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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

Jessica R. Schafer, Steven G. Hallett, and William G. Johnson*

In previous research conducted on nonweed species, the efficacy of glyphosate was shown to be greater in unsterile soils compared to sterile soils and soil microorganisms were found to play an important role in glyphosate efficacy. Conducting greenhouse studies in microbe-free soil may therefore produce unreliable data, leading to erroneous conclusions. The objective of this study was to determine the effect of soil microorganisms on the response of glyphosate-resistant and -susceptible biotypes of three problematic weeds of the midwestern United States: giant ragweed, horseweed, and common lambsquarters. A greenhouse dose–response study was conducted on each of the three weed species grown in sterile and unsterile field soil, and the dry weight response of roots and shoots was measured. The three weed species responded differently to glyphosate when grown in the sterile and unsterile soil; that is, in the presence and absence of soil microbes. Soil microbes influenced the response of the susceptible and resistant giant ragweed biotypes and the susceptible common lambsquarters, but not the tolerant common lambsquarters or either horseweed biotype. The different responses of the three species to glyphosate in the presence and absence of soil microbes demonstrates that rhizosphere interactions are fundamental to the mode of action of glyphosate. These findings suggest that the range of tolerance to glyphosate observed in weeds and the evolution of resistance in weed biotypes may also be influenced by rhizosphere interactions. The soil media used in dose–response screenings to identify susceptible and resistant weed biotypes is very important. Unsterile field soil should be incorporated into growth media when conducting dose–response screenings to avoid false-positive results. In addition, researchers performing glyphosate dose–response assays should be aware of these findings.
Nomenclature: Glyphosate; common lambsquarters, *Chenopodium album* L. CHEAL; giant ragweed, *Ambrosia trifida* L. AMBTR; horseweed, *Conyza canadensis* (L.) Cronq. ERICA.
Key words: Dose–response, glyphosate-resistance, glyphosate-tolerance, soil-borne plant pathogens.

Glyphosate is an environmentally benign, POST herbicide that provides broad-spectrum weed control in glyphosate-resistant crops (Cerdeira and Duke 2006; Duke and Powles 2008). Glyphosate has a unique and specific mode of action, blocking the enzyme 5-enolpyruvylshikimate-3-phosphate synthase within the shikimate pathway (Steinrücken and Amrhein 1980) and thus inhibiting the biosynthesis of aromatic amino acids (Duke and Powles 2008). Glyphosate use has increased drastically since 1996 due to the rapid adoption of glyphosate-resistant crop technology (Cerdeira and Duke 2006), and this has contributed to the evolution of glyphosate resistance in 21 different weed species worldwide (Heap 2012; Powles and Preston 2006).

In the United States, populations of glyphosate-resistant giant ragweed and horseweed are present in a total of 10 and 20 states, respectively (Heap 2012). In recent surveys, these weeds were identified by midwestern U.S. corn and soybean producers as two of the three most problematic weeds (Kruger et al. 2009). In 2005, 23% of the farmers surveyed in Indiana identified horseweed as a problematic winter annual, while 29% identified giant ragweed as a problematic summer annual (Gibson et al. 2005). Common lambsquarters was also identified as problematic by 14% of farmers surveyed, due to poor control with glyphosate (Gibson et al. 2005). Glyphosate-insensitive weeds have become increasingly common throughout the midwestern U.S. cropping systems.

Greenhouse dose–response screenings are the most common method of testing weeds for herbicide resistance. However, researchers have reported a differential response of weed species to glyphosate between greenhouse and field experiments. Stachler (2008) reported that when screening giant ragweed biotypes for resistance to glyphosate in the greenhouse, the GR_{50} (growth reduction of 50%) values for the sensitive population were higher than what would be expected under field conditions. Westhoven et al. (2008b) showed that the dose of glyphosate required to achieve a GR_{50} in a glyphosate-tolerant common lambsquarters biotype was 1.48 to 3.22 kg ae ha^{-1} in the greenhouse, but only 0.060 kg ae ha^{-1} in the field. Similarly, the GR_{50} of a glyphosate-susceptible common lambsquarters biotype was 0.57 kg ae ha^{-1} in the greenhouse and only 0.036 kg ae ha^{-1} in the field. Researchers conducting greenhouse dose–response studies to diagnose glyphosate resistance have used many different types of soil media, but most commonly use some type of commercial potting medium that is largely free of fungal plant pathogens (Ingram et al. 1993).

Environmental conditions play a large role in the activity of glyphosate. In particular environmental conditions that support active plant growth and photosynthetic translocation tend to support glyphosate efficacy (Sprankle et al. 1975). The uptake and translocation of radioactive ^{14}C-glyphosate was greater at 22 C than 35 C and increased as relative humidity increased in Florida beggarweed [Desmodium tortuosum (Sw.) DC.] (Sharma and Singh 2001). Visible glyphosate injury to Bermudagrass [*Cynodon dactylon* (L.) Pers.] (Jordan 1977) and ^{14}C-glyphosate translocation in cotton (*Gossypium hirsutum* L.) (Wills 1978) was greater at 100% relative humidity compared to 40% relative humidity. Also known, but less frequently cited, is that glyphosate efficacy is strongly influenced by root-invading soil microorganisms.

