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A Multistate Study of the Association Between Glyphosate Resistance and EPSPS Gene Amplification in Waterhemp (*Amaranthus tuberculatus*)

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Waterhemp is an increasingly problematic weed in the U.S. Midwest, having now evolved resistances to herbicides from six different site-of-action groups. Glyphosate-resistant waterhemp in the Midwest is especially concerning given the economic importance of glyphosate in corn and soybean production. Amplification of the target-site gene, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was found to be the mechanism of glyphosate resistance in Palmer amaranth, a species closely related to waterhemp. Here, the relationship between glyphosate resistance and EPSPS gene amplification in waterhemp was investigated. Glyphosate dose response studies were performed at field sites with glyphosate-resistant waterhemp in Illinois, Kansas, Kentucky, Missouri, and Nebraska, and relative EPSPS copy number of survivors was determined via quantitative real-time polymerase chain reaction (qPCR). Waterhemp control increased with increasing glyphosate rate at all locations, but no population was completely controlled even at the highest rate (3,360 g ae ha^{-1}). EPSPS gene amplification was present in plants from four of five locations (Illinois, Kansas, Missouri, and Nebraska) and the proportion of plants with elevated copy number was generally higher in survivors from glyphosate-treated plots than in plants from the untreated control plots. Copy number magnitude varied by site, but an overall trend of increasing copy number with increasing rate was observed in populations with gene amplification, suggesting that waterhemp plants with more EPSPS copies are more resistant. Survivors from the Kentucky population did not have elevated EPSPS copy number. Instead, resistance in this population was attributed to the EPSPS Pro106Ser mutation. Results herein show a quantitative relationship between glyphosate resistance and EPSPS gene amplification in some waterhemp populations, while highlighting that other mechanisms also confer glyphosate resistance in waterhemp.

Nomenclature: Glyphosate; common waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif; Palmer amaranth, *Amaranthus palmeri* S. Wats AMAPA; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Key words: Dose response, EPSPS gene amplification, glyphosate-resistant waterhemp, Pro106Ser mutation.

Glyphosate was first commercialized in 1974 and has since been considered one of the most ideal herbicides. It has broad-spectrum control, good translocation combined with a slow mode of action, and low mammalian toxicity and favorable environmental characteristics (Duke and Powles 2008). The addition of crop selectivity to this list endowed by glyphosate-resistant (GR) crop technology commercialized in 1996 has made glyphosate arguably the most economically important herbicide worldwide. However, the frequent and widespread use of glyphosate that followed has led to intense selection pressure on weeds to evolve resistance. To date, 30 weeds across 24 countries have evolved resistance to glyphosate (Heap 2014).

Few species have invaded agronomic fields and developed such a widespread distribution as quickly or efficiently as waterhemp. This success is attributed to waterhemp's dioecious strategy of sexual reproduction, C_4 photosynthetic pathway, prolonged emergence period, small seeds (which are adapted to conservation tillage), and propensity to evolve herbicide resistance (Tranel and Trucco 2009). In a production field setting, waterhemp competition

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can reduce soybean yields up to 43% (Hager et al. 2002), and season-long competition in corn can reduce yields as much as 74% (Steckel and Sprague 2004). Glyphosate was the fourth of six herbicides with distinct sites of action to which waterhemp has evolved resistance. The first reported glyphosate-resistant waterhemp population was identified in a Missouri field with a history of continuous GR soybean and multiple yearly glyphosate applications (Legleiter and Bradley 2008).

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimic acid pathway, preventing the biosynthesis of aromatic amino acids and all downstream products (Steinrücken and Amrhein 1980). Weeds have evolved a number of resistance mechanisms in an attempt to thwart glyphosate. Currently identified mechanisms of glyphosate resistance include reduced translocation (Lorraine-Colwill et al. 2003), which in most cases is likely due to vacuolar sequestration (Ge et al. 2010); target-site point mutations at Pro106 (Baerson et al. 2002; Wakelin and Preston 2006); and EPSPS gene amplification (Gaines et al. 2010; Salas et al. 2012; Wiersma et al. 2012).

