RESEARCH PROGRESS ON UNDERSTANDING HERBICIDE RESISTANCE IN WISCONSIN GIANT RAGWEED

Dave Stoltenberg, Stacey Marion, Courtney Glettner, and Vince Davis [1/]

Introduction

Giant ragweed is one of the most difficult to manage weed species in Midwestern cropping systems due to its biology and competitive ability. Adaptation to a wide range of soil environments, rapid vertical growth, and high biomass production make giant ragweed particularly competitive (Abul-Fatih et al. 1979; Harrison et al. 2007; Webster et al. 1994). An extended germination period characterized by the ability to germinate early and grow rapidly, combined with embryo dormancy that allows for prolonged emergence periods, contributes to the difficulty of managing giant ragweed (Gramig and Stoltenberg 2007; Harrison et al. 2001; Schutte et al. 2012). In Wisconsin, giant ragweed is found in both corn (Fickett et al. 2013a) and soybean (Fickett et al. 2013b) production fields. As the most competitive species relative to other common weed species in corn and soybean cropping systems (Fickett et al. 2013a,b), giant ragweed represents a serious threat to crop yield potential.

Herbicide resistance contributes further to the difficulty of giant ragweed management (Brabham et al. 2011; Norsworthy et al 2010, 2011; Vink et al. 2012; Westhoven et al. 2008). Glyphosate resistance in giant ragweed was first confirmed in Ohio in 2004 and has since been found in several other states (Heap 2014) including Wisconsin (Glettner 2013; Stoltenberg et al. 2012). Acetolactate synthase (ALS) inhibitor resistance in giant ragweed has also been found in several Midwestern states (Heap 2014), including recent confirmation in Wisconsin (Marion et al. 2013, 2014). In two instances (Minnesota and Ohio), giant ragweed has demonstrated multiple resistance to glyphosate and ALS inhibitors. Resistance to glyphosate, ALS inhibitors, or both of these herbicide modes of action, severely constrains herbicide options available to growers for effective management of giant ragweed and proactive resistance management.

Glyphosate inhibits the chloroplast enzyme *5*-enolpyruvylshikimate-*3*-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway (Shaner 2014). Inhibition of EPSPS disrupts the production of the aromatic amino acids tyrosine, phenylalanine, and tryptophan, ultimately causing plant death. Glyphosate resistance in weeds has been attributed to one or more of three mechanisms: 1) an altered EPSPS target site, 2) changes in vacuolar sequestration and/or reduced translocation of glyphosate to meristematic tissues where the EPSPS gene is primarily expressed, and 3) amplification of the EPSPS gene resulting in increased EPSPS gene expression (Sammons and Gaines 2014; Shaner et al. 2012).

Acetolactate synthase catalyzes the first common step in the biosynthesis of the branched chain amino acids leucine, isoleucine, and valine and is the primary target enzyme for five structurally distinct chemical classes of herbicides, including the sulfonylurea herbicides such as cloransulam-methyl (Shaner et al 2014). In most cases, resistance to ALS inhibitors has been attributed to reduced sensitivity of the ALS enzyme (Tranel and Wright 2002; Tranel et al 2014).

Our research objectives are to better understand herbicide resistance in Wisconsin giant ragweed and the potential of resistance to persist and spread. Research progress on glyphosate resistance in giant ragweed from Rock County and cloransulam-methyl resistance in giant ragweed from Columbia County is reported below.

[1/] Professor, Graduate Research Assistant, Graduate Research Assistant, and Assistant Professor, Dept. of Agronomy, 1575 Linden Drive, Univ. of Wisconsin-Madison, Madison, WI, 53706.

Glyphosate Resistance in Rock County Giant Ragweed

Background

In 2010, we identified a giant ragweed population that was suspected of surviving repeated exposure to glyphosate on a farm located in Rock County (Glettner 2013; Stoltenberg et al. 2012). Seeds collected from suspected glyphosate-resistant (R) and -sensitive (S) plants located on this farm were used for subsequent experiments to 1) quantify the whole-plant dose-response of R and S plants to glyphosate, 2) determine the role of glyphosate absorption and translocation in the plant, and the sensitivity of the glyphosate target site (EPSPS) in conferring resistance, and 3) determine if glyphosate resistance has affected the growth, development, and seed production of R plants relative to S giant ragweed plants.

