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Interactions between Glyphosate, *Fusarium* Infection of Common Waterhemp (*Amaranthus rudis*), and Soil Microbial Abundance and Diversity in Soil Collections from Missouri

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Greenhouse and laboratory experiments were conducted on common waterhemp and soil collected from 131 soybean fields in Missouri that contained late-season common waterhemp escapes. The objectives of these experiments were to determine the effects of soil sterilization on glyphosate-resistant (GR) and -susceptible (GS) common waterhemp survival, to determine the effects of soil sterilization and glyphosate treatment on infection of GR and GS common waterhemp biotypes by *Fusarium* spp., and to determine the soil microbial abundance and diversity in soils collected from soybean fields with differences in common waterhemp biotypes and herbicide and crop rotation histories. Common waterhemp biotypes were treated with 1.7 kg glyphosate ae ha^{-1} or left untreated once plants reached approximately 15 cm in height. Common waterhemp survival was visually assessed at 21 d after glyphosate treatment (21 DAT). To determine *Fusarium* infection frequency, a single intact common waterhemp root was harvested from each treatment at 0, 3, 7, 14, and 21 DAT and surface sterilized, and 10 to 15–mm common waterhemp root sections were plated on Komada culture medium. After 14 d incubation, fungal colonies were selected from colonized roots and maintained on potato dextrose agar medium amended with antibiotics before identification. Speciation of *Fusarium* isolates was conducted through microscopic examination of fungal characters and confirmed by sequencing and analysis of ribosomal DNA. Soil samples from 131 different collections were subjected to phospholipid fatty acid (PLFA) analysis and were conducted utilizing gas chromatography to determine the soil microbial community abundance and structure. Common waterhemp plants grown in sterile soils had the highest common waterhemp survival, regardless of biotype. After treatment with glyphosate, survival of GS common waterhemp grown in nonsterile soil was only 29% 21 DAT, whereas survival of GS common waterhemp grown in nonsterile soil was only 10%. Similarly, GR common waterhemp survival was reduced from 83 to 61% following treatment with glyphosate when grown in nonsterile compared to sterile soil. *Fusarium* spp. were recovered from only 12% of the assayed roots (223 treatments with *Fusarium* out of a total 1,920 treatments). The greatest occurrence of *Fusarium* root infection in both GR and GS common waterhemp occurred in nonsterile soils following a glyphosate treatment. Few differences in total PLFA were observed in field soil collected from locations with either GR or GS common waterhemp, and regardless of herbicide or crop history. This research supports previous findings that plant species are more sensitive to glyphosate in nonsterile than sterile soils and indicates glyphosate may predispose plants to soil-borne phytopathogens. This research also suggests that continuous use of glyphosate does not significantly affect soil microbial abundance or diversity.

Nomenclature: Glyphosate; common waterhemp, *Amaranthus rudis* Sauer; *Fusarium solani*; *Fusarium oxysporum*; soybean, *Glycine max* (L.) Merr.

Key words: Soil sterilization, soil microorganisms, soil phytopathogens, phospholipid fatty-acid analysis (PLFA), glyphosate resistance.

Glyphosate is a nonselective, foliar herbicide that is the world's best-selling herbicide; used in over 130 countries and in more than 150 crops (Baylis 2000; Woodburn 2000). In the soil, glyphosate is subject to several processes, including sorption and microbial degradation. Sorption to soil colloids and organic matter immobilizes a large portion of introduced glyphosate and is controlled by pH, soil mineralogy, and adsorbed phosphate (Borggaard and Gimsing 2008; Simonsen et al. 2008). Any remaining nonsorbed glyphosate may be degraded by the soil microbial community, which leads to increased microbial biomass and activity (Haney et al. 2000; Lancaster et al. 2010). In plants, glyphosate is

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leaf-absorbed and translocated to the roots, where it accumulates and may eventually be released into the rhizosphere (Coupland and Casely 1979). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, a key enzyme in the shikimate pathway. Inhibition of this enzyme prevents the plant from synthesizing the aromatic amino acids phenylalanine, tyrosine, and tryptophan. These amino acids are used for the synthesis of plant-growth regulating compounds, cell walls, and proteins, including those involved in plant defense (Hammond-Kosack and Jones 2000).

The insertion of the transgene that encodes for glyphosate resistance in genetically modified crops has greatly expanded glyphosate usage. An extensive reliance on glyphosate for weed control in glyphosate-resistant (GR) crop production systems has resulted in a concurrent increase in the number of GR weeds. Worldwide, there are currently 397 herbicide-resistant biotypes within 217 separate weed species that are resistant to one or more herbicides; 24 of which are resistant to glyphosate (Heap 2013). Within the United States, 14 species across 30 states contain GR weed species with 12 states specifically documenting glyphosate resistance in common waterhemp (Heap 2013).

In Missouri, common waterhemp is the most common species encountered in corn (*Zea mays* L.) and soybean production systems (Waggoner and Bradley 2011). The incidence of glyphosate resistance in common waterhemp is prevalent; 69% of the common waterhemp populations remaining in soybean fields at harvest were resistant to glyphosate, and these populations occurred across 41 counties in Missouri (Rosenbaum et al. 2012). Similar results have been reported with GR common waterhemp populations in Iowa (Owen 2013) and Illinois (Riggins et al. 2012).

Several studies have investigated the effects of glyphosate applications on soil microbial and phytopathogen populations such as *Fusarium* in the soil rhizosphere (Baley et al. 2009; Johal and Huber 2009; Johal and Rahe 1984; Liphadzi et al. 2005; Means and Kremer 2007; Sanogo et al. 2000, 2001; Smiley et al. 1992; Tesfamariam et al. 2009). The effect of glyphosate on soil microbial populations was investigated in common bean (*Phaseolus vulgaris* L.) (Johal and Rahe 1984; Lévesque et al. 1992, 1993) and in weed species (Close and Kniss 2011; Kawate et al. 1997; Lévesque et al. 1987; Schafer et al. 2012). Ten times more glyphosate was needed to kill bean seedlings grown in soil free of microorganisms compared to seedlings grown in nonsterile soil (Lévesque et al. 1992). Death of glyphosate-treated bean plants in nonsterile soil was at least partially attributed to parasitization by the fungal root-rot phytopathogens *Fusarium* and *Pythium* in the growth medium (Johal and Rahe 1984). Kawate et al. (1997) determined that glyphosate-treated henbit (*Lamium amplexicale* L.) and downy brome (*Bromus tectorum* L.) served as a reservoir for *Fusarium* and *Pythium* to proliferate compared to nontreated plants, but the pathogens did not readily infect roots of either weed species and were therefore not virulent. Lévesque et al. (1987) found similar results with common chickweed [*Stellaria media* (L.) Vill.]. *Fusarium* infection increased in chickweed after application with glyphosate, but common bean and cucumber (*Cucumis sativus* L.) sown into the common chickweed residue were not detrimentally affected by either the glyphosate application or *Fusarium* infection (Lévesque et al. 1987). Schafer et al. (2012) reported increased glyphosate toxicity in nonsterile soils on glyphosate-susceptible (GS) giant ragweed (*Ambrosia trifida* L.) and GS common lambsquarters (*Chenopodium album* L.), but not on GR horseweed [*Conyza canadensis* (L.) Cronq.] or glyphosate-tolerant common lambsquarters. Close and Kniss (2011) found that glyphosate efficacy on Canada thistle [*Cirsium arvense* (L.) Scop.] rhizomes was not affected when tested under field or greenhouse conditions and regardless of sterile or nonsterile field or greenhouse soil. Variation in response to glyphosate by multiple plant taxa may be associated with differential uptake, translocation, metabolism of the herbicide, differences in regulatory controls with which glyphosate interferes, and variances in the sensitivity of different plants to fungal root-rot pathogens, in particular, *Fusarium* and *Pythium* (Johal and Rahe 1984; Tesfamariam et al. 2009).