EPSPS gene amplification was first discovered in a weed closely related to waterhemp, Palmer amaranth (Gaines et al. 2010). Multiple studies in Palmer amaranth have demonstrated the positive correlation between glyphosate resistance and EPSPS genomic copy number, EPSPS transcript and protein expression, and EPSPS enzyme activity (Gaines et al. 2010; Ribeiro et al. 2014). Additional EPSPS proteins resulting from multiple EPSPS gene copies allow the plant to survive in the presence of glyphosate because the herbicide becomes overwhelmed by a massive amount of target-site protein (Powles 2010). Although the mechanism of gene amplification remains unknown, MITEs (miniature inverted-repeat transposable elements), a putative *Ac* (*Activator*) transposase, and repetitive sequence region were found to be associated with amplified EPSPS copies in GR Palmer amaranth individuals. Introns were also confirmed to be present in amplified gene copies, providing further evidence for a DNA-mediated mechanism of gene amplification (Gaines et al. 2013). Preliminary reports suggest that EPSPS gene amplification might be associated with glyphosate resistance in waterhemp as well (Bell et al. 2009; Shaner et al. 2011; Tranel et al. 2010). However, the extent to which elevated EPSPS copy number contributes to glyphosate resistance remains unclear. In addition to EPSPS gene amplification as a potential mechanism of resistance, a Pro106Ser point mutation (Bell et al. 2013; Nandula et al. 2013), and reduced glyphosate translocation were also found to confer resistance in some waterhemp populations (Nandula et al. 2013).

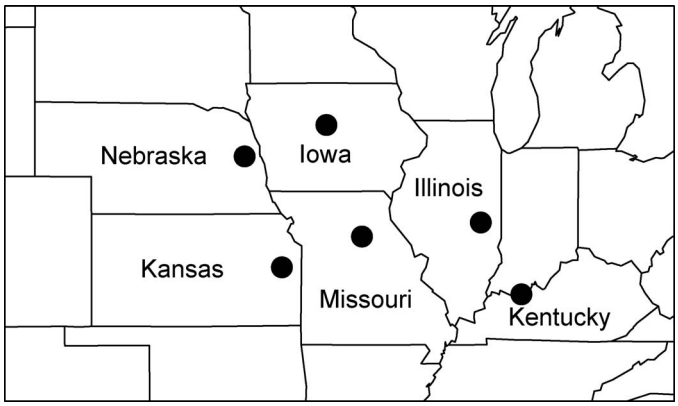

Figure 1. Map of the Midwest showing the locations of five glyphosate-resistant waterhemp populations investigated in this study plus an additional population (Iowa) included in a pilot study.

Although preliminary reports have linked glyphosate resistance and EPSPS gene amplification in waterhemp, the relationship between EPSPS copy number magnitude and glyphosate resistance under field conditions has not been examined. Is EPSPS gene amplification the primary GR mechanism in geographically diverse waterhemp populations? Do higher glyphosate rates select for plants with higher EPSPS gene copy numbers? With these questions in mind, the primary objective of this study was to determine EPSPS gene copy numbers in waterhemp plants surviving different rates of glyphosate at multiple field locations in the Midwest.

Materials and Methods

Field Studies. Field studies were conducted in Douglas County, Illinois, Franklin County, Kansas, Hancock County, Kentucky, Randolph County, Missouri, and Dodge County, Nebraska at sites with suspected or confirmed GR waterhemp (Figure 1). Field studies were established as randomized complete block designs with 0X, 0.5X, 1X, 2X, and 4X rates of formulated glyphosate (1X = 840 g ae ha^{-1}) with three or four replications. Rates were chosen based on the results of a pilot study performed on GR waterhemp populations in Illinois and Iowa the previous year (Chatham et al. 2012). Herbicide applications were made in mid-June to mid-July 2013 when the majority of waterhemp plants were 8 to 12 cm in height. Studies were carried out using standard small-plot research procedures using pressurized CO_2-back-

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pack sprayers; however, specific application equipment and sprayer calibration varied by location. Plot sizes ranged from 10 to 20 m^2, and were planted with either soybean or no crop. Two to four wk after treatment, counts and/or visual observations of glyphosate activity were taken and leaf samples were obtained from at least four survivors in each plot (replication) for each treatment at each location. Leaf samples were taken from newly emerging leaves approximately 1 to 2 cm in length, stored at 4 C, and shipped on ice to the University of Illinois.

Examination of Resistance Mechanisms. *Sample preparation.* The CTAB (hexadecyltrimethylammonium bromide) DNA isolation method described previously by Doyle and Doyle (1990) was used to extract DNA from leaf samples. The concentration and purity of each sample was examined using a spectrophotometer (NanoDrop 1000 Spectrophotometer, Thermo Fisher Scientific, 81 Wyman St., Waltham, MA 02451), and each sample was diluted to 10 ng μl^{-1} for all subsequent procedures.

EPSPS Gene Amplification. Samples were tested for elevated EPSPS copy number compared to a one-copy reference gene (*CPS*, which encodes the large subunit of carbamoylphosphate synthetase) using quantitative real-time PCR as described previously (Délye et al. 2015; Ma et al. 2013). A threshold EPSPS copy number value was set to the maximum relative copy number observed for any of the glyphosate-sensitive controls used in the study. Samples with a relative EPSPS copy number above this threshold (1.5) were considered to have elevated copy number. GR samples previously shown to have elevated EPSPS copy number were included as positive controls and were consistently above the threshold.

