CASE HISTORIES OF WEED RESISTANCE TO GLYPHOSATE

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Introduction

Weed management has changed dramatically in the last 10 years. Since the introduction of glyphosate-resistant (Roundup Ready) soybean in 1996, varieties with the Roundup Ready trait have been grown on an increasing number of acres, totaling more than 65 million acres in the US in 2004 (Anonymous, 2005). Corn hybrids with the Roundup Ready trait have also increased in popularity since their introduction in 1998, and were planted on more than 8 million acres in the U.S. in 2004 (Anonymous, 2005). With this dramatic increase in Roundup Ready trait acreage, glyphosate use for in-crop weed management has also increased, and will likely increase further with expected increases in Roundup Ready corn and alfalfa acreage. Although glyphosate offers the perceived benefits of safe, simple, and effective weed management to growers, it is subject to many of the same pitfalls as other herbicides, including resistant weeds.

About the time that Roundup Ready technology came to the market, strong arguments were proposed against the likelihood of weeds developing resistance to glyphosate (Bradshaw et al., 1997). However, resistance to glyphosate has occurred (Heap, 2005). The first confirmed case of weed resistance to glyphosate was rigid ryegrass in Australia (Pratley et al., 1996). Since then, resistance has been confirmed in seven other weed species (Heap, 2005), including several species in the U.S. In 2000, glyphosate-resistant horseweed was confirmed in Delaware (Van Gessel, 2001) and has since been found in 13 other states (Heap, 2005). More recently, resistance of common ragweed in Missouri (Bradley, 2005a) and Palmer amaranth in Georgia (Culpepper, 2005) to glyphosate has been confirmed.

Potential resistance of other important weed species to glyphosate has also recently been reported including common waterhemp in Missouri (Bradley, 2005b), common lambsquarters in Ohio (Loux and Stachler, 2005), and giant ragweed in Ohio (Stachler et al., 2005). In Wisconsin, weed resistance to glyphosate has not been confirmed, but since 2002, there have been many reports of variable or inconsistent responses of common lambsquarters to glyphosate in Roundup Ready soybean fields (Boerboom, 2004). Re-treatment of common lambsquarters escapes with glyphosate has typically resulted in adequate control, but the cause of less than optimal control, and in some cases poor control, has yet to be fully explained.

Resistance to herbicides other than glyphosate has been selected for in several weed species found in Wisconsin, including resistance to ALS (acetolactate synthase) inhibitors in eastern black nightshade (Volenberg et al., 2000), giant foxtail (Volenberg et al., 2001), and green foxtail (Volenberg et al., 2002), resistance to ACCase (acetyl-coenzyme A carboxylase) inhibitors in giant foxtail (Stoltenberg and Wiederholt, 1995) and large crabgrass (Wiederholt and Stoltenberg, 1995), and resistance to triazine herbicides in several broadleaf weed species (Stoltenberg, 1995).

The development of weed resistance has typically been associated with reliance on a single herbicide chemistry over time, i.e. a high level of herbicide selection intensity (Volenberg et al., 2000, 2001, 2002; Stoltenberg and Wiederholt, 1995; Wiederholt and Stoltenberg, 1995). That is, repeated exposure of a weed community to a specific herbicide chemistry (or related chemistries)

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has selected for weeds resistant to that chemistry. Once such herbicide use has selected for resistant individuals, continued herbicide use (i.e. continued selection intensity) favors resistant plants over susceptible plants. Over time, the frequency of resistant plants in a weed population increases, representing a potentially serious long-term weed management problem.

Although herbicide selection intensity is a critical factor in the development of weed resistance, predicting what species will develop resistance, when it will happen, where it will happen, and the rate of spread of resistance once it has occurred, has been very difficult. This has been due in large part to many factors (including herbicide selection intensity) that play a role in the selection for herbicide-resistant weeds (Stoltenberg, 2004).

Can we learn from some of these occurrences of glyphosate-resistant weeds, such that we can minimize the potential for resistance to glyphosate developing in Wisconsin? In the case of confirmed weed resistance to glyphosate, what has been the glyphosate selection intensity (e.g. frequency of use, rate, and other weed management factors)? How does the selection intensity in these cases compare with how we're using glyphosate in Wisconsin? If we consider some cases of weed resistance to glyphosate, it may help us assess the potential for selection of glyphosate-resistant weeds in Wisconsin.