It is important to note that similar results have also been observed in response to herbicides other than glyphosate. An increase in pathogenicity or colonization has been observed in response to applications of acifluorfen, imidazolinone herbicides, and chlorimuron-ethyl (Sanogo et al. 2001; Zhang et al. 2011). When chlorimuron-ethyl was applied for 5 to 10 yr in a continuously cropped soybean field, the diversity and evenness of the soil microbial community decreased while the presence of *Fusarium* spp. intensified (Zhang et al. 2011). Sanogo et al. (2001) also indicated there was an increase in disease levels in response to applications of acifluorfen and imazethapyr. Conversely, Souza

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et al. (2013) observed no differences in microbial biomass with conventional and imidazolinone-group herbicide applications that could be attributed to the specific use of imazapyr.

The potential of glyphosate altering the soil environment after application has been evaluated in the literature resulting in variable responses. Zaboloy et al. (2012) evaluated the potential effects of glyphosate treatment on microbial community structure and function of soils and found little to no effect on the microbial community. On the contrary, Lane et al. (2012) reported that glyphosate application caused a significant decrease in the total microbial biomass in soybean rhizosphere soil that had no previous exposure to glyphosate; however, no significant changes were observed in the overall microbial community structure. A variety of other studies found no differences in CO_2 release from soil, soil enzymes, or PLFA from typical use rates of glyphosate on the soil microbial community (Accinelli et al. 2005, 2007; Araujo et al. 2003; Gomez et al. 2009; Weaver et al. 2007). Bacterial soil communities were altered in one study conducted by Ratcliff et al. (2006), but only where glyphosate was applied at 100 times the field use rate. Other studies have shown inconsistent results pertaining to the effects of glyphosate on the soil microbial community, indicating that additional research is necessary to determine the functional consequences of glyphosate on the microbial diversity of treated soils (Gimsing et al. 2004; Lupwayi et al. 2007; Powell et al. 2009).

Given the prevalence of GR common waterhemp across the midwestern United States, more information is needed on the effects of glyphosate application on soil microbial populations, and whether this effect is correlated to GR development in common waterhemp. The interactions between glyphosate, pathogen infection, and soil microorganisms have been studied in a number of crops and a select number of weed species, but not common waterhemp. The objectives of this research were to determine the interactions that occur between glyphosate application, *Fusarium* infection of common waterhemp, and soil microbial abundance and diversity in soil collections from Missouri. A greenhouse study was conducted to evaluate if soil microorganisms influence the efficacy of glyphosate on common waterhemp biotypes with the use of soil collected from fields with a previous history of GR or GS common waterhemp. Laboratory experiments were conducted to identify and determine the abundance of plant pathogens present on common waterhemp roots from the initial greenhouse experiment, and also to identify the total soil microbial biomass differences in 131 Missouri soil collections with differences in common waterhemp biotype, crop-rotation history, and herbicide-use history.

Materials and Methods

Soil Treatment Experiment. Soil was collected from 10 fields throughout Missouri; five sites with previously confirmed GR common waterhemp populations, and five sites with GS common waterhemp populations (Rosenbaum et al. 2012). The five GR sites were cropped continuously in soybeans and glyphosate was the only herbicide applied for a period of three or more years, whereas the five GS sites had some form of crop and/or herbicide rotation for a period of three or more years. Topsoil was randomly sampled from uniform surfaces within each landscape to a depth of 6 to 10 cm, yielding a final 13 to 23–kg sample. One-half of the soil from each of the locations was autoclaved at 120 C for 1 hr to kill all living microorganisms; the remaining half was left non-sterilized. 'Weston 2006' GR and 'Bradford 2006' GS common waterhemp biotypes (Legleiter and Bradley 2008) were planted (approximately 0.1 to 0.2 g of seed or 300 to 600 seeds) onto separate 19 by 28–cm greenhouse flats containing sterilized potting soil (General Purpose Potting Media by Premier Tech Horticulture, Hummert Supplies, Earth City, MO) that had been autoclaved at 120 C for 1 hr. Flats were maintained in a greenhouse at 25 to 30 C, watered and fertilized as needed, and provided with artificial lighting from metal halide lamps (600 μmol photon m^{-2} s^{-1}) to provide a 16-h-photoperiod day. Once seedlings (< 2-cm in height) emerged from the sterilized potting soil, 10 common waterhemp plants per biotype were transplanted into 19 by 28–cm greenhouse flats containing a 5-cm depth of either sterile or a nonsterile 3 : 1 mixture of field soil to sterile vermiculite. Approximately 7 d following the transplant, the common waterhemp plants reached 10 to 15 cm in height and flats were treated with glyphosate at 1.7 kg ae ha^{-1}, which represents twice the labeled use rate for common waterhemp control, or left untreated. Glyphosate applications were made with a compressed air, laboratory spray chamber calibrated to deliver 220 L ha^{-1} carrier volume at a pressure of 234 kPa with the use of a 8001EVS nozzle (Teejet Technologies, Wheaton, IL). Ammonium sulfate was added to all treatments at 2.9 kg ha^{-1}.

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At 21 d after treatment (DAT), the number of plants remaining in each greenhouse flat was counted to determine the percent common waterhemp survival in response to each herbicide treatment; plants with green tissue were counted as survivors and any new growth or obvious regrowth from the treatment was also considered survival in the visible evaluations. Soil and glyphosate treatments were arranged in a completely randomized design with four replications, and the experiment was conducted twice.