Alternative Target-Site Resistance. Select samples without gene amplification were screened for the Pro106Ser mutation (Bell et al. 2013; Nandula et al. 2013) using a derived cleaved amplified polymorphic sequences (dCAPS) assay designed according to methods described by Délye et al. (2015) and performed as described previously (Chatham et al. 2015.

EPSPS Sequencing. A portion of the EPSPS gene was sequenced from two plants each that tested homozygous positive, homozygous negative, or heterozygous for the Pro106Ser mutation to confirm the accuracy of the dCAPS assay. PCR was performed using primers EPSF1, originally designed for qPCR (Gaines et al. 2010), and eps106wt-R3, originally designed for use in the dCAPS assay mentioned above. After confirming the presence of the correct amplicon via agarose gel electrophoresis (1% agarose; 0.5 $\mu g\ ml^{-1}$ ethidium bromide), the PCR product was purified (E.Z.N.A. Cycle Pure Kit, Omega Bio-Tek, Inc., 400 Pinnacle Way, Suite 450, Norcross, GA 30071) and sequenced (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Inc., 850 Lincoln Centre Drive, Foster City, CA 94404) with the EPSF1 primer. Products were further analyzed by the W. M. Keck Center for Comparative and Functional Genomics (1201 W. Gregory Dr., Urbana, IL 61801) using an AB 3730xl DNA analyzer (Applied Biosystems, Inc.). Returned sequences were aligned to waterhemp EPSPS sequences from glyphosate-susceptible lines in GenBank (FJ869881 and FJ869880) with MEGA6 (Tamura et al. 2013).

Statistical Analysis. Statistical analysis of the correlation between glyphosate rate and relative EPSPS copy number was carried out in R (v3.0.3) (R Development Core Team 2014) using bootstrapping of the correlation coefficient (r) with 99,999 resamples and treating location as a random effect. The resampled distributions were compared to the real copy number data to determine how many times the correlation coefficient from the original data set was observed by chance alone in the resampled distributions.

Results and Discussion

Waterhemp Control. Dose response studies confirmed the presence of GR waterhemp at each field location. Less than 90% control was observed at 840 g glyphosate ha^{-1} and complete control was not seen even at 3,360 g ha^{-1}. In contrast, 840 g glyphosate ha^{-1} typically provides 90% to 100% control of glyphosate-susceptible waterhemp populations (Wait et al. 1999; Young et al. 2001). Because of the variability among locations in how the experiments were conducted, the dose-response data were not statistically analyzed. However, visual inspection of the data (Figure 2) reveals a general trend of increasing waterhemp control with increasing glyphosate rate at all locations. This pattern of control with glyphosate has been seen previously

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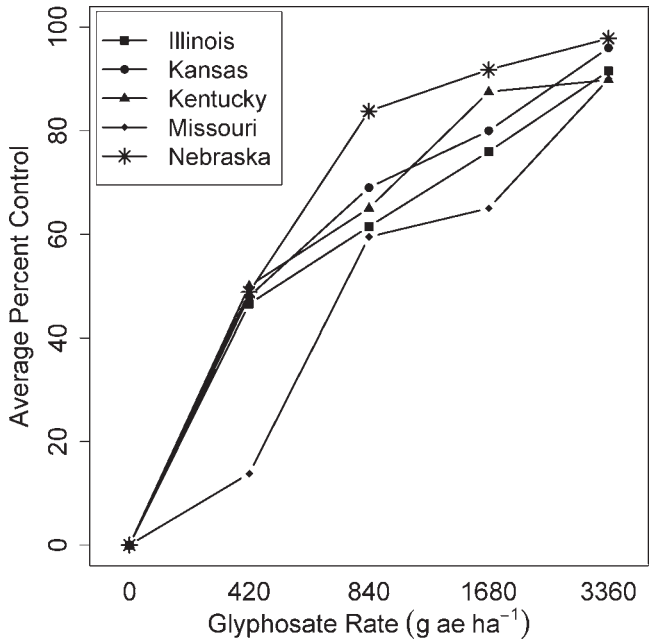

Figure 2. Results of the dose-response studies performed at each field location showing percent control with increasing glyphosate rate. Average percent control values are based on counts or visual observations of glyphosate activity and averaged for each replicate at each location.

with GR waterhemp (Legleiter and Bradley 2008; Patton et al. 2012).

EPSPS Gene Amplification. Copy number analysis revealed that EPSPS gene amplification was present at four (Illinois, Kansas, Missouri, Nebraska) of the five locations studied (Figure 3). When a pilot study was conducted in 2012, gene amplification was also observed at the same location in Illinois and at a location in Iowa (Chatham et al. 2012). Counting the Iowa location, EPSPS gene amplification was observed in five of six locations investigated. Because the Iowa location was not included in 2013 (due to weather events) it is not discussed further, and the Kentucky location, where EPSPS gene amplification was not observed, is discussed in a following section.