The effect of soil microbes have been investigated in detail in model species, such as beans (*Phaseolus vulgaris* L.) (Johal and Rahe 1984; Lévesque and Rahe 1992), but not in weeds. Lévesque and Rahe (1992) reported that between 15- and 20-fold

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more glyphosate was required to kill bean seedlings in autoclaved soil free of plant pathogens, as compared to seedlings in unsterile soil. When the fungal pathogens *Pythium* sp. and *Fusarium* sp. were added to sterile soil, injury caused by glyphosate was greater on bean seedlings (Johal and Rahe 1988). *Pythium* isolates enhanced glyphosate activity by 20- to 50-fold in sunflower (*Helianthus annuus* L. cv. Sunwheat 101) seedlings, and 6- to 30-fold in pepper (*Capsicum frutescens* L. cv. California Wonder) seedlings (Descalzo et al. 1997). These findings suggest the cause of death in plants treated with glyphosate involves more than the direct metabolic consequences of aromatic amino acid depletion and the predisposition of plants to pathogens can be very important.

The aromatic amino acids produced by the shikimate acid pathway serve as precursors for a suite of phenolic compounds including auxins, phenolic phytoalexins, and lignins, all of which are important for defense against soil-borne plant pathogens (Altman and Campbell 1977). Glyphosate can reduce the synthesis of these compounds, predisposing the plant to pathogens and other stresses (Altman and Campbell 1977; Pline-Srnic 2005). Sublethal doses of glyphosate increased the susceptibility of bean seedlings to bean anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams.-Scrib.] and Pythium root rot (caused by *Pythium* sp.) (Johal and Rahe 1988).

The interaction among plants, glyphosate, and soil microorganisms has been studied in a number of crops, but not in weeds. This is surprising since the soil biotic environment has a strong impact upon the activity of this important herbicide. Gaining a better understanding of these interactions will shed more light on the performance of glyphosate in the field. It will also assist in the development of more reliable dose–response screenings and give insight into the ability of various weed species to withstand a glyphosate application. It is possible, for example, that the evolution in the plant–microbe relationship may play a role in the evolution of resistance to glyphosate. The objective of this research was to investigate if soil microorganisms influence the efficacy of glyphosate in weed species. A greenhouse dose–response study was conducted to characterize the response of known glyphosate-resistant and -susceptible giant ragweed and horseweed and glyphosate-tolerant and -susceptible common lambsquarters in the presence and absence of soil microorganisms.

Materials and Methods

Soil Sterilization Procedure. Field soil used throughout this study was collected in August 2009 from Throckmorton-Purdue Agricultural Center in Lafayette, Indiana. Topsoil was taken to a depth of 20 cm from a field in which glyphosate-resistant corn had been grown in the previous season. The soil was dried in the greenhouse (27/14 C day/night), ground to remove large debris, and packaged into 11.35-L plastic storage containers (Sterilite Corporation, Townsend, MA). Half of the field soil containers were sterilized using gamma irradiation (γ-irradiation) (STERIS Isomedix Services, STERIS Corporation, Mentor, OH) and the others were not sterilized. Soil was confirmed sterile by soil serial dilution plating technique according to the Miles-Misra method (Corry 1982). Soil samples were diluted in sterile distilled water and spread onto potato dextrose agar (Difco^{TM} potato dextrose agar, Becton, Dickinson and Company, Sparks, MD) and incubated at 25 C for 72 h, after which colony-forming units per gram of soil were calculated. The γ-irradiated soil was not completely sterile but contained extremely low microbial numbers, very few microbial species, and no fungi. The dry soils were stored at 10 C in the dark until use to prevent microbial activity.

We chose γ-irradiation as our method for soil sterilization because it was reported to cause less change to the nutrient balance of the soil then other sterilization methods, such as autoclaving (Berns et al. 2008). Nonetheless, soil samples were analyzed (A&L Great Lakes Laboratories, Fort Wayne, IN) for nitrate, ammonium, phosphorus, potassium, magnesium, and manganese concentrations. Phosphorus, magnesium, and manganese concentrations were unaltered by γ-irradiation, compared to the unsterile soil. Ammonium concentrations were increased, and nitrate concentrations decreased when soil was γ-irradiated. A number of studies have reported that a decrease in nitrate after γ-irradiation maybe due to the fact that nitrifying bacteria are more sensitive to γ-irradiation than ammonifying bacteria (McNamara et al. 2003; Thompson 1990).