***Fusarium* Colonization Experiment.** From each flat in the soil treatment experiment, a single intact living common waterhemp plant was collected 0, 3, 7, 14, and 21 DAT. Loosely adhering soil on roots was removed by vigorous shaking. The aboveground portion of the common waterhemp plant was severed at the soil line and discarded, and roots were used in subsequent assays for detecting *Fusarium* colonization. Common waterhemp roots were surface sterilized in a 10% NaOCl solution (Clorox Regular Bleach, The Clorox Company, Oakland, CA) for 2 to 3 min, followed by two consecutive, 1-min rinse intervals with 50 to 100 ml sterile water. Roots were blotted dry with sterile paper towels and cut into 2-cm segments. Eight root segments from each plant were placed onto a single agar plate containing Komada selective growth medium (Komada 1975). The samples were inspected daily up to 28 d after incubation for mycelium appearance, which usually occurred within 7 to 14 d. After incubation, putative *Fusarium* colonies from root segments were counted (data not shown). Hyphal tips from putative *Fusarium* colonies were transferred aseptically to potato dextrose agar (PDA +++) amended with 50 μg ml^{-1} each of chloramphenicol (Sigma-Aldrich, St. Louis, MO), streptomycin sulfate (Sigma-Aldrich), and tetracycline (Sigma-Aldrich).

Initially, morphology for each species was conducted with the use of the micro- and macroconidia of each sample and was identified by microscopic evaluation of *Fusarium* cultures grown on carnation-leaf agar (Fisher et al. 1982; Nelson et al. 1983). To prepare *Fusarium* colonies for DNA extraction, hyphal tips were transferred onto a sterilized cellophane (Amersham Biosciences, Piscataway, NJ) square overlaid on PDA +++. Mycelia were scraped from the cellophane and placed into a sterile 1.5 ml microcentrifuge tube. The tube was centrifuged at max speed (17,000 rotations per minute) for 5 min and stored at −80 C until further use.

Fusarium colonies were identified through DNA extraction, amplification, purification and sequencing as described by Gardes and Bruns (1993). Genomic DNA from 408 unknown isolates was extracted with the use of the EasyDNA Kit (Invitrogen Corp., Carlsbad, CA). PCR amplification of ribosomal (r)DNA regions internal transcribed spacer (ITS) ITS1 5.8S rRNA, and ITS2 was performed on 348 DNA extracts using the universal primers ITS4 and ITS5. PCR reactions were 50 μl in volume and consisted of 35.75 μl of cold DNA H_2O, 10 μl PCR buffer (Bioline Inc., Taunton, MA), 2 μl DNA template, 1 μl of each primer, and 0.25 μl of *Taq* polymerase (Bioline Inc., Taunton, MA). Thermal cycling conditions involved an initial denaturation step at 94 C for 1 min followed by 32 cycles of 94 C for 30 s, 55 C for 1 min, 72 C for 1 min and a final extension step at 72 C for 2 min. Amplicon presence and size were confirmed with gel electrophoresis. Amplicons were purified with a Qiaquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sent to the University of Missouri DNA Sequencing Facility (Columbia, MO) for cleanup, electrophoresis, and fluorometric analysis. The consensus sequence for each isolate was manually aligned from one to two sequencing reactions with each primer. A basic local alignment search tool (BLAST) search of GenBank was performed with each consensus sequence (Zhang et al. 2000). For each sample, one or two of the most similar sequences based on maximal identity percentage were downloaded for comparison. All sequences were aligned with the use of the sequencing alignment tool in BLAST and adjusted by visual examination. In all cases, the molecular analysis confirmed the speciation made based on observed morphological characteristics.

Soil Microbial Abundance and Diversity Experiment. Soil samples were collected in the spring of 2011 from 131 field locations. The locations were selected based on the presence of GR or GS common waterhemp and known crop- and herbicide-use history (Rosenbaum et al. 2012). Fields from soil collections were located within 10 Missouri Major Land Resource Areas, including Iowa and Missouri Heavy Till Plain, Central Claypan Areas, Missouri and Iowa Deep Loess Hills, Central Mississippi Valley Wooded Slopes, and Cherokee Prairies. These locations represent major soil groups typically cultivated to corn and soybean in Missouri (U.S. Department of Agriculture–Natural Resources Conservation Service [NRCS]