At least one plant that survived glyphosate treatment but did not have EPSPS gene amplification was found at each glyphosate rate at each location (Figure 3). If EPSPS gene amplification was solely responsible for resistance in these populations, one would expect to see elevated EPSPS copy number in all plants surviving glyphosate, particularly at the higher glyphosate rates. Although some survivors might have escaped exposure to glyphosate and not truly be resistant, their presence at all four locations—and even at the highest rate—is difficult to ignore. Alternative mechanisms of resistance are a more likely explanation for these anomalies.

To further examine whether EPSPS gene amplification was associated with resistance, a chi-square goodness-of-fit test was performed on the copy number data obtained from the four locations at which amplification was observed (Table 1). The proportion of plants with elevated copy number sampled from untreated control plots was used as the expected proportion and the proportion of plants with elevated copy number in all glyphosate treatments was used as the observed proportion. In Illinois, Kansas, and Nebraska waterhemp populations, the proportion of surviving plants with elevated EPSPS copy number in the glyphosate-treated plots was significantly different from that in the untreated control plots ($P < 0.001$), confirming that gene amplification is associated with glyphosate resistance in waterhemp. However, in the Missouri population, the proportion of plants with elevated copy number in the glyphosate treated plots was not statistically different from what was expected based on the untreated control ($P = 0.386$). Although not statistically significant, data from the Missouri population suggested a general trend of increasing copy number with rate (Figure 3). The lack of significance here might be due to the high background frequency of plants with elevated copy number in the population (75% based on plants from control plots).

An examination of the raw EPSPS copy number data revealed that copy number magnitude might vary by location (Figure 3), and the combined data for all treatments at each location (Figure 4a) showed an obvious difference in the copy number distributions for each population. The populations from Illinois, Kansas, and Missouri clearly had individuals with EPSPS gene amplification; the average copy number of plants with gene amplification at these locations was 4.1, 4.0, and 3.9, respectively. These averages are similar to those found in the original GR population (MO1) (Legleiter and Bradley 2008) in the first accounts of EPSPS gene amplification in waterhemp (Bell et al. 2009). However, the EPSPS copy number distribution from the Nebraska population was distinct from those of the Illinois, Kansas, and Missouri populations (Figure 4a). The magnitude of EPSPS copy number in Nebraska was lower, with an average copy number of 2.1 among plants with elevated EPSPS copy number.

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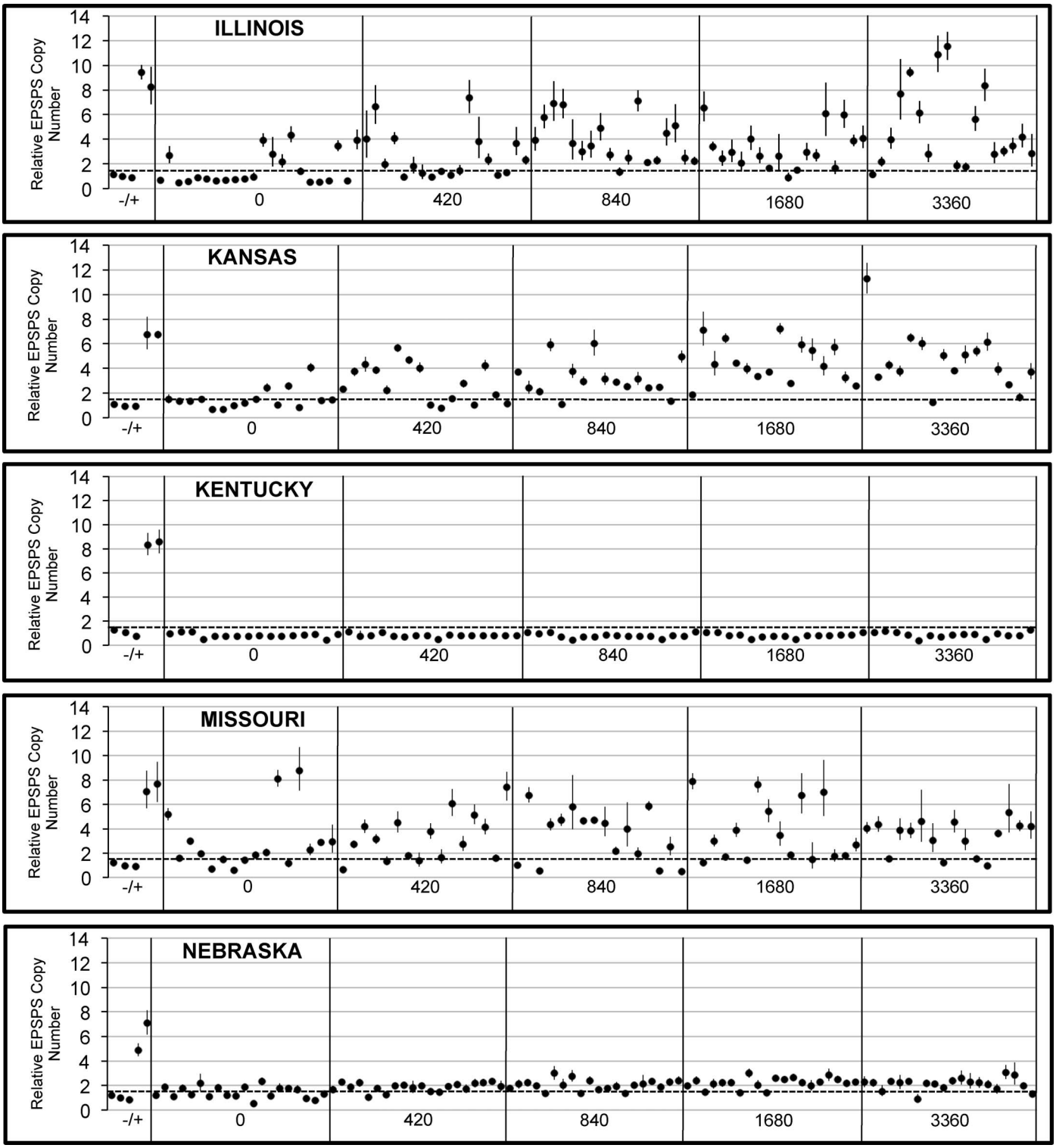