Therefore, plant tissue nitrogen content of both biotypes of each weed was also tested to determine if differences in plant nitrogen was influenced by γ-irradiated soil. Whole plant samples were cut at the soil line and dried for 5 d in a forced air dryer at 50 C. Dried plant samples were ground and analyzed for total Kjeldahl nitrogen (Isaac and Johnson 1976). Where differences in tissue nitrogen content were found, they showed that plants grown in the γ-irradiated soil generally had greater nitrogen concentration. However, in our studies nitrogen concentration did not influence plant response to glyphosate, unlike findings reported by other researchers (Mithila et al. 2008).

Greenhouse Dose–Response Studies. Biotypes of glyphosate-resistant and glyphosate-susceptible giant ragweed (Stachler 2008), horseweed (Davis et al. 2008), and glyphosate-tolerant and glyphosate-susceptible common lambsquarters (Westhoven et al. 2008a) were characterized previously through greenhouse dose–response studies. Tolerance, referring to the common lambsquarters biotype, is defined as an inherent ability to survive and reproduce after a herbicide application, implying that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant (WSSA 1998). The biotypes of each weed species exhibiting the highest level of glyphosate resistance or tolerance and susceptibility were used throughout this study. Seeds of each species were stored at 4 C, sown in commercial potting soil (Sun Gro Redi-earth plug and seedling mix, Sun Gro Horticulture, Bellevue, WA), and grown in the greenhouse. Plants were grown under natural lighting supplemented with high-pressure sodium lamps that provided 400 μmol m^{-2} s^{-1} photosynthetic photon flux density with a 16-h photoperiod. Greenhouse day and night temperatures were maintained at 28 and 17 C. Plants were watered daily and fertilized weekly with a commercial 24–8–16 fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food, Scotts Miracle-Gro Products Inc., Marysville, OH). At seedling transplant, roots were dipped into a 0.05% sodium hypochlorite (bleach) solution (Clorox Regular Bleach, The Clorox Company, Oakland, CA) for surface disinfection and washed before being planted into individual sterilized 106.5-cm^3 cones

(Ray Leach Cone-tainer, Stuewe & Sons, Inc., Tangent, OR) containing either sterile or unsterile field soil.

Giant Ragweed. The glyphosate-resistant giant ragweed seed was collected from Noble County, Indiana, and the glyphosate-susceptible biotype from Darke County, Ohio (Stachler 2008). Giant ragweed seeds were pregerminated by burying wire mesh bags containing seed in a field soil : sand mixture at 4 C for 4 to 6 wk (Westhoven et al. 2008a). After dormancy was broken, seeds were imbibed on moist paper towels for 24 h (Brabham et al. 2011) and then planted approximately 1 cm deep in commercial potting soil and transplanted as described in Greenhouse Dose–Response Studies upon the appearance of the first true leaves. Glyphosate was applied at the three- to four-node growth stage.

Horseweed. The glyphosate-resistant and glyphosate-susceptible horseweed seed populations were collected from locations in Indiana and Ohio, respectively (Davis et al. 2008). Horseweed seed was planted on the surface of commercial potting soil. Seeds were bottom watered daily and transplanted when four to five true leave were visible. Glyphosate was applied when plants were approximately 5 to 8 cm in diameter.

Common Lambsquarters. The glyphosate-tolerant biotype of common lambsquarters was collected from Ripley County, Indiana, and the glyphosate-susceptible biotype from Jefferson County, Indiana (Westhoven et al. 2008b). Common lambsquarters seed was soaked in 95.9% sulfuric acid (Mallinckrodt Baker, Inc., Phillipsburg, NJ) for 15 min, washed with running water for 45 min, and dried for 24 h at approximately 24 C (Westhoven et al. 2008a). Seeds were planted 0.5 cm deep into commercial potting soil and transplanted when plants reached the two- to three-node growth stage. Glyphosate was applied at the seven- to eight-node growth stage.

Glyphosate Application. Each dose–response experiment was conducted as a randomized complete block design with a three-way factorial arrangement; factors were field soil, biotype, and glyphosate rate. Weed species were evaluated in separate experiments. Each experiment included four replications of each treatment. The experiment was run three times with giant ragweed and horseweed, and twice with common lambsquarters. When plants grown in sterile and unsterile field soil reached the appropriate growth stage they were treated with 0, 0.21, 0.42, 0.84, 1.68, 3.36, 6.72, or 13.44 kg ae ha^{-1} of glyphosate (giant ragweed) and (common lambsquarters), and with an additional rate of 26.88 kg ha^{-1} of glyphosate for horseweed. Recommended rates for control of these weeds fall in the range of 0.84 to 1.68 kg ha^{-1} of glyphosate. Glyphosate treatments were prepared from a mixture of technical-grade solution of the isopropylamine salt of glyphosate and a solution of the formulation blank of Glyphomax Plus (Glyphomax Plus®, DOW AgroSciences LLC, Indianapolis, IN), as described by Smith and Hallett (2006). A fixed concentration of the formulation blank solution equivalent to the concentration found in a 0.84 kg ha^{-1} rate of the formulated Glyphomax Plus was used in all treatments to ensure that the concentration of adjuvants remained constant as glyphosate concentrations varied (Smith and Hallett 2006). All treatments were applied with 2.8 kg ha^{-1} ammonium sulfate (AMS) (N-PAK, Winfield Solutions, LLC, St. Paul, MN) and 0.5% v/v nonionic surfactant (NIS) (Preference, Winfield Solutions, LLC). The control treatment received the fixed concentration of formulation blank, AMS, and NIS without glyphosate. The treatments were applied using a laboratory compressed-air spray chamber calibrated to deliver 190 L ha^{-1} carrier volume at a pressure of 230 kPa using a 8002EVS nozzle (TeeJet Technologies, Wheaton, IL). At 21 d after glyphosate treatment (DAT) for giant ragweed and common lambsquarters and 14 DAT for horseweed, living shoot biomass and root tissue were harvested separately and dried for 5 d in a forced air dryer at 50 C. Individual plant shoot and root tissue were weighed separately to determine plant dry weight.

