been conducted recently to better understand the biology and physiology of these glyphosate-resistant weed species. Information from such research is important to better predict the rate of spread of resistance to glyphosate in confirmed cases, to assess the likelihood of additional or new occurrences of weed resistance to glyphosate, and to improve management strategies to reduce the selection for weed resistance to glyphosate.

Weed Resistance to Glyphosate

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, an enzyme in the aromatic amino acid biosynthesis pathway, also known as the shikimate pathway (Devine et al. 1993). The three essential amino acids phenylalanine, tyrosine, and tryptophan are products of the shikimate pathway. Furthermore, many aromatic secondary products such as lignins, alkaloids, flavonoids, and benzoic acids that are important for plant growth and development are also products of this pathway. In addition to plants, the shikimate pathway is found in fungi and bacteria, but the pathway does not occur in animals. As much as 20% of the carbon fixed by photosynthesis, flows through this highly regulated pathway.

EPSP synthase is a nuclear-encoded, chloroplast-localized enzyme that catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to form EPSP and inorganic phosphate (Devine et al. 1993). Glyphosate is the only herbicide known to inhibit this enzyme. The inhibition of EPSP synthase by glyphosate is complex, but accumulated data indicate that glyphosate binds to the region of EPSP synthase that binds the PEP moiety, forming an enzyme-S3P-glyphosate complex, which prevents the reaction from proceeding normally. Inhibition of the pathway leads to an accumulation of the intermediates shikimate and S3P. Although resistance to herbicides has been due to an altered site of action in many weed species, resistance can be associated with several other mechanisms, including altered herbicide absorption, translocation, and metabolism, as well as herbicide sequestration (Powles et al. 1997).

Rigid ryegrass

Some the first research to address the mechanism of resistance to glyphosate focused on rigid ryegrass from Australia (Pratley et al. 1996, 1999; Feng et al. 1999). A resistant population occurred in an area that had been continuously cropped since 1981, with glyphosate applied annually, typically with 2,4-D, for preplant weed management in a mixed rotation of summer and winter crop species. Glyphosate applied at labeled recommended rates had failed to control the resistant population. The resistant biotype exhibited nearly 10-fold resistance to glyphosate compared to susceptible biotypes (Pratley et al. 1999). Additional research indicated that both absorption and translocation of glyphosate were similar between resistant and susceptible biotypes (Feng et al. 1999). Metabolism of glyphosate to aminomethyl-phosphonic acid (AMPA) occurred to a limited extent in resistant and susceptible biotypes, but did not differ greatly between biotypes. Consequently, Feng et al. (1999) concluded that neither uptake, translocation, nor metabolism played a major role in rigid ryegrass resistance to glyphosate.

More extensive research (Lorraine-Colwill et al. 1999, 2001) on the mechanism of resistance in another glyphosate-resistant rigid ryegrass population from Australia (Powles et al. 1998) has led to different conclusions than those of Feng et al. (1999). This second population of rigid ryegrass occurred within rows of trees in which glyphosate had been applied two or three times annually for 15 years. The resistant plants exhibited 7- to 11-fold resistance to glyphosate compared to susceptible plants (Powles et al. 1998). In subsequent experiments (Lorraine-Colwill et al. 2001), no differences in absorption of glyphosate occurred between susceptible and

resistant plants. Furthermore, no differences were observed between the biotypes in EPSP synthase activity in the presence or absence of glyphosate. However, in contrast to Feng et al. (1999), results of Lorraine-Colwill et al. (2001) indicated that altered translocation of glyphosate may confer resistance to glyphosate. In most plants, glyphosate is readily translocated in the phloem to growing points. However in resistant rigid ryegrass plants, glyphosate was translocated primarily in the xylem. Since transpiration is the driving force for water movement in plants, such a resistance mechanism would result in glyphosate accumulation in mature leaf tissue rather than in the growing points, thus providing an effective mechanism of resistance.

Goosegrass

In contrast to glyphosate-resistant ryegrass from Australia, resistance of goosegrass from Malaysia to glyphosate appears to be due to an altered EPSP synthase (Dill et al. 2000). Research results indicated that uptake and translocation of glyphosate did not differ between resistant and susceptible biotypes, and that glyphosate was not metabolized by either biotype. Rather, a mutation of proline to serine within the ESPS synthase coding region of the resistant biotype was concluded to be the underlying basis of resistance. As such, this mechanism of resistance represents the first report of an altered EPSP synthase conferring resistance to glyphosate in weeds. Prior to this occurrence, plants with glyphosate-resistant EPSP synthase had never been found in nature (Bradshaw et al. 1997). It had been postulated that any potential alterations in weed EPSP synthase that significantly decreased glyphosate binding (i.e. that conferred resistance to glyphosate), would also be deleterious to phosphoenolpyruvate (PEP) binding and potentially have a negative impact on weed fitness and the ability to survive (Bradshaw et al. 1997). Interestingly, altered sites of action typically confer relatively high levels of resistance to herbicides (Volenberg et al. 2000, 2001, 2002a,b), but in the case of glyphosate-resistant goosegrass, resistance was only 2- to 3-fold compared to susceptible plants.