Figure 3. Raw gene copy number data showing relative 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) copy number of each surviving plant at each glyphosate rate (g ae ha^{-1}) in glyphosate-resistant waterhemp populations from Illinois, Kansas, Kentucky, Missouri, and Nebraska. Relative EPSPS copy number data of three sensitive (−) and two resistant (+) controls are shown to the left of each graph. Solid vertical lines bound the copy number data from each glyphosate rate. Dashed lines represent the threshold EPSPS copy number value (1.5); samples with relative copy number above the threshold were considered to have EPSPS gene amplification.

Differences in background frequencies of plants with high EPSPS copy numbers (e.g., the Missouri population relative to other populations) and in the copy number magnitudes (e.g., the Nebraska population relative to the other populations) could be explained by temporal differences in the GR evolutionary process at each location. Anecdotal reports indicate that the Missouri population was

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Table 1. Percentage of waterhemp plants with elevated 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) copy number in the untreated control and glyphosate treatments for each population studied and the corresponding chi-square goodness of fit P values.

	Percentage of plants with elevated EPSPS copy number		
State	Untreated	Glyphosate	χ^2 P value
Illinois	32	85	< 0.001
Kansas	31	89	< 0.001
Missouri	75	80	0.386
Nebraska	45	86	< 0.001

suspected to be resistant in 2008, whereas the Nebraska population was not known to be resistant until 2012 (Illinois and Kansas locations were identified as GR in 2010). Because resistance evolution is a gradual process, and because of varying lag times between observation and reporting of resistance, it is impossible to accurately chronologically compare resistance evolution at the different locations. Nevertheless, our EPSPS copy number data are consistent with a longer and shorter GR evolutionary history in the Missouri and Nebraska populations, respectively. For example, longer-term selection for glyphosate resistance in the Missouri population might have given this population more time to accumulate the gene amplification mechanism as well as any alternate mechanism that might confound the correlation between glyphosate resistance and gene copy number.

Among the four populations that have EPSPS gene amplification, a general trend of increasing gene copy number with increasing glyphosate rate was observed in the raw copy number data (Figure 3). This relationship is more clearly depicted when data are combined among populations with gene amplification (Illinois, Kansas, Missouri, and Nebraska; Figure 4b). However an examination of the interaction between median relative EPSPS copy number and glyphosate rate for these four locations shows that, although this relationship exists, it seems to vary slightly by population (Figure 4c).

Although a general trend of increasing gene copy number with increasing rate can be seen in the combined EPSPS copy number data (Figure 4b) and individually by location (Figures 3 and 4c.), statistical characterization of the trend was not straightforward; the data set did not meet the assumptions required to perform parametric statistics.