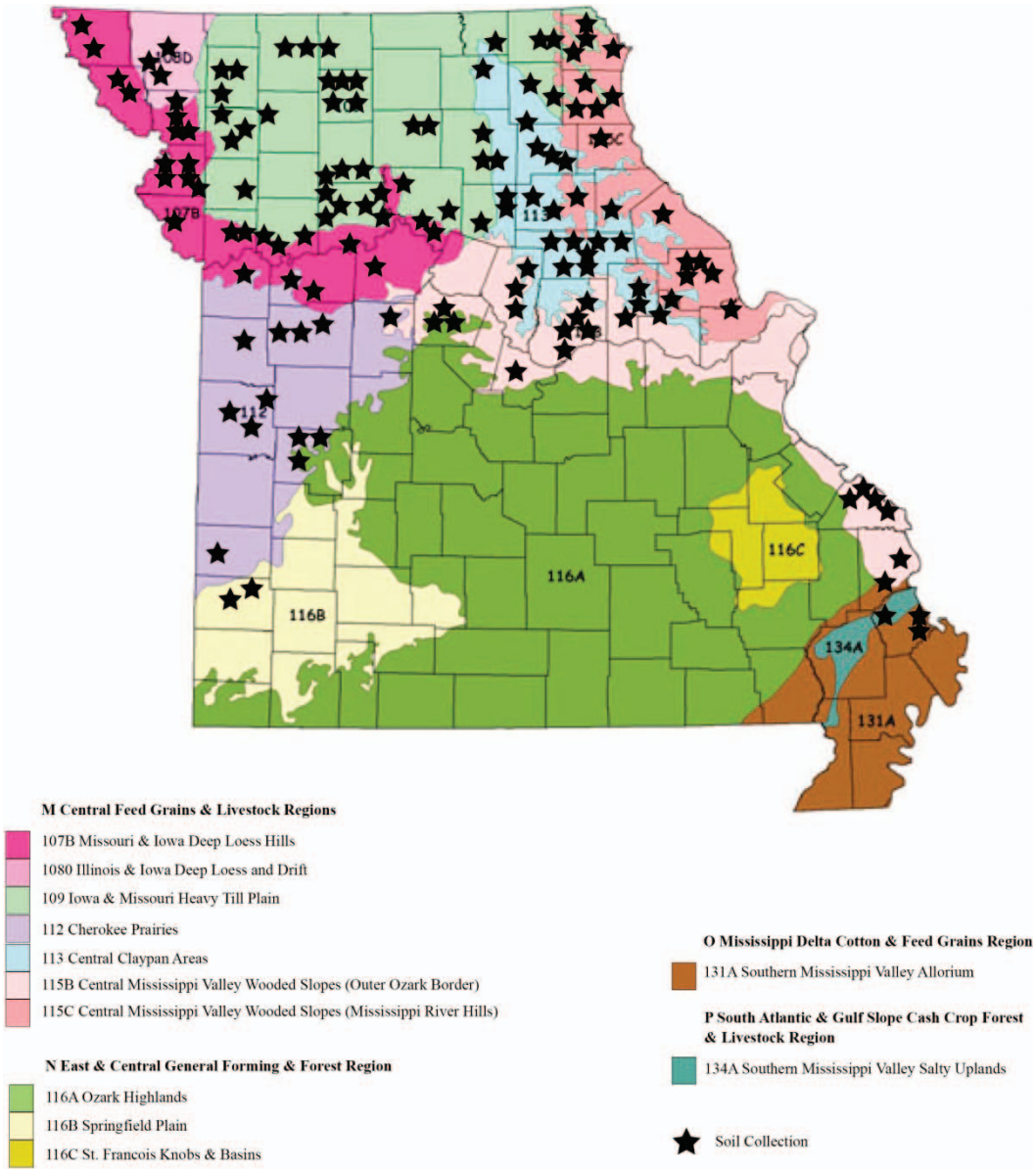

Figure 1. Predominant soil types and location of soils (U.S. Department of Agriculture, Natural Resources Conservation Service 2013) sampled in Missouri for use in the soil microbial abundance and diversity experiment.

2006). Details of the various soils are presented in Figure 1. Soils were sampled from the surface to 10-cm depth at each site. For analysis, each soil sample was air dried and ground to pass through a 2-mm sieve. Carbon : nitrogen (C : N) ratios were calculated by dry combustion of total nitrogen and total organic carbon (Nelson and Sommers 1996) with a LECO analyzer (TruSpec CN Analyzer, St. Joseph, MI).

All soils were freeze-dried in a production grade freeze drier (Lyph-Lock 12 model, Labconco Corp., Kansas City, MO) at −20 C for 24 h. The soil microbial community was determined in each of the 131 field sites with the use of phospholipid fatty-acid (PLFA) analysis as described by Bligh and Dyer (1959) and Peterson and Klug (1994). Gas-chromatograph peak responses were translated with the use of internal standards. For example, peaks corresponding to fatty-acid carbon chain lengths of 12 to 20 are indicative of microorganisms, bacteria markers correspond to fatty-acid carbon lengths of 12 to 19 carbons, fungal markers correspond to fatty-acid carbon chain lengths of 16 and 18 carbons, and the ratio of total saturated to total monounsaturated fatty acids use the ratio of the sum of 14 to 20 carbons to the sum of 16 to 17 carbon chain lengths (Unger 2009). For further details regarding markers utilized in this study refer to Unger et al. (2009, 2013). The PLFA analysis determines the amount of living microbial biomass

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Table 1. Analysis of variance for survival of glyphosate-resistant and -susceptible common waterhemp biotypes with treatment differences including soil sterilization, herbicide treatment, soil collection, and common waterhemp biotype, 21 DAT.[a]

Variables	AMATA biotype	Glyphosate treatment	Soil treatment	Soil collection	Soil treatment/ glyphosate
	% survival P value[b]				
AMATA biotype	0.01	0.01	0.03	0.25	0.13
Glyphosate treatment	0.01	0.01	0.01	0.74	–
Soil treatment	0.03	0.01	0.01	0.17	–
Soil collection	0.25	0.74	0.17	0.77	0.32

[a] Abbreviations: AMATA = common waterhemp; DAT = days after treatment.
[b] Orthogonal contrast of R vs. S soil locations were not different ($P > 0.45$) and were combined with soil collection; P values ≥ 0.05 are not different.

and includes the percentage of total PLFA, bacteria, and fungi within the living microbial biomass.

Statistical Analysis. Across all three experiments, data were subjected to analysis of variance with the use of SAS PROC MIXED (SAS 9.3, SAS Institute Inc., Cary, NC) with each treatment combination considered an environment sampled at random. Fixed effects for the greenhouse study included soil collection, soil sterilization treatment, glyphosate treatment, and common waterhemp biotype; replication was utilized as the random effect. Data from the greenhouse study was combined across experiment timings (experiment was conducted in time twice) and was specifically evaluated for treatment differences at 21 DAT. The fixed effects for the laboratory experiment evaluating *Fusarium* colonization included soil sterilization, glyphosate treatment, and common waterhemp biotype with replication as the random effect. To evaluate treatment differences in the *Fusarium* colonization experiment, because there were no differences between experiments, including root collection timings ($P > 0.45$; data not shown), data were combined across experiment timings (experiment was conducted in time twice) and root collection timings (0, 3, 7, 14, and 21 DAT). The soil microbial abundance and diversity experiment effects included site characteristics of common waterhemp biotype, herbicide-use history, and crop-rotation history, and were evaluated for treatment differences within individual site characteristics. Where *F* values were significant ($P \leq 0.05$), means were separated with the use of Fisher's Protected LSD. Transformations of the data did not improve the model; therefore nontransformed means are reported.

Results and Discussion

Soil Treatment Experiment. No differences in common waterhemp survival 21 DAT were observed between any of the 10 soils evaluated, regardless of the cropping history and common waterhemp biotype at the soil collection site. These results are derived from an orthogonal contrast which provided a P value for all treatment variables combined into one contrast to determine treatment differences specifically between soil locations. Therefore all common waterhemp survival data were combined across soil locations ($P > 0.45$; data not shown). Additionally, there was no significant effect for the specific variable of soil collections on common waterhemp survival regardless of the individual or multivariable interactions evaluated 21 DAT ($P > 0.12$; Table 1). These findings suggest there are no differences between soils from locations containing GR or GS common waterhemp, and that soil microbial–glyphosate interactions are not influenced by origin of soil. With the exception of soil collection, treatment differences were observed for all remaining tested variables, including individual and multivariable interactions for common waterhemp biotype, glyphosate application, and soil sterilization (Table 1).