Horseweed

The occurrence and spread of glyphosate-resistant horseweed in the eastern U.S. (VanGessel 2001) likely represents the greatest immediate concern or impact to corn and soybean production in the upper Midwest. Within 3 years of using only glyphosate for weed control in no-tillage continuous glyphosate-resistant soybean, glyphosate failed to control horseweed in several fields in the mid-Atlantic region (VanGessel 2001). Seedlings originating from seed of one population collected in Delaware exhibited 8- to 13-fold resistance to glyphosate compared to susceptible seedlings. Extensive research focusing on the mechanism of resistance has shown that resistance does not appear to be based on differential glyphosate uptake, metabolism, differential gene expression of EPSP synthase, or amplification of EPSP synthase (Heck et al. 2002). However, one physiological distinction has been observed between resistant and susceptible plants. Although similar amounts of glyphosate were absorbed by leaves, susceptible horseweed plants translocated about 2-fold more glyphosate to roots compared to resistant plants (Heck et al. 2002). It is not clear whether this differential translocation confers resistance to glyphosate at the whole plant level, but it appears to be a characteristic associated with the resistance locus.

In 2000, glyphosate-resistant horseweed was estimated to occur at more than 100 sites across more than 10,0000 acres in Delaware (Heap 2003). Since then, its occurrence has increased rapidly. In 2001, glyphosate-resistant horseweed was estimated to occur in Tennessee at more than 500 sites and more than 100,000 acres (Heap 2003). In 2002, glyphosate-resistant horseweed was confirmed in New Jersey, Maryland, Ohio, and Indiana (Heap 2003). This rapid increase in occurrence of glyphosate-resistant horseweed is likely due in part to the widespread

adoption of no-tillage glyphosate-resistant soybean production systems, but it is also likely due to horseweed biology. The reproduction, dispersal, and germination ecology of horseweed make it a species likely to infest adjacent and distant fields (Holm et al. 1997). Horseweed produces a large number of small seeds, whose wind dispersal is the most likely means for the spread of resistance (VanGessel 2001). Horseweed seeds are able to germinate and establish in non-disturbed soils, providing the potential to colonize both no-tillage crop fields and non-disturbed, non-crop sites. Furthermore, outcrossing can occur between horseweed plants, increasing the potential for movement of resistance traits within and among horseweed populations (VanGessel 2001). Subsequent research has confirmed this potential. The resistance trait for glyphosate was transmitted in crosses between resistant and susceptible horseweed plants (Heck et al. 2002). Further analysis indicated that a nuclear-encoded dominant allele confers horseweed resistance to glyphosate (Heck et al. 2002).

Common waterhemp

Inconsistent control of common waterhemp populations in Iowa (Hartzler et al. 2002; Owen 2002) and in Illinois and Missouri (Smeda and Schuster 2002) with glyphosate, have raised concerns about resistance or tolerance to glyphosate in these weed populations. Research to characterize potential resistance in these populations has been difficult, in part to the variable response of these populations to glyphosate, and also to the low percentage of surviving plants following exposure to glyphosate. Even after three generations of selection with glyphosate, only a low percentage of plants survived glyphosate applied at rates recommended for control (Smeda and Schuster 2002). However, a relatively high percentage of plants propagated from shoot cuttings of putatively resistant plants initiated new growth 2 weeks after treatment with glyphosate at rates of 0.75 lb ae/acre or greater; even plants treated with glyphosate at rates as high as 3.0 lb ae/acre survived and formed reproductive structures (Smeda and Schuster 2002). These results and those of Hartzler et al. (2002) and Owen (2002) suggest that individual common waterhemp plants within populations are resistant to glyphosate, but many aspects of common waterhemp resistance to glyphosate are not clear. Although the trait for resistance to glyphosate appears to be heritable (Owen 2002), the alleles that confer resistance to glyphosate have yet to be identified. Furthermore, a specific physiological mechanism of common waterhemp resistance to glyphosate has yet to be identified (Owen 2002).

Conclusions

Herbicide-resistant weed species have become an increasing problem in many cropping systems. Worldwide, resistance to herbicides has been confirmed in 156 weed species and a total of 261 biotypes (Heap 2003). The spread of weed resistance to herbicides has been relatively rapid in corn and soybean cropping systems, due in part to management factors associated with these crops (Owen 2001). While very few of these occurrences of herbicide-resistant weeds in corn and soybean have been unmanageable, they highlight the potential risks associated with typical weed management practices. The occurrence of glyphosate-resistant horseweed in soybean cropping systems in the mid-Atlantic states (VanGessel 2001) and the rapid increase in its occurrence across the eastern corn belt (Heap 2003) are important examples of this risk. Although research is increasing our understanding of many aspects of glyphosate-resistant weeds, the physiological mechanism of resistance is not clearly understood for several biotypes. However, it appears that resistance to glyphosate is conferred by more than one mechanism in weeds, including altered EPSP synthase, suggesting that there may be fewer constraints to the evolution of weed resistance to glyphosate (Gressel 1996) than have been proposed (Bradshaw et al. 1997).

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