Case Histories

Glyphosate-Resistant Horseweed

Within 3 years of using only glyphosate for weed control in a no-tillage, glyphosate-resistant soybean production system, glyphosate failed to control horseweed in several fields in the mid-Atlantic region (Van Gessel, 2001). Glyphosate applied preplant and in Roundup Ready soybean was the sole control method in 1998, 1999, and 2000. In 2000, several horseweed plants were not affected by glyphosate applied at 1.5 lb ae/acre, and were not controlled with an additional treatment of glyphosate applied at 1.5 lb ae/acre. Progeny of surviving plants exhibited 8- to 13-fold resistance to glyphosate compared to susceptible seedlings.

Glyphosate-resistant horseweed has spread rapidly since 2001. Since then, it has been found in 13 other states (Heap, 2005). The rapid increase in occurrence of glyphosate-resistant horseweed is likely due in part to the widespread adoption of no-tillage, Roundup Ready soybean production systems, but it is also likely due to horseweed biology. The reproduction, dispersal, and germination characteristics 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 (Van Gessel, 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. Also, outcrossing can occur between horseweed plants, increasing the potential for movement of resistance traits within and among horseweed populations.

What have been the management implications following the discovery and spread of glyphosate-resistant horseweed in the eastern corn belt? The rapid spread of horseweed has resulted in the need to treat all horseweed as glyphosate-resistant plants (Van Gessel et al., 2004). In Delaware, Maryland, and Virginia, extension specialists do not recommend the use of glyphosate for preplant weed control in Roundup Ready soybeans (Van Gessel et al., 2004). Since glyphosate will likely be used at least once after soybeans are planted, it is their recommendation to vary the mode of action of herbicides used for controlling horseweed, e.g. dicamba or 2,4-D based programs. This is particularly critical for management of horseweed, as

it has evolved resistance to many herbicide chemistries (triazine, amide, bipyridilium, imidazolinone, and sulfonylurea herbicides) in more than 10 countries worldwide, and is considered one of the 10 most important herbicide-resistant weeds (Heap, 2005). Horseweed plants in Ohio have developed multiple-resistance to glyphosate and ALS-inhibiting herbicides. For this reason, the use of herbicides that contain the active ingredient cloransulam for preplant horseweed control is not recommended by extension specialists in the mid-Atlantic region (Van Gessel et al., 2004).

Van Gessel et al. (2004) recommend that a diversity of weed management tactics be used for controlling horseweed. Risk for selecting glyphosate-resistant horseweed is greater when soybeans are planted on a frequent basis. Planting soybeans one out of two years appears to lessen the risk of developing horseweed resistance to glyphosate compared to continuous soybean, but this does not eliminate the risk. They discourage planting Roundup Ready soybeans more than three out of six years. If soybeans are planted more frequently than three out of six years, periodic use of tillage is recommended; specifically, tillage in two out of six years is encouraged to minimize the impact of developing glyphosate-resistant biotypes. They have found that spring tillage is a very effective option for controlling horseweed. As a general rule, Van Gessel et al. (2004) recommend not planting Roundup Ready crops in the same field two years in a row. The use of non-glyphosate based weed control programs (a soil-applied preemergence herbicide program followed by an appropriate postemergence herbicide when needed) should be used periodically in combination with tillage and crop rotation when and where possible.

Glyphosate-Resistant Common Ragweed

In 2002, a population of common ragweed was discovered in central Missouri that was inadequately controlled following several applications of glyphosate over six years (Pollard et al., 2004). This population was identified in a no-tillage soybean field that had been in continuous soybean production (with some double-cropped wheat) for many years and in Roundup Ready soybean since 1996 (Bradley, 2005a). In greenhouse experiments, progeny of suspected resistant plants were nearly 10-fold resistant to glyphosate compared to known susceptible plants. Under field and greenhouse conditions, glyphosate-resistant plants typically grow slower and exhibit shortened internodes, reduced stature, and an overall bushy appearance compared to susceptible plants (Sellers et al., 2004). Although differences in morphology between resistant and susceptible plants have been shown to affect interception of glyphosate spray solution, this has not fully accounted for the reduced glyphosate activity on resistant plants. Most of the glyphosate-resistant common ragweed plants have remained concentrated in a 50-acre area within the field of origin, but recent evaluation has shown that resistant plants have spread along a roadside at least 200 feet away from the infested field (Smeda et al., 2005).

Following the discovery of glyphosate-resistant common ragweed, management recommendations from University of Missouri extension specialists have included tank mixing glyphosate with another mode of action herbicide in burndown treatments (e.g. 2,4-D) when glyphosate is to be applied in the subsequent crop, tank mixing glyphosate with another mode of action herbicide (e.g. lactofen or Cobra) for standard in-crop glyphosate use, alternating glyphosate use with other herbicide modes of action between years, and other integrated weed management practices (Bradley, 2005a).