Treatment differences were evaluated by multivariable interactions within soil sterilization, glyphosate application, and common waterhemp biotype (Table 1). Mean common waterhemp survival 21 DAT for each biotype in response to soil sterilization and glyphosate treatment is presented in Table 2. Regardless of the treatment variable, greater than 95% common waterhemp survival occurred 21 DAT where no glyphosate was applied, indicating that soil microbial populations alone cannot solely explain common waterhemp death or survival (Table 2). If soil microbes were the primary cause of plant death, a significant reduction in common waterhemp survival would also have occurred in the nonglyphosate treatments. Therefore, these results suggest that the reduction in survival of common waterhemp is dependent upon

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a combination of both the activity of glyphosate targeting EPSP synthase and the presence of soil microorganisms. When combined across all other factors, 73% of GR common waterhemp survived a 2× rate of glyphosate compared to only 25% survival in GS common waterhemp (Table 2). Although complete death is expected in confirmed GS common waterhemp 21 DAT, a low level of the GS biotype survived glyphosate treatment. This can be partially attributed to the dioecious nature of common waterhemp and high level of variability within a given population of this species (Bradley et al. 2009; Chandi et al. 2013; Nordby et al. 2007). This inconsistency in the level of herbicide activity in common waterhemp has also been noted in previous research (Foes et al. 1998; Horak and Peterson 1995).

Greater common waterhemp survival was observed in sterile soil compared with nonsterile soil; sterilizing soils increased GR and GS common waterhemp survival by 5 and 10%, respectively ($P \leq 0.03$; Table 1). Overall, the absence of microorganisms in the soil increased the survival of common waterhemp following a glyphosate application by 18% when compared to nonsterile soil media in the same treatment. Survival of GS common waterhemp grown in nonsterile soil and treated with glyphosate was only 13% by 21 DAT, whereas survival of GS common waterhemp grown in sterile soil was 38%. Similarly, GR common waterhemp survival was reduced from 78 to 67% when grown in nonsterile compared to sterile soil.

A similar response to glyphosate treatment and soil sterilization has been reported with giant ragweed, common lambsquarters, and common bean, suggesting that soil microorganisms play a role in the efficacy of glyphosate (Johal and Rahe 1984; Lévesque and Rahe 1992; Lévesque et al. 1992, 1993; Schafer et al. 2012). Schafer et al. (2012) reported an 88% increase in glyphosate efficacy on giant ragweed when plants were grown in nonsterile soil. *Pythium* (soil-borne pathogen) was isolated from these plant roots, yet no change in glyphosate efficacy in glyphosate-tolerant common lambsquarters and GR or GS horseweed biotypes was observed.

Overall, the plant–microbe interactions within the rhizosphere are extremely, complex as microbial communities are dissimilar across plant communities (Garbeva et al. 2004). Our findings suggest that plants treated with glyphosate may be more susceptible to colonization by *Fusarium* or, alternatively, that plants colonized by *Fusarium* are more

Table 2. Influence of soil sterilization, herbicide treatment, and soil collection on mean common waterhemp survival of glyphosate-resistant and -susceptible biotypes.[a]

Variables	R AMATA	S AMATA	Glyphosate	No glyphosate	Sterile	Nonsterile	R soil collection	S soil collection	Nonsterile/ glyphosate	Nonsterile/ no-glyphosate	Sterile/ glyphosate	Sterile/no glyphosate
	% survival 21 DAT[b]											
R AMATA	–	–	73	97	87	82	83	86	67	98	78	96
S AMATA	–	–	25	99	66	56	63	62	13	99	38	99
Glyphosate	73	25	–	–	58	40	48	49	–	–	–	–
No glyphosate	97	99	–	–	98	98	98	98	–	–	–	–
Sterile	87	66	58	98	–	–	79	77	–	–	–	–
Nonsterile	82	56	40	98	–	–	67	71	–	–	–	–
R soil collection	83	63	48	98	79	67	–	–	37	98	59	98
S soil collection	86	62	49	98	77	71	–	–	43	99	57	98

[a] Abbreviations: AMATA = common waterhemp, DAT = days after treatment, LSD = least significant difference, R = resistant, S = susceptible.
[b] Orthogonal contrasts of GR vs. GS soil locations were not different ($P > 0.45$). LSD (0.05) for all two-way interactions = 5; LSD (0.05) for all three-way interactions = 7.

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Table 3. Influence of soil and glyphosate treatment on the percentage of glyphosate-resistant and -susceptible common waterhemp roots infected with *Fusarium* species.

Soil treatment	Glyphosate treatment	Common waterhemp biotype	
		GR	GS
		Infection (%)[a]	
Nonsterile	Glyphosate	23 a	25 a
	Nontreated	12 b	16 b
Sterile	Glyphosate	2 c	5 c
	Nontreated	3 c	6 c

[a] Means followed by the same letter are not different within individual columns, LSD (0.05).

susceptible to glyphosate. One would have to determine this with the use of multiple sampling times to determine when *Fusarium* colonization occurs in weeds in nonsterile soils and if colonization increases soon after glyphosate is exuded from plant roots following application. Other researchers have observed that variation in glyphosate efficacy occurs in some weedy species and that the presence/absence of root pathogens could be exploited to increase efficacy (Johal and Rahe 1984; Kawate et al. 1987; Lévesque and Rahe 1992; Lévesque et al. 1987; Schafer et al. 2012).

***Fusarium* Colonization Experiment.** *Fusarium* root infection of common waterhemp plants was evaluated in a total of 1,920 treatments. Across all 1,920 treatments, *Fusarium* infection was low and *Fusarium* species were recovered from only 12% of the assayed roots (223 treatments). The low incidence of *Fusarium* root infection observed in this study may be attributed to the lower susceptibility of common waterhemp to *Fusarium*. Kawate et al. (1997) also observed low *Fusarium* colonization on the roots of downy brome and henbit after glyphosate treatment. Because of this low level of root infection, the results were combined across *Fusarium* species (Table 3). Within the infected root samples, the most predominant species identified through molecular analysis included *Fusarium solani* (the group that includes the causal agent of sudden death syndrome in soybean; Li et al. 2008), which comprised 54.9% of the infected samples, *Fusarium oxysporum* (likely the causal agent of vascular wilt; Kistler et al. 1991), which comprised 25.8% of the infected samples, and other miscellaneous species including *Fusarium acuminatum*, *Fusarium avenaceum*, *Fusarium chlamydosporum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium proliferatum*, *Fusarium sporotrichioides*, and *Fusarium tricinctum*, which comprised 19.3% of the infected samples (Table 4).