Statistical Analysis. Dose–response shoot and root dry weight of individual plants were converted to a percentage of the untreated control. Prior to analysis percentages of untreated control data were checked for normality and constant variance in SAS. Normality assumptions were met, therefore data were not transformed. An interaction between experiments was absent, so data from all experiments were pooled for each weed species. Untransformed dry weight data were fit to a dose–response curve using a nonlinear regression model with the drc package in R software (R 2.10, Kurt Hornik, http://www.R-project.org) (Knezevic et al. 2007). The dose–response curve for each weed species was constructed using the three-parameter log-logistic model in Equation 1.

$$Y = d/1 + \exp\{b[\log x - \log(e)]\} \quad [1]$$

In Equation 1, the parameter d is the upper limit, b is the relative slope around e, and e is the dose producing a response halfway between the upper limit (d) and the lower limit (c) fixed at zero. A lack-of-fit test indicated that the model accurately described the data for each weed species. Growth reductions for dry weights were calculated as GR_{50} and GR_{90}, indicating a 50% or 90% decrease in plant growth compared to the untreated control. To identify significant differences among treatments, dose–response curves between treatments were compared at the GR_{50} and GR_{90} using the selective index (SI) in Equation 2.

$$SI(x, y) = GR_x/GR_y \quad [2]$$

The ratio between the growth reduction (GR_x) for one curve and GR_y for another curve was calculated at $\alpha = 0.05$ (Ritz and Streibig 2007).

Results and Discussion

Greenhouse Dose–Response Studies. The dose–response curves for each weed biotype produced a characteristic sigmoid S-shaped curves, fitting the models adequately. As the glyphosate rate increased, the percentage of dry weight decreased for both the susceptible and resistant biotypes. Previously identified resistant or tolerant biotypes used in our experiments showed a decreased response to glyphosate, compared to the susceptible biotypes.

Table 1. Regression parameter estimates of dose–response curves for shoot dry weight tissue using a three-parameter logistic model (Equation 1) in dose–response study conducted in sterile and unsterile field soil.[a]

Weed species	Biotype	Soil	Regression parameters		GR_{50} (SE)	GR_{90} (SE)
			b	*d*	kg ae ha $^{-1}$	
Giant ragweed	Susceptible	Sterile	1.11	102.16	2.6 (0.5)	—
	Susceptible	Unsterile	1.32	99.12	0.3 (0.1)	—
	Resistant	Sterile	0.63	98.30	3.0 (1.0)	—
	Resistant	Unsterile	0.65	99.84	0.7 (0.2)	—
Horseweed	Susceptible	Sterile	0.68	98.39	0.9 (0.4)	22.3 (16.4)
	Susceptible	Unsterile	0.65	99.91	0.8 (0.4)	22.7 (17.9)
	Resistant	Sterile	1.25	87.22	7.4 (2.4)	42.4 (47.3)
	Resistant	Unsterile	1.45	91.89	6.8 (1.6)	30.8 (22.2)
Common lambsquarters	Susceptible	Sterile	1.32	98.74	0.5 (0.1)	2.8 (0.9)
	Susceptible	Unsterile	2.41	100.10	0.1 (0.1)	0.3 (0.1)
	Tolerant	Sterile	1.66	97.51	0.6 (0.1)	2.1 (0.6)
	Tolerant	Unsterile	1.40	96.72	0.4 (0.1)	2.1 (0.6)

[a] Abbreviations: *b*, slope of the curve; *d*, upper response limit; GR_{50}, glyphosate dose to reduce dry weight by 50%; GR_{90}, glyphosate dose to reduce dry weight by 90%; SE, standard error.