Glyphosate-Resistant Palmer Amaranth

The most recent addition to the list of glyphosate-resistant weeds is Palmer amaranth (Culpepper, 2005; Heap, 2005). It is known to infest about 500 acres of cotton in central Georgia and has demonstrated resistance to extremely high rates of glyphosate applied in the field under excellent growing conditions. Numerous field and greenhouse studies completed in 2005 indicated probable Palmer amaranth resistance to glyphosate, but heritability studies to determine whether the resistance trait is passed on to progeny have been completed and confirm resistance of this population to glyphosate. This development is considered a serious threat to future cotton production throughout the region. Palmer amaranth is considered a very troublesome weed in cotton production systems due its high competitive ability with neighboring crop plants and its potential to interfere with cotton harvest. Palmer amaranth resistance to glyphosate is also of considerable concern because this species commonly hybridizes with other closely related pigweed species, including redroot pigweed and smooth pigweed (Stubbendieck et al., 1994).

Since the introduction of cotton with the Roundup Ready trait in 1997, glyphosate has been the most effective tool to manage Palmer amaranth; most alternative control options are much less effective than glyphosate in controlling typical (susceptible) populations (Culpepper, 2005). Consequently, farmers have relied heavily on glyphosate to control weeds in cotton (Culpepper, 2005). About 94% of Georgia's 1.21 million acres of cotton were Roundup Ready in 2005. The long-term effects of glyphosate-resistant Palmer amaranth on cotton production are unknown, but if this weed species can no longer be controlled with glyphosate, alternative herbicide chemistries and aggressive tillage may once again have to be used for management, increasing both time and costs to growers (Culpepper, 2005).

Potential Resistance to Glyphosate – Common Lambsquarters

Although the variable response of common lambsquarters to glyphosate has received widespread attention in the Midwest including Wisconsin (Boerboom, 2004) over the last few years, it probably has been more of a prominent issue in Ohio than elsewhere. Confirmation and characterization of common lambsquarters resistance to glyphosate has been difficult. As early as 2003, Ohio State University weed scientists characterized several common lambsquarters populations as having reduced sensitivity to glyphosate; subsequent research on additional common lambsquarters biotypes found several of these resistant to glyphosate (Loux and Stachler, 2005). However, these biotypes demonstrated a relatively low level of resistance to glyphosate, especially compared to the high level of resistance exhibited by most ALS-inhibitor resistant weed species. For glyphosate-resistant common lambsquarters, a glyphosate rate of two to four times the labeled rate (0.75 lb ae/acre) is typically needed to obtain the same response as for susceptible or sensitive biotypes. Smaller resistant plants are typically more easily controlled than larger resistant plants, but some small plants may survive treatment with four times the labeled rate (Loux and Stachler, 2005).

Ohio State University weed scientists have concluded that some common lambsquarters populations in Ohio are developing resistance to glyphosate, and expect no abatement of this trend due to the selection pressure resulting from the over-reliance on glyphosate in weed management programs (Loux and Stachler, 2005). However, they acknowledge that several other factors have likely contributed to the variable response of common lambsquarters response to glyphosate, including glyphosate rate, adjuvant use, plant stage of growth (size and age), and environmental conditions. Results from 2004 field experiments indicated that common lambsquarters may be more sensitive to glyphosate formulation and surfactant rate, and possible surfactant type, than other weed species. In a recent summary of their greenhouse, growth

chamber, and field research, Loux et al. (2005) indicated that there appears to be reduced sensitivity of some common lambsquarters biotypes to rates of glyphosate up to 3 lb ae/acre, but that such expression of this response varies among studies. Results of a single 2005 field study did not corroborate those from greenhouse and growth chamber studies. They concluded that reduced sensitivity of some common lambsquarters biotypes to glyphosate may be an evolved response, and could be a contributing factor to poor performance of postemergence glyphosate treatments occasionally observed in growers' fields (Loux et al., 2005).