Regardless of common waterhemp biotype, 23% of roots were infected with *Fusarium* when grown in nonsterile soil treated with glyphosate (Table 3). In a nonsterile soil, *Fusarium* root infection was reduced by 11 and 9% for GR and GS common waterhemp, respectively, in nontreated compared to glyphosate-treated plants. *Fusarium* infection of common waterhemp roots was lower in sterilized ($< 7\%$) compared to nonsterilized (12 to 25%) soil for all treatments. *Fusarium* colonization 21 d after glyphosate application was greater in nonsterile soils than in sterile soils, and applying glyphosate increased colonization compared to no glyphosate. These results are similar to responses observed in the literature, suggesting that a greater level of plant infection occurs in the presence of glyphosate and soil microorganisms (Johal and Rahe 1984; Kawate et al. 1997; Lévesque and Rahe 1992; Lévesque et al. 1987, 1992; Schafer et al. 2012). Previous reports suggest that inert ingredients in glyphosate-formulated products (i.e., Roundup herbicide) slightly inhibit soil microbial activity; however, it is concluded that these effects are largely overcome by the microbial community in the presence of glyphosate (Haney et al. 2002). Furthermore, infection by soil-borne pathogens such as *Fusarium* or *Pythium* suppressed the ability of glyphosate-treated plants to synthesize plant defense compounds and may have contributed to the overall herbicidal efficacy of glyphosate (Johal and Huber 2009; Schafer et al. 2012). For example, phenylalanine is a precursor for certain phytoalexins, which are important plant defense compounds; therefore the induction of glyphosate activity toward plant roots could be influenced by soil microorganisms (Johal and Huber 2009; Liu et al. 1995).

The results from this study also suggest that allowing GR common waterhemp to spread and proliferate could increase *Fusarium* populations in future corn and soybean production systems. However, these data suggest that *Fusarium* spp. in decaying plant roots are not obligate phytopathogens, but more likely saprophytic and opportunistic pathogens. Overall, variation in phytopathogen infection will most likely be determined by the interaction of a number of characteristics within an individual crop field such as soil properties, plant properties, environmental conditions, competing organisms, different inoculum levels, initial microbial community, and composition of living plant root exudates (Garbeva et al. 2004; Lévesque et al. 1987).

Table 4. Influence of soil and glyphosate treatment on the *Fusarium* isolates recovered from common waterhemp roots.

Soil treatment	Glyphosate treatment	*Fusarium* species	Isolates recovered		
			No. of isolates	Within treatment	All isolates[a]
				——————% of total isolates ——————	
Nonsterile	Glyphosate	*Fusarium acuminatum*	2	1.6	0.9
		Fusarium avenaceum	6	4.8	2.6
		Fusarium equiseti	3	2.4	1.3
		Fusarium oxysporum	30	24.2	12.9
		Fusarium solani	78	62.9	33.5
		Fusarium tricinctum	5	4.0	2.1
Nonsterile	Nontreated	*F. acuminatum*	2	2.9	0.9
		F. avenaceum	2	2.9	0.9
		Fusarium chlamydosporum	1	1.4	0.4
		F. equiseti	2	2.9	0.9
		F. oxysporum	23	32.9	9.9
		F. solani	34	48.6	14.6
		F. tricinctum	6	8.6	2.6
Sterile	Glyphosate	*F. equiseti*	6	35.3	2.6
		F. oxysporum	3	17.6	1.3
		Fusarium poliferatum	1	5.9	0.4
		F. solani	7	41.2	3.0
Sterile	Nontreated	*F. acuminatum*	1	4.5	0.4
		Fusarium culmorum	1	4.5	0.4
		F. equiseti	3	13.6	1.3
		F. oxysporum	4	18.2	1.7
		F. poliferatum	1	4.5	0.4
		F. solani	9	40.9	3.9
		Fusarium sporotrichioides	2	9.1	0.9
		F. tricinctum	1	4.5	0.4

[a] Data include *Fusarium* incidence recovered from 12% of the assayed roots (233 treatments). Isolates were not tested for virulence.

Soil Microbial Abundance and Diversity Experiment. No differences were observed for soil microbial properties including total PLFA, bacteria, protozoa, and fungi content, or in the bacteria : fungi ratio among sites characterized by common waterhemp biotype, glyphosate-use history, and crop-rotation history for all 131 soils evaluated (Table 5). In addition, no differences were observed in the saturated to monounsaturated fatty acid ratio in response to the glyphosate-use history or crop-rotation history between any of the 131 soils evaluated (Table 5). Similarly, few differences in soil microbial community abundance and structure in response to glyphosate applications have been documented within the literature. In one Missouri study, no shifts were observed in microbial community structure in response to increased antibiotics applied to the soil system (Unger et al. 2013). Therefore this study suggests that soil microbial communities are robust and the use of synthetic organic compounds (xenobiotics) in agriculture, including herbicides or antibiotics, may not readily diminish important primary soil functions or properties. A number of others have revealed no shifts in the soil microbial community abundance and structure occurred after the addition of glyphosate (Accinelli et al. 2005; Gomez et al. 2009; Weaver et al. 2007). Accinelli et al. (2005) observed that incorporated corn residues did not affect or stimulate glyphosate mineralization in sandy or sandy loam soils. Gomez et al. (2009) observed increased dehydrogenase activity and decreased microbial biomass following the application of glyphosate to the soil. However, after incubation, the initial inhibitory effect of the herbicide observed in the Gomez et al. (2009) study had been diluted, and it was determined that no harmful effects should be expected in the short term when glyphosate is applied at doses equivalent to or higher than those usually applied in the field. Additionally, no significant differences between the soil and soybean rhizosphere microbial communities because of treatment with glyphosate were observed in a Mississippi silt loam soil (Weaver et al. 2007).

No differences were observed in soil organic matter (SOM), C : N ratio, or nitrogen content among any of the 131 soils evaluated (Table 5). These results correspond with previous research suggesting rhizosphere microbial activity, optimum plant growth, soil microbial ecology, and soil

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Table 5. Analysis of microbial PLFA and carbon and nitrogen content of soil collected from 131 Missouri soybean fields previously characterized with GR or GS common waterhemp and varying site characteristics across common waterhemp biotypes, herbicide-rotation and crop-rotation histories.[a]

	Microbial PLFA[b]						Carbon and nitrogen content[b]		
	Total PLFA	Bacteria	Protozoa	Fungi	Bacteria: fungi	Sat: Monounsat	SOM	TN	C : N
	ng g^{-1} oven-dried soil	%							
Waterhemp biotype									
Site w/R waterhemp	1971	1696	9.28	111	18.39	2.34	3.5	0.18	11.13
Site w/S waterhemp	1955	1675	9.05	105	19.45	2.63	3.7	0.20	10.64
P value	0.91	0.86	0.89	0.56	0.59	0.03	0.43	0.15	0.17
Glyphosate-use history									
Continuous glyphosate	1934	1660	9.79	106	19.88	2.37	3.5	0.19	10.90
Some herbicide rotation	1825	1574	7.56	97	18.33	2.55	3.4	0.18	11.12
P value	0.44	0.48	0.22	0.38	0.53	0.21	0.63	0.39	0.59
Crop-rotation history									
Continuous soybean	1967	1689	9.28	107	19.86	2.43	3.4	0.18	10.71
Some corn : soybean	1993	1716	9.54	112	17.36	2.50	3.5	0.18	11.24
P value	0.86	0.84	0.88	0.65	0.23	0.64	0.51	0.97	0.19