Giant Ragweed. Both biotypes grown in the sterile soil had more shoot dry weight at 21 days after glyphosate treatment, compared to plants grown in the unsterile soil. The dose of glyphosate required to achieve GR_{50} of the susceptible biotype grown in the sterile (SS) and unsterile (SU) soil were 2.6 and 0.3 kg ha^{-1}, respectively (Table 1). The resistant biotype GR_{50} values were 3.0 and 0.7 kg ha^{-1} when grown in the sterile (RS) and unsterile (RU) soil, respectively (Table 1). A difference was observed when comparing the dose–response curves at the GR_{50} of the SS and SU ($P = 0.0008$), yet was not observed in comparing the RS and RU ($P = 0.0796$) (Table 2). Interestingly, the GR_{50} values of SS and RS were not different ($P = 0.7314$) (Table 2), revealing that the response to glyphosate between the two biotypes was similar when grown in the sterile soil. The GR_{90} values for giant ragweed are not presented because they could not be accurately predicted from the model generated because the highest rate of glyphosate used, 13.44 kg ha^{-1}, did not kill 90% of the plants in all treatments. The shoot dry weight response of the SS was similar to the response of the RS at glyphosate rates up to 3.36 kg ha^{-1} and significant injury and death were observed in the SS at a rate of 6.72 kg ha^{-1} (Figure 1). A higher rate of glyphosate was required to kill the susceptible biotype when grown in sterile soil (Figure 2). Surviving SS plants previously treated with a 0.84 and 1.68 kg ha^{-1} of glyphosate were able to continue growing from axillary buds and set seed, yet SS plants that survived a rate higher than 3.36 kg ha^{-1} were severely stunted and did not continue to grow or produce seed. The shoot dry weight accumulation of SU plant decreased after a glyphosate application of only 0.42 kg ha^{-1} and plant death occurred at a rate of 3.36 kg ha^{-1} (Figure 2). At a commonly used rate of glyphosate in the field (0.84 kg ha^{-1}) the susceptible biotype was able to survive when grown in sterile soil, visually comparable to the resistant biotype grown in sterile soil (Figure 3). This may lead to inaccurate conclusions when screening giant ragweed biotypes for glyphosate-resistance in the greenhouse using commercial potting soil free of soil microbes.

Table 2. Selective index (Equation 2) tests of GR_{50} and GR_{90} values based on shoot dry weight percent of control for dose–response curves.[a]

Weed species	Comparisons	GR_{50} (SE) estimate	P value[b]	GR_{90} (SE) estimate	P value[b]
		Dry weight (% control)		Dry weight (% control)	
Giant ragweed	SS vs. SU	**9.6 (2.55)**	**0.0008**	—	—
	SS vs. RS	0.9 (0.33)	0.7314	—	—
	SS vs. RU	**4.0 (1.43)**	**0.0354**	—	—
	SU vs. RS	**0.1 (0.03)**	**<0.0001**	—	—
	SU vs. RU	**0.4 (0.14)**	**0.0001**	—	—
	RS vs. RU	4.5 (2.01)	0.0796	—	—
Horseweed	SS vs. SU	1.1 (0.7)	0.8881	1.0 (1.1)	0.9861
	SS vs. RS	**0.1 (0.1)**	**<0.0001**	0.5 (0.7)	0.5006
	SS vs. RU	**0.1 (0.1)**	**<0.0001**	0.7 (0.7)	0.7109
	SU vs. RS	**0.1 (0.1)**	**<0.0001**	0.5 (0.7)	0.5267
	SU vs. RU	**0.1 (0.1)**	**<0.0001**	0.7 (0.8)	0.7389
	RS vs. RU	1.1 (0.4)	0.8477	1.4 (1.8)	0.8371
Common lambsquarters	SS vs. SU	4.0 (2.1)	0.1593	8.5 (4.0)	0.0602
	SS vs. TS	0.9 (0.3)	0.8292	1.3 (0.6)	0.5653
	SS vs. TU	1.2 (0.4)	0.6302	1.3 (0.6)	0.5849
	SU vs. TS	**0.2 (0.1)**	**<0.0001**	**0.2 (0.1)**	**<0.0001**
	SU vs. TU	**0.3 (0.2)**	**<0.0001**	**0.2 (0.1)**	**<0.0001**
	TS vs. TU	1.2 (0.4)	0.5003	1.0 (0.4)	0.9698

[a] Abbreviations: GR_{50}, glyphosate dose to reduce dry weight by 50%; GR_{90}, glyphosate dose to reduce dry weight by 90%; SE, standard error; SS, susceptible biotype grown in sterile soil; SU, susceptible biotype grown in unsterile soil; RS, resistant biotype grown in sterile soil; RU, resistant biotype grown in unsterile soil; TS, tolerant biotype grown in sterile soil; TU, tolerant biotype grown in unsterile soil.

[b] Values of significance within each weed species are in bold at $\alpha = 0.05$.