Confirmation of Glyphosate Resistance

Whole-plant dose-response of Rock County R and S giant ragweed plants to glyphosate was determined in repeated greenhouse experiments using eight replications of doses that ranged from 0 to 16.8 kg ae ha^{-1} and included 20 g L^{-1} ammonium sulfate (AMS). Shoot mass was harvested, dried, and weighted 28 days after treatment. The glyphosate ED_{50} value (the effective dose that reduced dry shoot mass 50% relative to non-treated plants) was 6.5-fold greater for R plants (0.86 ± 0.24 kg ae ha^{-1}) than for S plants (0.13 ± 0.02 kg ae ha^{-1}) (Figures 1 and 2).

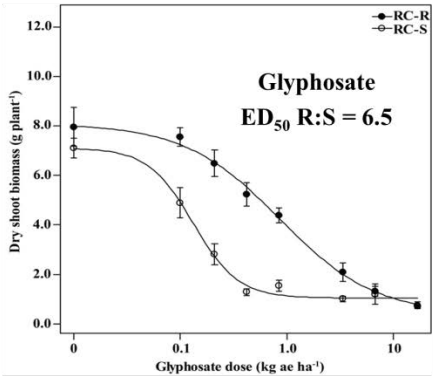

Figure 1. Dry shoot biomass for resistant (R) and sensitive (S) giant ragweed plants from Rock County (RC) 28 days after treatment with glyphosate. Vertical bars represent standard error of the mean. Data were pooled from repeat experiments for analysis.

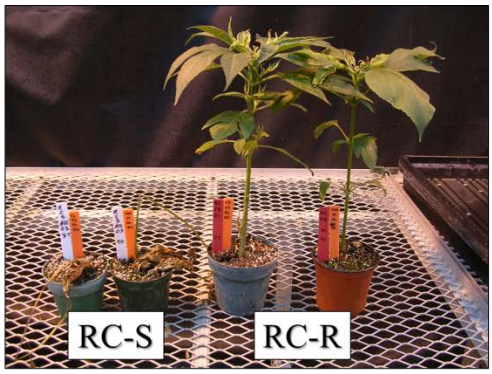

Figure 2. Glyphosate-resistant (R) and sensitive (S) Rock County (RC) giant ragweed plants 28 day after treatment with 3.36 kg ae ha^{-1} glyphosate (3.0 lb ae $acre^{-1}$) under greenhouse conditions.

Glyphosate Absorption and Translocation

Glyphosate absorption into plants and translocation to meristematic tissues were estimated by treating 5- to 6-node plants with 0.84 kg ha^{-1} glyphosate plus 2.8 kg ha^{-1} AMS (the third oldest leaf was covered) and subsequently applying ^{14}C-labeled glyphosate to the third oldest leaf. Plants were harvested 0, 6, 24, 48, and 72 hours after treatment (HAT) and sectioned into the treated leaf, tissue above the treated leaf (excluding the shoot apical meristem), the shoot apical meristem (the uppermost 1 cm of shoot including emerging leaves), aboveground tissue below treated leaf, and roots. Treatments were replicated four times and two experiments were conducted. ^{14}C was quantified using liquid scintillation spectroscopy. We found that glyphosate absorption did not differ between R and S plants, with absorption reaching 57 and 59% of applied

^{14}C 72 HAT for R and S plants, respectively (data not shown). Translocation of ^{14}C-glyphosate did not differ between R and S plants for any plant part (data not shown).

Glyphosate Target Site Sensitivity

Glyphosate target site (EPSPS enzyme) sensitivity was estimated by measuring in vivo shikimate accumulation in excised leaf discs exposed to a range of glyphosate concentrations. Shikimate was extracted from tissue and quantified spectrophotometrically. Each glyphosate concentration was replicated three times and experiments were repeated in time. Glyphosate EC_{50} values (the effective concentration that increased shikimate accumulation 50% relative to nontreated leaf tissue) were 4.6- to 5.4-fold greater for R plants than S plants (Figure 3). However, at high glyphosate concentrations (>1,000 µM), shikimate accumulation in R plants was similar to or greater than S plants.