[a] Abbreviations: C = carbon; GR = glyphosate resistant; GS = glyphosate susceptible; Monounsat = monounsaturated; PLFA = phospholipid fatty acid analysis; R = resistant; S = susceptible; Sat = saturated;; SOM = soil organic matter; TN = total nitrogen.
[b] Means followed by P value ≥thinsp;0.05 are not different.

quality are not impacted by locations with continuous use of glyphosate, and/or the crop rotation history of the field (Means et al. 2007). Means et al. (2007) observed little effect of glyphosate on general rhizosphere microbial activity measured by enzyme activity and CO_2 respiration in an experiment conducted under fairly constant SOM and soil N levels. Therefore, plant–microbe–soil interactions may not be readily affected by specific microbial components or functions within the overall microbial community that were assessed in this study following a history of GR common waterhemp, glyphosate usage, and/or lack of crop rotation.

Differences were observed between the saturated to monounsaturated fatty acid ratio. This ratio indicates that under stress such as soil temperature increase or soil moisture decrease, the degree of unsaturated fatty acid decreases and that of saturated fatty acid increases; thus the ratio will also increase (Peterson and Klug 1994). The ratio is a general indicator for the status of bacterial community in the environment and can provide input regarding whether some impact by an environmental factor (glyphosate treatment and/or glyphosate resistant common waterhemp) is shown by the microbes (Peterson and Klug 1994). The site characteristic of common waterhemp biotype resulted in differences in this ratio, but this biomarker was not different for the other site characteristics including glyphosate-use history or crop-rotation history (Table 5). A site with GR common waterhemp provided a lower saturated to monounsaturated ratio than a site with GS common waterhemp. These results suggest that the soil microbial community structure and abundance in locations where a GR common waterhemp biotype has evolved may benefit from some growth factor released in the root exudates that influences the soil microbial community. In addition, there may be slight differences between common waterhemp biotypes in the amount and/or rate of release of glyphosate from the roots into soil, potentially affecting microbial growth and activity. However, further research into these interactions is necessary to understand the significance of this response within the soil microbial community.

Overall, the plant rhizosphere relationships with soil microorganisms are not completely understood from the standpoint of glyphosate effects on weed–rhizosphere microbial community interactions. This research supports previous findings with other plant species that indicate a greater sensitivity to glyphosate when grown in nonsterile relative to sterile soils, demonstrating that glyphosate may predispose plants to diseases incited by soil-borne phytopathogens. Few differences were observed across all site characteristics for soil microbial abundance and diversity, and therefore suggest crop- and herbicide-use history and

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common waterhemp biotype do not readily alter soil microbial communities in the soils evaluated in our study. Based on the results of this study, future research is needed to understand when and how glyphosate influences *Fusarium* colonization of common waterhemp.

Literature Cited

Accinelli C, Koskinen WC, Becker JM, Sadowsky MJ (2007) Environmental fate of two sulfonamide antimicrobial agents in soil. J Agric Food Chem 55:2677–2682

Accinelli C, Koskinen WC, Seebinger JD, Vicari A, Sadowsky MJ (2005) Effects of incorporated corn residue on glyphosate mineralization and sorption in soil. J Agric Food Chem 53:4110–4117

Araujo ASF, Monteiro RTR, Abarkeli RB (2003) Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52:799–804

Baley GJ, Campbell KG, Yenish J, Kidwell KK, Paulitz TC (2009) Influence of glyphosate, crop volunteer and root pathogen on glyphosate-resistant wheat under controlled environmental conditions. Pest Manag Sci 65:288–299

Baylis AD (2000) Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Manag Sci 56:299–308

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 3: 911–917

Borggaard OK, Gimsing AL (2008) Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci 64:441–456

Bradley K, Johnson B, Smeda R, Boerboom C (2009) Integrated pest management: practical weed science for the field scout—corn and soybeans. University of Missouri Extension Publ. IPM1007. 71 p

Chandi A, Milla-Lewis SR, Jordan DL, York AC, Burton JD, Zuleta MC, Whitaker JR, Culpepper AS (2013) Use of AFLP markers to assess genetic diversity in Palmer amaranth (*Amaranthus palmeri*) populations from North Carolina and Georgia. Weed Sci 61:136–145

Close TM, Kniss AR (2011) Glyphosate efficacy on Canada thistle (*Cirsium arvense*) grown in field and greenhouse soil. Proc West Soc Weed Sci 64:43

Coupland D, Casely JC (1979) Presence of ^{14}C activity in root exudates and guttation fluid from *Agropyron repens* treated with ^{14}C-labelled glyphosate. New Phytol, 83:17–22

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151–153

Foes MJ, Liu L, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 46:514–520

Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gimsing AL, Borggaard OK, Jacobsen OS, Aamand J, Sorensen J (2004) Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils. Appl Soil Ecol 27:233–242

Gomez E, Ferreras L, Lovotti L, Fernandez E (2009) Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina. Eur J Soil Biol 45:163–167

Hammond-Kosack K, Jones JDG (2000) Responses to plant phytopathogens. Pages 1102–1156 *in* Buchanan BB, Gruissem W, and Jones RL, eds. Biochemistry and Molecular Biology of Plants. ASPP, Rockville, MD

Haney RL, Seseman SA, Hons FM, Zuberer DA (2000) Effect of glyphosate on soil microbial activity and biomass. Weed Sci. 48:89–93

Haney RL, Senseman SA, Hons FM (2002) Effects of Roundup Ultra on microbial activity and biomass from selected soils. J Environ Qual 31:730–735

Heap I (2013) The International Survey of Herbicide-Resistant Weeds. http://www.weedscience.com. Accessed March 10, 2013

Horak MJ, Peterson DE (1995) Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol 9:192–195

Johal GS, Huber DM (2009) Glyphosate effects on diseases of plants. Eur J Agron 31:144–152

Johal GS, Rahe JE (1984) Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74:950–955

Kawate MK, Colwell SG, Ogg AG Jr, Kraft JM (1997) Effect of glyphosate treated henbit (*Lamium amplexicaule*) and downy brome (*Bromus tectorum*) on *Fusarium solani* f. sp. *pisi* and *Pythium ultimum*. Weed Sci 45:739–743