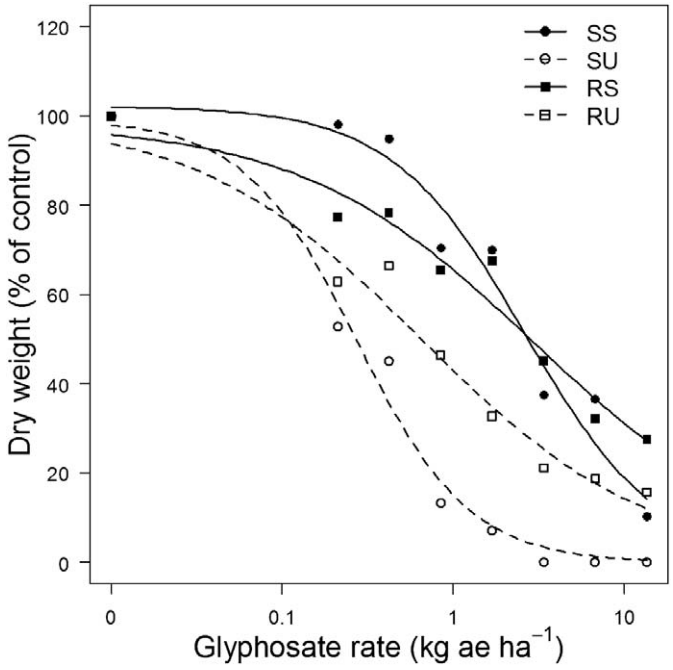

Figure 1. Response (21 d after glyphosate treatment [DAT]) of glyphosate-resistant and glyphosate-susceptible biotypes of giant ragweed grown in sterile or unsterile field soil. Model fit P = 0.6617 using Equation 1. SS, susceptible biotype grown in sterile soil; SU, susceptible biotype grown in unsterile soil; RS, resistant biotype grown in sterile soil; RU, resistant biotype grown in unsterile soil.

The effect of the sterile and unsterile soil on the response of each biotype to glyphosate was less evident in the root dry weight data. This was partly due to difficulty of harvesting root tissue because some tissue was lost during root washing and was not accounted for in the dry weight measurement. The GR_{50} of the SS and SU root tissue were 1.1 and 0.2 kg ha^{-1}, respectively (Table 3). Roots of the SU were necrotic and macerated at 21 DAT, while the roots of the SS appeared healthy following a glyphosate application when compared to treatment controls (Figure 4). The GR_{50} of the RS and RU were 1.2 and 1.1 kg ae ha^{-1}, respectively

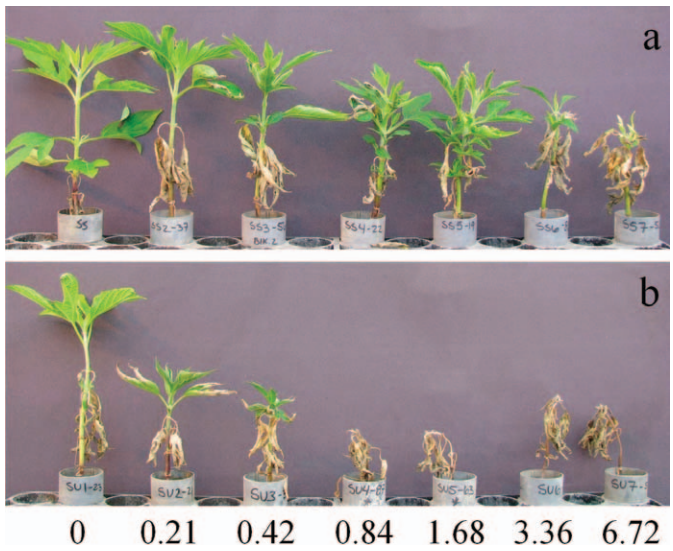

Figure 2. Control of susceptible giant ragweed biotype grown in sterile field soil (a) and unsterile field soil (b) 21 d after glyphosate treatment (DAT) with 0, 0.21, 0.42, 0.84, 1.68, 3.36, or 6.72 kg ae ha^{-1} of glyphosate.

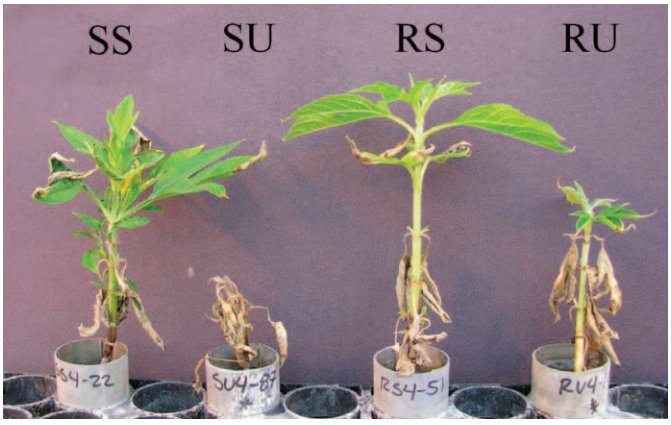

Figure 3. Response of shoot tissue 21 d after glyphosate treatment (DAT) of giant ragweed biotypes to glyphosate at 0.84 kg ae ha^{-1} when grown in sterile and unsterile field soil. SS, susceptible biotype grown in sterile field soil; SU, susceptible biotype grown in unsterile field soil; RS, resistant biotype grown in sterile field soil; RU, resistant biotype grown in unsterile field soil.