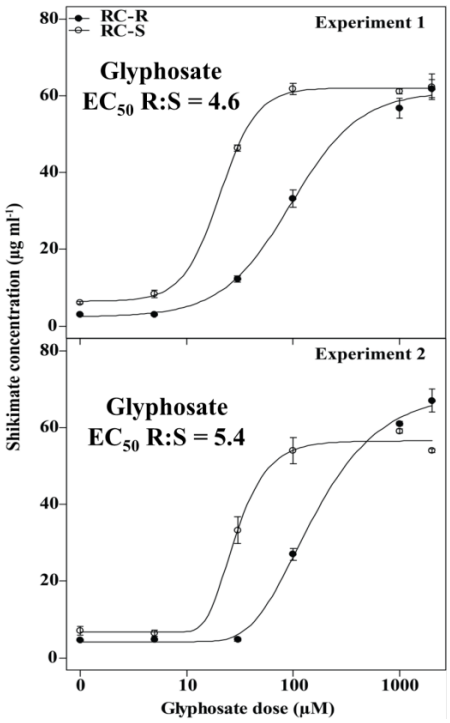

Figure 3. Shikimate concentration in leaf tissue of glyphosate-resistant (R) and -sensitive (S) giant ragweed plants from Rock County (RC) at glyphosate doses ranging from 0 to 2,000 µM after 24 hour incubation under continuous light. Vertical bars indicate standard error of the mean.

To further investigate target site sensitivity to glyphosate, we performed partial sequence analysis of the EPSPS gene extracted from apical meristematic tissue of 4- to 5-node plants. Three replicates from each accession were sequenced. Sequence results showed no missense mutations in the Pro_{106} codon in R plants that would confer resistance to glyphosate (data not shown).

Growth and Seed Production of Glyphosate-Resistant Plants

In greenhouse experiments, plant height, leaf area, and dry shoot biomass were similar between the R and S plants during vegetative growth to the onset of flowering (data not shown). However, seed production of R plants was greater than S plants (Table 1). The percentage of intact-viable seeds, intact-nonviable seeds, and empty involucres did not differ between R and S plants.

Table 1. Seed production of glyphosate-resistant and -sensitive giant ragweed from Rock County under noncompetitive conditions in the greenhouse. Data from repeated experiments were pooled for analysis.

		Seed fate category[†]		
Plant type	Seed yield	Intact-viable	Intact-nonviable	Empty involucre
	seeds/plant	% of seeds produced		
Glyphosate-resistant	812 a[‡]	75 a	13 a	12 a
Glyphosate-sensitive	425 b	65 a	14 a	21 a

[†] Intact-viable and intact nonviable: involucres contain fully formed seeds with viability of embryo determined by tetrazolium assay; empty involucre: no seed or not fully formed seed.

[‡] Means followed by the same letter within a column do not differ at the 5% level of significance as determined by a Student's t-test.

Summary: Glyphosate Resistance in Rock County Giant Ragweed

- Whole-plant dose-response experiments showed that the Rock County giant ragweed was 6.5-fold resistant (R) to glyphosate compared to sensitive (S) plants based on ED_{50} values (Figures 1 and 2). Both accessions were sensitive to cloransulam-methyl (data not shown).
- The ^{14}C-glyphosate results showed that glyphosate resistance in Rock County giant ragweed is not conferred by reduced glyphosate absorption into the plant or translocation to meristematic tissues (data not shown).
- The similar or greater shikimate accumulation in leaf discs from R plants than S plants at high glyphosate concentrations (Figure 3) and the lack of missense mutations in the Pro_{106} codon of R plants (data not shown) suggest that resistance is not likely due to an altered EPSPS target site. However, the possibility remains that the EPSPS target site is less sensitive in R plants compared to S plants at lower glyphosate concentrations. Current research is investigating other possible mechanisms that may confer resistance.
- Glyphosate resistance has not negatively affected the growth and development of R plants relative to S plants in Rock County giant ragweed. The greater seed production and similar viability of R plants relative to S plants suggests that in the absence of selection by glyphosate, the frequency of the resistance trait for glyphosate may increase in the giant ragweed field population over time.

Cloransulam-methyl Resistance in Columbia County Giant Ragweed

Background

A giant ragweed population with suspected resistance to acetolactate synthase (ALS) inhibitors was identified in a long-term corn-soybean rotation that included cloransulam-methyl (FirstRate herbicide) use in soybean for broadleaf weed management (Marion et al. 2013, 2014). After four rotation cycles (8 years total), field observations suggested that several giant ragweed plants had survived repeated exposure to cloransulam-methyl. Our objectives were to 1) confirm and quantify the whole-plant response of suspected resistant (R) and sensitive (S) plants to cloransulam-methyl, 2) if confirmed, quantify the sensitivity of the target site enzyme (acetolactate synthase, ALS) to cloransulam-methyl, and 3) determine if resistance has affected the relative competitive ability of R and S plants.

Confirmation of Cloransulam-methyl Resistance

The cloransulam-methyl ED_{50} value (the effective dose of herbicide that reduced shoot mass 50% relative to non-treated plants) for R plants was 30 times that of S plants (Figure 4). A high level of variability among R plants in response to treatment with cloransulam-methyl was attributed to incomplete segregation of the resistance trait within the sampled population. Resistant plants treated with cloransulam-methyl at up to 10 times the labeled rate showed little or no injury symptomology compared to non-treated control plants (Figure 5).