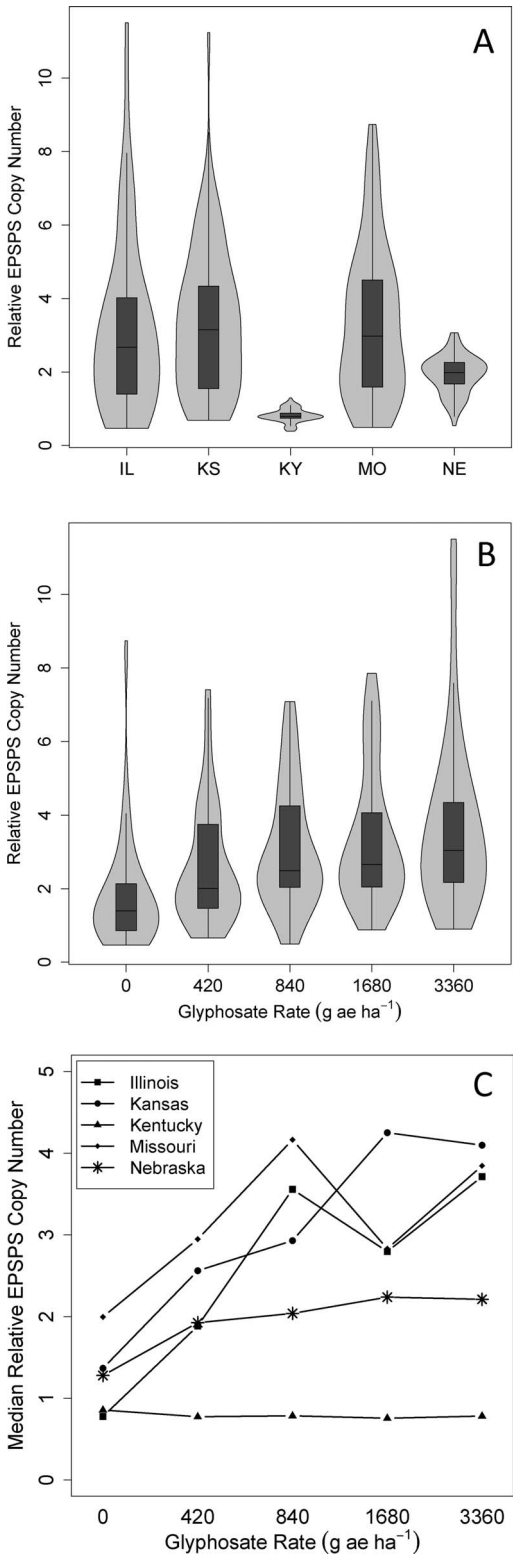

Figure 4. (A) Violin plots combining a standard box plot with a kernel density plot to represent the distributions of relative 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) copy number combined for all treatments of each waterhemp population; (B) Violin plots representing the distributions of the relative EPSPS copy number data at each glyphosate treatment level for the waterhemp populations in which gene amplification was present (Illinois, Kansas, Missouri, Nebraska); and (C) Median relative EPSPS copy number of each population at each glyphosate rate.

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Both the original data and the linear model residuals were not normally distributed (Shapiro-Wilk $P < 0.001$) and the variances were not equal among locations (Bartlett's test, $P < 0.001$) or treatments (Bartlett's test $P < 0.001$). These violations of the parametric ANOVA assumptions were also obvious from visual inspection of the data (Figures 4a and 4b). Combined with the unequal sample sizes, these results warranted a nonparametric statistical approach to analyze the data. A significant correlation was found between glyphosate rate and EPSPS copy number ($r = 0.3$, $P < 0.001$, 99999 resamples) in the combined data from the populations with EPSPS gene amplification. These results indicate that gene amplification is associated with glyphosate resistance in waterhemp and suggests that higher gene copy number survivors were present at higher rates. To further explore this hypothesis, bootstrapping was performed again on the combined data, without the data from control treatments in each state. The increase in gene copy number between the control and the first glyphosate treatment could have contributed largely to the observed r value. Without the 0X data, a smaller yet still significant correlation was observed ($r = 0.20$, $P < 0.001$, 99999 resamples), confirming that a quantitative relationship between EPSPS copy number and glyphosate rate exists in these populations. These results indicate that higher glyphosate rates select for individuals with higher average EPSPS copy number, suggesting that plants with more EPSPS copies have a higher level of resistance than those with fewer copies. Gaines et al. (2010) suggested that a similar pattern of increasing EPSPS copy number with increasing glyphosate rate exists in Palmer amaranth.

Kentucky Population. There is a clear connection between EPSPS gene amplification and glyphosate resistance in several of the waterhemp populations studied; however, there is also evidence for alternate resistance mechanisms. Because none of the survivors from the Kentucky population had elevated gene copy number, the samples were screened with a dCAPS assay designed to detect the Pro106Ser mutation, which previously was found to be associated with glyphosate resistance in waterhemp (Bell et al. 2013; Nandula et al. 2013). The Pro106Ser mutation was present in the population, and found in 69% percent of samples from the control plots. However, only 6% of plants from the control were homozygous for the mutation, with both alleles having the serine substitution. At the 2X and 4X rates, 100% of survivors had the mutation, and at the 4X rate, 67% were homozygous for the mutation (Figure 5). The strong relationship between glyphosate rate and the proportion of survivors with the mutation suggests that the Pro106Ser point mutation is responsible for glyphosate resistance in the Kentucky population. Glyphosate survivors from the Illinois, Kansas, Missouri, and Nebraska populations that did not have elevated EPSPS copy number were also examined, but the Pro106Ser mutation was not found in any of these samples. Several samples from the Kentucky population of each genotype were sequenced and results confirmed that the dCAPS assay was working correctly (data not shown).