(Table 3). Root tissue damage and necrosis were not observed in the resistant biotype, regardless of the presence or absence of soil microbes within the soil (Figure 4). In comparing GR_{50} estimates, the SU was found to be different than both the RS and RU, while the SS root tissue responded similar to the RS and RU (Table 4).

From these data and observations we can hypothesize that there was a lack of microbial root infection following glyphosate application in the RS and SS treatments, and this had a large effect on the survival of plants. Soil microorganisms play an important role in the activity of glyphosate on giant ragweed, presumably by causing root infection, therefore aiding in plant death.

Horseweed. The resistant biotype had a greater amount of biomass than the susceptible biotype across all glyphosate rates, but biotypes grown in the sterile soil and unsterile soil responded similarly (Figure 5). The GR_{50} values of the susceptible biotype grown in sterile soil (SS) and unsterile soil (SU) were 0.9 and 0.8 kg ha^{-1} (Table 1), respectively; which is comparable to a field use rate. The GR_{90} values for the SS and SU were 22.3 and 22.7 kg ha^{-1}, respectively (Table 1). The resistant biotype required the highest dose of glyphosate to achieve both a GR_{50} and GR_{90}. The GR_{50} values of the resistant biotype were 7.4 and 6.8 kg ha^{-1} of glyphosate when grown in the sterile (RS) and unsterile soil (RU), respectively (Table 1). The GR_{90} values for the RS and RU were 42.4 and 30.8 kg ha^{-1}, respectively (Table 1). Comparisons of the dose–response curves at the GR_{50} and GR_{90} indicated that differences were only observed at the GR_{50} between biotypes, with all biotype differences having a $P < 0.0001$ (Table 2); therefore, soil microbes did not play a role in glyphosate efficacy in this species.

The root dry weight responses for horseweed showed the same trends as for shoot dry weights. Differences in root dry weight within each biotype grown in the sterile and unsterile soil were not observed (Table 4), indicating that the roots responded similarly to glyphosate in the presence and absence of soil microbes.

These results indicate that soil microorganisms did not play a role in glyphosate efficacy on horseweed in comparison to giant ragweed. The soil used in this study may not have

Table 3. Regression parameter estimates of dose–response curves for root dry weight tissue using a three-parameter logistic model (Equation 1) in dose–response study conducted in sterile and unsterile field soil.[a]

Weed species	Biotype	Soil	Regression parameters		GR_{50} (SE)	GR_{90} (SE)
			b	*d*	kg ae ha^{-1}	
Giant ragweed	Susceptible	Sterile	0.65	100.74	1.1 (0.5)	—
	Susceptible	Unsterile	0.86	99.28	0.2 (0.1)	—
	Resistant	Sterile	0.35	100.32	1.2 (0.8)	—
	Resistant	Unsterile	0.56	101.38	1.1 (0.5)	—
Horseweed	Susceptible	Sterile	0.64	99.62	0.3 (0.2)	7.7 (7.1)
	Susceptible	Unsterile	0.69	99.87	0.5 (0.2)	10.8 (9.1)
	Resistant	Sterile	0.62	98.05	2.3 (1.4)	80.1 (80.4)
	Resistant	Unsterile	1.77	96.88	5.3 (1.2)	18.3 (10.8)
Common lambsquarters	Susceptible	Sterile	0.70	99.72	0.2 (0.1)	4.4 (2.5)
	Susceptible	Unsterile	2.34	99.98	0.2 (0.0)	0.4 (0.1)
	Tolerant	Sterile	1.07	99.94	0.6 (0.1)	4.5 (1.9)
	Tolerant	Unsterile	1.36	97.30	0.4 (0.1)	2.0 (0.6)

[a] Abbreviations: *b*, slope of the curve; *d*, upper response limit; GR_{50}, glyphosate dose to reduce dry weight by 50%; GR_{90}, glyphosate dose to reduce dry weight by 90%; SE, standard error.

contained high enough populations of soil microbes that are pathogenic to horseweed, which may partly explain the results obtained from this study. But, since the same soil was used to test all species, we hypothesize that horseweed does not interact with soil microbes in the same way, and a synergistic relationship with glyphosate and soil microbes may be absent from this species.