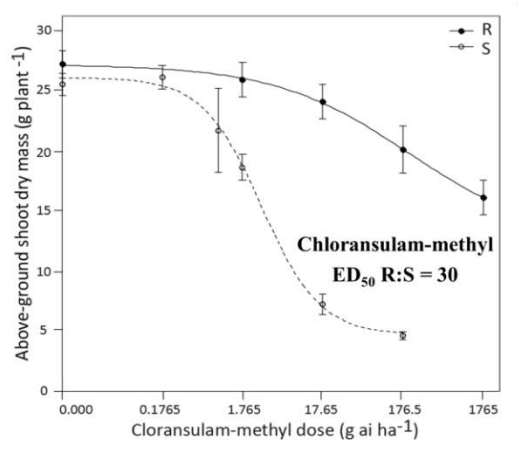

Figure 4. Shoot dry mass for resistant (R) and sensitive giant ragweed from Columbia County 28 days after treatment with cloransulam-methyl (FirstRate). Vertical bars represent standard error of the mean. Data were pooled from four experiments for analysis.

Figure 5. Columbia County giant ragweed plants 28 days after treatment with cloransulam-methyl under greenhouse conditions. The labeled rate of cloransulam-methyl (17.6 g ai ha^{-1} or 3.0 oz product $acre^{-1}$) is designated by 1X. Non-treated plants are designated by 0X.

Cloransulam-methyl Target Site Sensitivity

Cloransulam-methyl EC_{50} values [the effective concentration of herbicide that inhibited target enzyme (ALS) activity 50% relative to non-treated plants] were 10.6- to 13.6-fold greater for R than S plants across experiments (Figure 6). Differential ALS inhibition in response to cloransulam-methyl treatment suggests a less sensitive target site in R compared to S plants.

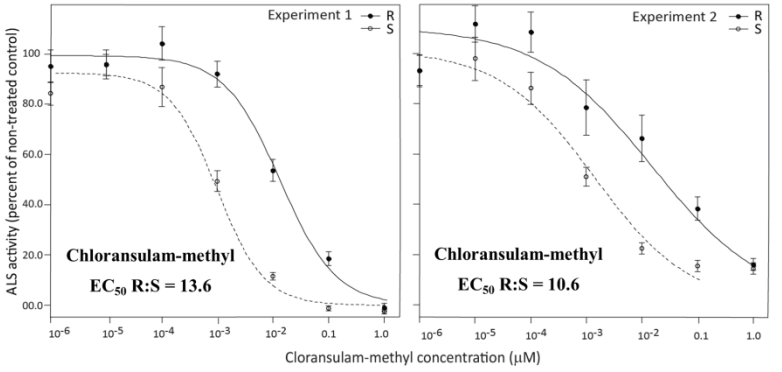

Figure 6. Acetolactate synthase (ALS) enzyme activity (expressed as a percent of control) in response to cloransulam-methyl concentration in R and S giant ragweed plants from Columbia County, Wisconsin.

Competitive Ability of Cloransulam-methyl Resistant Plants

Experiments conducted under competitive conditions in the greenhouse showed that average dry shoot biomass accumulation was less for cloransulam-methyl R (120 ± 5 g plant^{-1}) than S (168 ± 7 g plant^{-1}) plants. However, shoot height over time did not differ between R and S plants (data not shown), nor did total seed mass, total seed number, and seed viability (Table 2).

Table 2. Seed production of cloransulam-methyl-resistant and -sensitive giant ragweed from Columbia County under competitive conditions in the greenhouse. Data from repeated experiments were pooled for analysis.

			Seed fate category[†]		
Plant type	Seed yield		Intact-viable	Intact-nonviable	Empty involucre
	g/plant	seeds/plant	% of seeds produced		
Cloransulam-resistant	20 a[‡]	430 a	75 a	17 a	9 a
Cloransulam-sensitive	20 a	451 b	74 a	17 a	9 a

[†] Intact-viable and intact-nonviable: involucres contain fully formed seeds with viability of embryo determined by tetrazolium assay; empty involucre: no seed or not fully formed seed.

[‡] Means followed by the same letter within a column do not differ at the 5% level of significance as determined by a Welch's t-test.