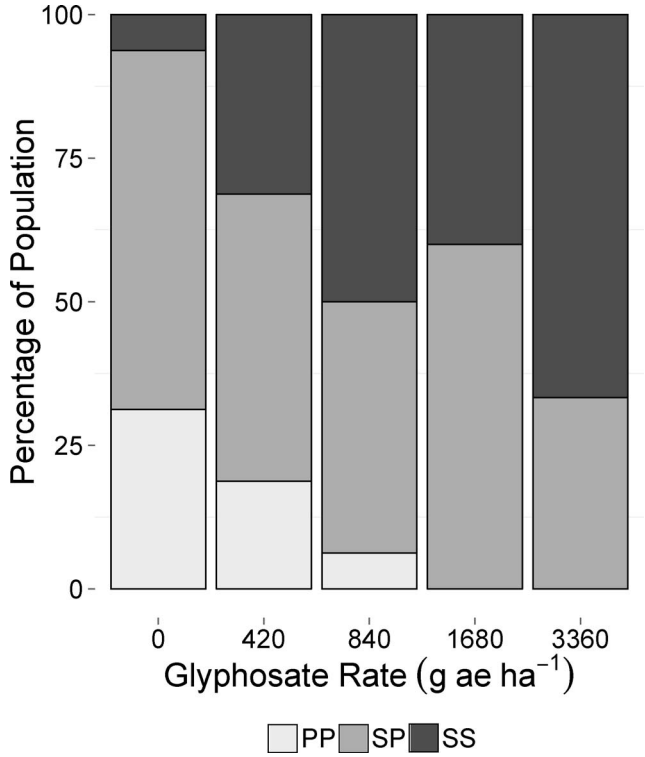

Figure 5. Percentage of survivors from the Kentucky waterhemp population with the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) Pro106Ser mutation at each glyphosate rate. PP individuals are homozygous with two proline alleles, SS individuals are homozygous with two serine alleles, and SP individuals are heterozygous.

Alternate Mechanisms. The only other mechanism of glyphosate resistance reported in waterhemp and not examined herein is altered translocation/uptake (Nandula et al. 2013). Examination of this mechanism would require whole plant specimens of the glyphosate survivors. It is possible that altered translocation might be present in some of the populations studied here. This might explain why

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some plants without gene amplification survived glyphosate treatment, especially at the higher rates. Multiple mechanisms of resistance in some of these populations might have confounded the results, making examination of the relationship between EPSPS gene amplification and glyphosate resistance more difficult. Therefore, the correlation between rate and gene copy number might be underestimated here relative to what would be expected in a more homogeneous population with gene amplification as the only existing mechanism of glyphosate resistance. The potential for multiple resistance mechanisms might also have contributed to the lack of a significant chi-square goodness of fit P value in the Missouri population.

In conclusion, results from this study confirm that an association between glyphosate resistance and EPSPS gene amplification exists in some waterhemp populations. Gene amplification was found in five of six populations studied (including the Iowa population in the 2012 pilot study). Given the locations of the populations studied, EPSPS gene amplification appears to be a common and widespread mechanism of resistance (Figure 1). Survey studies carried out in Illinois and Missouri both found gene amplification present in the majority of GR waterhemp populations (Chatham et al. 2015; Schultz et al. 2015). Among waterhemp populations with elevated EPSPS copy number, a positive correlation between copy number and level of resistance was observed. Gene copy number magnitude varied by location, but overall was significantly lower than copy numbers observed in Palmer amaranth. Not all the populations studied had gene amplification; the Pro106Ser mutation was found to be primarily responsible for resistance in the Kentucky population. Although EPSPS gene amplification appears to be the primary mechanism of resistance in waterhemp, it is clear that other mechanisms exist. Further investigation of these mechanisms and their interplay when combined is required to more fully understand glyphosate resistance in waterhemp.