Loux and Stachler (2005) recommend that the simplest and most effective method for avoiding problems with postemergence common lambsquarters control may be to include an herbicide that provides residual control in preplant or preemergence burndown treatments in soybean. Their suggestions for maximizing glyphosate activity on lambsquarters include the following:

- Apply glyphosate when common lambsquarters are less than 6 inches tall
- Increase glyphosate rate to at least 1.1 lb ae/acre if plants are taller than 6 inches
- Increase glyphosate rate to at least 1.1 lb ae/acre and add surfactant when plants are under stress associated with non-favorable environmental conditions
- Consider use of additional surfactant for spray volumes more than 15 gpa
- Include 2,4-D ester with glyphosate applied preplant

Potential Resistance to Glyphosate – Common Waterhemp

Inconsistent control of common waterhemp populations with glyphosate has been the focus of much research, including populations found in Iowa (Hartzler et al., 2002; Owen, 2002) and Illinois and Missouri (Smeda and Schuster, 2002). Results from these studies suggest that individual common waterhemp plants within populations are resistant to glyphosate, but as noted above for common lambsquarters, these results have also been variable, making it difficult to confirm resistant to glyphosate at the population level.

However, common waterhemp with potential resistance to glyphosate has recently been found in two soybean fields in northwest Missouri (Bradley, 2005b). The potentially resistant weeds were found in fields planted to Roundup Ready soybeans continuously since 1996, and where glyphosate had been the sole herbicide used. Problems developed with common waterhemp control over the last three years at one of these sites, and the grower continued to use glyphosate at greater rates. In greenhouse studies, waterhemp plants (from seed collected in 2004) continued to grow after being treated with glyphosate at rates as high as 6 lb ae/acre. Studies have been initiated to determine whether a resistance trait is present in seeds collected from plants that survive glyphosate treatment. This development of glyphosate-resistant common waterhemp is of considerable concern in Missouri as this weed species is their No. 1 problem in corn and soybean production.

Potential Resistance to Glyhosate - Giant Ragweed

In Ohio, giant ragweed control with glyphosate in soybean has reportedly become more variable in recent years, indicating a potential change in the sensitivity of giant ragweed populations to glyphosate (Stachler et al., 2005). Several separate samples of giant ragweed seed were collected in 2004 from a field where glyphosate had been the sole herbicide used for at least four years, and where glyphosate appeared to less effective over time. The results of recent studies using these seed samples suggest that a giant ragweed biotype with reduced sensitivity to glyphosate has been selected (Stachler et al., 2005). This biotype survived treatment with

glyphosate at rates up to 3 lb ae/acre in greenhouse studies, and multiple treatments with glyphosate totaling more than 4.5 lb ae/acre in field studies. Plants that survived treatment in the field with glyphosate at rates up to 3 lb ae/acres produced viable seeds. Stachler et al. (2005) concluded that reduced sensitivity of this giant ragweed biotype to glyphosate may be an evolved response (suggesting a resistance trait or traits), which could likely occur in other fields with similar glyphosate selection intensity.

Summary

The number of glyphosate-resistant weed species has steadily increased to eight species since the introduction of soybean, corn, and cotton cultivars with Roundup Ready traits in the 1990's (Heap, 2005). Perhaps the most notable case is glyphosate-resistant horseweed, which has spread to 13 other states since its discovery in no-tillage soybean production systems in Delaware in 2001 (Van Gessel, 2001). Common ragweed resistance to glyphosate has occurred in a long-term soybean production system in Missouri (Bradley, 2005a; Smeda et al., 2005). Also of note is the recent confirmation of glyphosate-resistant Palmer amaranth in Georgia (Culpepper, 2005); although this weed species is rare in the upper Midwest, it readily hybridizes with species that are common in our cropping systems such as redroot pigweed and smooth pigweed (Stubbendieck et al., 1994). The variable response of other troublesome weed species such as common lambsquarters, common waterhemp, and giant ragweed to glyphosate has also become an important issue in several states across the Midwest (Boerboom, 2004; Bradley, 2005b; Loux and Stachler, 2005, Stachler et al., 2005).

These cases of weed resistance to glyphosate suggest that there may be far fewer constraints to the evolution of weed resistance to glyphosate than were proposed not that long ago (Bradshaw et al., 1997). These cases of weed resistance or variable response to glyphosate do not appear to be extraordinary. Although it is very difficult to quantify the selection intensity or pressure associated with glyphosate use in these cases, it seems that intensive use of glyphosate over time is a common factor. With the popularity of Roundup Ready soybean, the apparent increasing acreage planted to Roundup Ready corn, and the recent introduction of Roundup Ready alfalfa, the potential exists for an increasing intensity of selection for glyphosate-resistant weeds in Wisconsin. To sustain glyphosate as a useful, weed management tool, it seems that these cases suggest that selection associated with glyphosate must be reduced by integration with other weed management practices.

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