Summary: Cloransulam-methyl Resistance in Columbia County Giant Ragweed

- Whole-plant experiments confirmed a high level of cloransulam-methyl resistance in giant ragweed from Columbia County Wisconsin (Figures 4 and 5).
- In vivo ALS enzyme bioassays suggested that the mechanism of cloransulam-methyl resistance in Columbia County giant ragweed is an altered ALS enzyme (Figure 6). Current research is conducting ALS gene amplification and sequencing to identify point mutations that may confer resistance.
- Despite a difference in shoot biomass produced between Columbian County R and S giant ragweed plants, the lack of difference in seed production and seed viability (Table 2) suggests that the frequency of the resistance trait is likely to persist over time in the field population.

Acknowledgments

This research was funded in part by the University of Wisconsin-Madison College of Agricultural and Life Sciences, and the Wisconsin Soybean Marketing Board.

References

Abul-Fatih HA, Bazzaz FA, Hunt R (1979) The biology of *Ambrosia Trifida* L. III. Growth and biomass allocation. New Phytol 83:829-838

Brabham CB, Gerber CK, Johnson WG (2011) Fate of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in the presence and absence of glyphosate. Weed Sci 59:506-511

Fickett ND, Boerboom CM, Stoltenberg DE (2013a) Predicted corn yield loss due to weed competition prior to postemergence herbicide application on Wisconsin farms. Weed Technol 27:54-62

Fickett ND, Boerboom CM, Stoltenberg DE (2013b) Soybean yield loss potential associated with early-season weed competition across 64 site-years. Weed Sci 61:500-507

Glettner CE (2013) Physiology, growth, and fecundity of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in Wisconsin. M.S. Thesis. Madison, WI: University of Wisconsin-Madison 100 p

Gramig GG, Stoltenberg DE (2007) Leaf appearance base temperature and phyllochron for common grass and broadleaf weed species. Weed Technol 21:249-254

Harrison SK, Regnier EE, Schmoll JT, Harrison JM (2007) Seed size and burial effects on giant ragweed (*Ambrosia trifida*) emergence and seed demise. Weed Sci 55:16-22

Harrison SK, Regnier EE, Schmoll JT, Webb JE (2001) Competition and fecundity of giant ragweed in corn. Weed Sci 49:224-229

Heap I (2014) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org

Marion SM, Davis VM, Stoltenberg DE. 2014. Characterization of Wisconsin giant ragweed resistance to cloransulam-methyl. Proc North Central Weed Sci Soc 69:30

Marion S, Glettner C, Trower T, Davis V, Stoltenberg D (2013) Acetolactate synthase (ALS) inhibitor resistance in Wisconsin giant ragweed. Wisc Crop Manager 20:52-53

Norsworthy JK, Jha P, Steckel LE, Scott RC (2010) Confirmation and control of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Tennessee. Weed Technol 24:64-70

Norsworthy JK, Riar D, Jha P, Scott RC (2011) Confirmation, control, and physiology of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Arkansas. Weed Technol 25:430-435

Sammons RD, Gaines TA (2014) Glyphosate resistance: State of knowledge. Pest Manag Sci 70:1367-1377

Schutte BJ, Regnier EE, Harrison SK (2012) Seed dormancy and adaptive seedling emergence timing in giant ragweed (*Ambrosia trifida*). Weed Sci 60:19-26

Shaner DL, ed (2014) Herbicide Handbook. Lawrence, KS: Weed Sci Soc America 513 p

Shaner DL, Lindenmeyer RB, Ostlie MH (2012) What have the mechanisms of resistance to glyphosate taught us? Pest Manag Sci 68:3-9

Stoltenberg D, Yerka M, Glettner C, Stute J, Trower T. 2012. Giant ragweed resistance to glyphosate in Wisconsin. Wisc Crop Manager. 19:43-44

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700-712

Tranel PJ, Wright TR, Heap IM (2014) Mutations in herbicide-resistant weeds to ALS inhibitors. http://www.weedscience.com

Vink JP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2012) Glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in Ontario: Dose response and control with postemergence herbicides. Am J Plant Sci 3:608-617

Webster TM, Loux MM, Regnier EE, Harrison SK (1994) Giant ragweed (*Ambrosia trifida*) canopy architecture and interference studies in soybean (*Glycine max*). Weed Technol 8:559-564

Westhoven AM, Davis VM, Gibson KD, Weller SC, Johnson WG (2008) Field presence of glyphosate-resistant horseweed (*Conyza canadensis*), common lambsquarters (*Chenopodium album*), and giant ragweed (*Ambrosia trifida*) biotypes with elevated tolerance to glyphosate. Weed Technol 22:544-548