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Literature Cited

Baerson SR, Rodriguez DJ, Tran M, Feng Y, Viest NA, Dill GM (2002) Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiol 129:1265–1275

Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). Weed Sci 61:460–468

Bell MS, Tranel PJ, Riggins CW (2009) Glyphosate resistance in waterhemp: inheritance and EPSPS copy number. North Central Weed Science Society Abstract 64. [CD-ROM Computer File]. Las Cruces, NM: North Central Weed Science Society

Chatham LA, Riggins C, Owen MD, Tranel P (2012) Association of EPSPS gene amplification with glyphosate resistance in waterhemp. Page 41 *in* Proceedings of the North Central Weed Science Society. Las Cruces, NM: North Central Weed Science Society

Chatham LA, Wu C, Riggins CW, Hager AG, Young BG, Roskamp GK, Tranel PJ (2015) EPSPS gene amplification is present in the majority of glyphosate-resistant Illinois waterhemp (*Amaranthus tuberculatus*) populations. Weed Technol 29:48–55

Délye C, Duhoux A, Pernin F, Riggins CW, Tranel PJ (2015) Molecular mechanisms of herbicide resistance. Weed Sci 63: 91–115

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Gaines TA, Wright AA, Molin WT, Lorentz L, Riggins CW, Tranel PJ, Beffa R, Westra P, Powles SB (2013) Identification of genetic elements associated with EPSPS gene amplification. PloS one 8:e65819

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci U S A 107:1029–1034

Ge X, d'Avignon DA, Ackerman JJH, Sammons RD (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag Sci 66:345–348

Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci 50:607–610

Heap IM (2014) International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed October 3, 2014

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587

Lorraine-Colwill DF, Powles SB, Hawkes TR, Hollinshead PH, Warner SAJ, Preston C (2003) Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. Pestic Biochem Physiol 74:62–72

Ma R, Kaundun SS, Tranel PJ, Riggins CW, McGinness DL, Hager AG, Hawkes T, McIndoes E, Riechers DE (2013) Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. Plant Physiol 163:363–377

Nandula VK, Ray JD, Ribeiro DN, Pand Z, Reddy KN (2013) Glyphosate resistance in tall waterhemp (*Amaranthus*

https://doi.org/10.1614/WS-D-14-00149.1

tuberculatus) from Mississippi is due to both altered target-site and nontarget-site mechanisms. Weed Sci 61:374–383
Patton BP, Witt W, Martin JR (2012) Multiple resistance issues within Kentucky waterhemp populations. *in* Proceedings of the Weed Science Society of America. Lawrence, KS: Weed Science Society of America. http://wssaabstracts.com/public/9/abstract-176.html. Accessed June 25, 2014 [Abstract 176]
Powles SB (2010) Gene amplification delivers glyphosate-resistant weed evolution. Proc Natl Acad Sci U S A 107:955–956
R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. http://www.R-project.org. Accessed March 26, 2014
Ribeiro DN, Pan Z, Duke SO, Nandula VK, Baldwin BS, Shaw DR, Dayan FE (2014) Involvement of facultative apomixes in inheritance of *EPSPS* gene amplification in glyphosate-resistant *Amaranthus palmeri*. Planta 239:199–212
Salas RA, Dayan FE, Pan Z, Watson SB, Dickson JW, Scott RC, Burgos NR (2012) EPSPS gene amplification in glyphosate-resistant Italian Ryegrass (*Lolium perenne* ssp. multiflorum) from Arkansas, USA. Pest Manag Sci 68:1223–1230
Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336–345
Shaner DL, Lindenmeyer RB, Ostlie MH (2011) What have the mechanisms of resistance to glyphosate taught us? Pest Manag Sci 68:3–9
Steckel LE, Sprague CL (2004) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci 52:359–364
Steinrücken HC, Amrbein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94:1207–1212
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis. Version 6.0. Mol Biol Evol 30:2725–2729
Tranel PJ, Riggins CW, Bell MS, Hager AG (2010) Herbicide resistances in *Amaranthus tuberculatus*: a call for new options. J Agric Food Chem 59:5808–5812
Tranel PJ, Trucco F (2009) 21st-century weed science: a call for *Amaranthus* genomics. Pp 53–81 *in* Stewart CN Jr, ed. Weedy and Invasive Plant Genomics. Ames, IA: Blackwell
Wait JD, Johnson WG, Massey RE (1999) Weed management with reduced rates of glyphosate in no-till, narrow-row, glyphosate-resistant soybean (*Glycine max*). Weed Technol 13:478–483
Wakelin AM, Preston C (2006) A target-site mutation is present in a glyphosate-resistant *Lolium rigidum* population. Weed Res 46:432–440
Wiersma AT, Chisholm ST, Godar AS, Stahlman PW, Leach J, Westra P (2012) Gene amplification of EPSP synthase in glyphosate resistant *Kochia scoparia*. Page 97 *in* Proceedings of the Western Society of Weed Science. Las Cruces, NM: Western Society of Weed Science
Young BG, Young JM, Gonzini LC, Hart SE, Wax LE, Kapusta G (2001) Weed management in narrow- and wide-row glyphosate-resistant soybean (*Glycine max*). Weed Technol 15:112–121

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