Kistler HC, Momol EA, Benny U (1991) Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331–336

Komada H (1975) Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev Plant Prot Res (Tokyo) 8:114–125

Lancaster SH, Hollister EB, Senseman SA, Gentry TJ (2010) Effects of repeated glyphosate application on soil microbial community composition and the mineralization of glyphosate. Pest Manag Sci 66:153–159

Lane M, Lorenz N, Saxena J, Ramsier C, Dick R (2012) Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. Pedobiologia 55:153–159

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

Lévesque CA, Rahe JE (1992) Herbicide interaction with fungal root pathogens, with special reference to glyphosate. Annu Rev Phytopathol 30:579–602

Lévesque CA, Rahe JE, Eaves DM (1987) Effects of glyphosate on *Fusarium* spp. : its influence on root colonization of weeds, propagule density in the soil, and crop emergence. Can J Microbiol 33:354–360

Lévesque CA, Rahe JE, Eaves DM (1992) The effect of soil heat treatments and microflora on the efficacy of glyphosate in seedlings. Weed Res 32:363–373

Lévesque CA, Rahe JE, Eaves DM (1993) Fungal colonization of glyphosate-treated seedlings using a new root plating technique. Mycol Res 97:299–306

Li S, Hartman GL, Domier LL, Boykin D (2008) Quantification of *Fusarium solani* f. sp. *glycines* isolates in soybean roots by colony forming unit assays and real time quantitative PCR. Theor Appl Genet 117:343–352

Liphadzi KB, Al-Khatib K, Bensch CN, Stahlman PW, Dille JA, Todd T, Rice CW, Horak MJ, Head G (2005) Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system. Weed Sci 53:536–545

Liu L, Punja ZK, Rahe JE (1995) Effect of Pythium spp. and glyphosate on phytoalexin production and exudation by bean (*Phaseolus vulgaris* L.) roots grown in different media. Physiol Mol Plant Pathol 47:391–405

Lupwayi NZ, Hanson KG, Harker KN, Clayton GW, Blackshaw RE, O'Donovan JT, Johnson EN, Gan Y, Irvine RB, Monreal MA (2007) Soil microbial biomass, functional diversity and enzyme activity in glyphosate-resistant wheat–canola rotations under low-disturbance direct seeding and conventional tillage. Soil Biol Biochem 39:1418–1427

Means NE, Kremer RJ (2007) Influence of soil moisture on root colonization of glyphosate-treated soybean by *Fusarium* species. Commun Soil Sci Plant Anal 38:1713–1720

Means NE, Kremer RJ, Ramsier C (2007) Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere. J Environ Sci Health B 42:125–132

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. Pages 961–1010 *in* Sparks DL, ed. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series 5, Soil Science Society of America, Madison, WI

Nelson PE, Toussoun TA, Marasas WFO (1983) *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, PA. Pp 14, 142–150

Nordby D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. Purdue Extension. GWC-13

Owen MD (2013) Update of herbicide resistance in common waterhemp: an Iowa perspective. Proc Weed Sci Soc Am 53:265

Petersen SO, Klug MJ (1994) Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Appl Environ Microbiol 60:2421–2430

Powell JR, Levy-Booth DJ, Gulden RH, Asbil WL, Campbell RG, Dunfield KE, Hamill AS, Hart MM, Lerat S, Nurse RE, Pauls KP, Sikkema PH, Swanton CJ, Trevors JT, Klironomos JN (2009) Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. Appl Ecol 46:388–396

Ratcliff AW, Busse MD, Shestak CJ (2006) Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl Soil Ecol 34:114–124

Riggins C, Hager A, Tranel P (2012) Three years of testing Illinois waterhemp populations for multiple resistance to glyphosate, PPO inhibitors, and ALS inhibitors. Proc North Cent Weed Sci Soc 67:122

Rosenbaum KK, Riley EB, Wait JD, Bradley KW (2012) Correlation between field management practices and glyphosate resistance in waterhemp populations from Missouri. Proc Weed Sci Soc Am 52:261

Sanogo S, Yang XB, Lundeen P (2001) Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Dis 85:773–779

Sanogo S, Yang XB, Scherm H (2000) Effects of herbicides on *Fusarium solani* f. sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. Phytopathology 90:57–66

Schafer JR, Hallett SG, Johnson WG (2012) Response of giant ragweed (*Ambrosia trifida*), horseweed (*Conyza canadensis*), and common lambsquarters (*Chenopodium album*) biotypes to glyphosate in the presence and absence of soil microorganisms. Weed Sci 60:641–649

Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH (2008) Fate and availability of glyphosate and AMPA in agriculture soil. J Environ Sci Heath Part B 43:365–375

Smiley RW, Ogg AG Jr, Cook RJ (1992) Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. Plant Dis 76:937–942

Souza RA, Babjuia LC, Silva AP, de Fatima Guimaraes M, Arias CM, Hungria M (2013) Impact of the *ahas* transgene and of herbicide associated with the soybean crop on soil microbial communities. Transgen Res. DOI: 10. 1007/s11248-013-9691-x

Tesfamariam T, Bott S, Cakmak I, Romheld V, Neumann G (2009) Glyphosate in the rhizosphere-role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. Eur J Agron 31:126–132

Unger IM, Goyne KW, Kennedy AC, Kremer RJ, McLain JET, Williams CF (2013) Antibiotic effects on microbial community characteristics in soils under conservation management practices. Soil Sci Soc Am J 77:100–112

Unger IM, Kennedy AC, Muzika RM (2009) Flooding effects on soil microbial communities. Appl Soil Ecol 42:1–8

[USDA-NRCS] U.S. Department of Agriculture, Natural Resources Conservation Service (2006) Land Resource Regions and Major Land Resource Areas of the United States, the Caribbean, and the Pacific Basin. USDA Handbook 296

Waggoner BS, Bradley KW (2011) A survey of weed incidence and severity in response to management practices in Missouri soybean production fields. Proc North Cent Weed Sci Soc 66:80

Weaver MA, Krutz LJ, Zablotowicz RM, Reddy KN (2007) Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Manag Sci 63:388–393

Woodburn AT (2000) Glyphosate: production, pricing and use worldwide. Pest Manag Sci 56:309–312

Zabaloy MC, Gomez E, Garland JL, Gomez MA (2012) Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. Appl Soil Ecol 61:333–339

Zhang X, Li X, Zhang C, Li X, Zhang H (2011) Ecological risk of long-term chlorimuron-ethyl application to soil microbial community: an in situ investigation in a continuously cropped soybean field in northeast China. Environ Sci Pollut Res 18:407–415

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol, 7 (1–2): 203–214

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