Common Lambsquarters. The presence or absence of soil microbes contributed to the level of tolerance to glyphosate in the susceptible biotype but not the tolerant biotype. The GR_{50} values for the tolerant biotype grown in the sterile soil (TS) and the tolerant biotype grown in the unsterile soil (TU) were 0.6 and 0.4 kg ha^{-1}, respectively (Table 1). The GR_{90} values for both the TS and TU were 2.1 kg ha^{-1} (Table 1). Comparisons of the TS and TU dose–response curves at the GR_{50} and GR_{90} revealed that the tolerant biotype responded the same to glyphosate regardless of the soil it was grown in (Table 2). The GR_{50} values for the susceptible biotype grown in the sterile soil (SS) and the susceptible biotype grown in the unsterile soil (SU) were 0.5 and 0.1 kg ha^{-1}, respectively (Table 1). The GR_{90} values for the SS and SU were 2.8 and 0.3 kg ha^{-1}, respectively (Table 1). The GR_{50} and GR_{90} values for the SS were similar to a greenhouse dose–response study conducted on the same susceptible common lambsquarters biotype grown in commercial potting media; Westhoven et al. (2008b) reported a GR_{50} and GR_{90} of 0.57 and 2.39 kg ha^{-1}, respectively. In the field, this same biotype exhibited decreased GR_{50} and GR_{90} values of 0.036 and 0.19 kg ha^{-1}, respectively (Westhoven et al. 2008b). Dry weight of the SU was reduced 75% by the lowest glyphosate rate used, 0.21 kg ha^{-1}, yet dry weight of the SS was reduced only 40% by the same glyphosate rate (Figure 6). The SS had a greater amount of dry weight than the SU throughout all glyphosate rates tested (Figure 6). However, differences were not found between the SS and SU dose–response curve at the GR_{50} ($P = 0.1593$) and GR_{90} ($P = 0.0602$) (Table 2). Due to the levels of control of the SU at the lowest rate of glyphosate used in this study, the dose–response curve lacked data points to accurately predict the GR_{50} values to separate the SS from the SU.

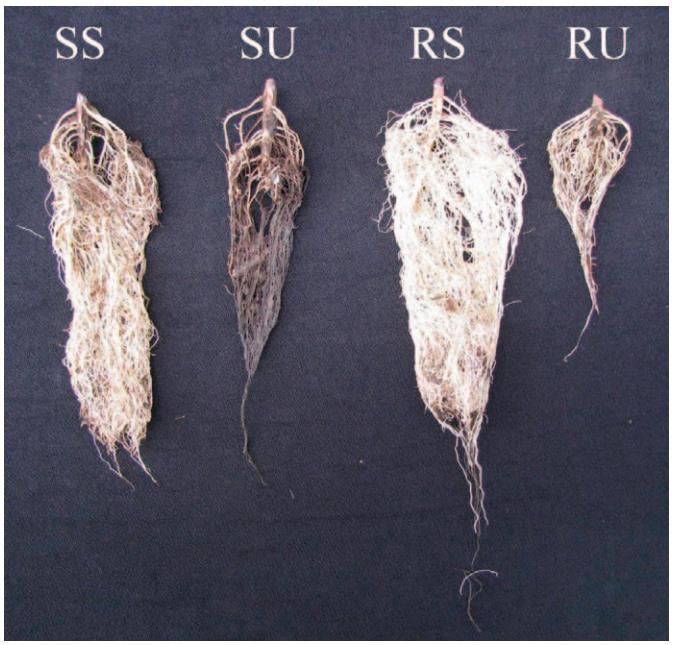

Figure 4. Giant ragweed root necrosis at 21 d after glyphosate treatment (DAT) of 0.84 kg ae ha^{-1} of glyphosate. SS, susceptible biotype grown in sterile field soil; SU, susceptible biotype grown in unsterile field soil; RS, resistant biotype grown in sterile field soil; RU, resistant biotype grown in unsterile field soil.

Interestingly, when comparing the dose–response curve at the GR_{50} and GR_{90}, SS responded to glyphosate similar to both TS and TU. The level of glyphosate tolerance in the tolerant biotype was not affected by soil microbes, yet the susceptible biotype was more tolerant to glyphosate when grown in the absence of soil microbes. Glyphosate dose–response screenings conducted on common lambsquarters grown in sterile soil or potting media could therefore give a false resistance diagnosis.

The root dry weight data for common lambsquarters demonstrated that each biotype growing in the sterile soil required a greater amount of glyphosate to cause root damage, shown by the GR_{90} for each biotype. The SS and SU had the same GR_{50} values of 0.2 kg ha^{-1}, while the GR_{90} values were 4.4 and 0.4 kg ha^{-1} (Table 4). The TS and TU GR_{50} values were 0.6 and 0.4 kg ha^{-1}, and the GR_{90} values were 4.5 and 2.0 kg ha^{-1}, respectively (Table 4). The greater GR_{90} values for both the SS and TS are hypothesized to be due to root regrowth at the crown of the stem. It was observed at 21 DAT that biotypes grown in sterile soil were able to regrow lateral roots

Table 4. Selective index (Equation 2) tests of GR_{50} and GR_{90} values based on root dry weight percent of control for dose–response curves.[a]

Weed species	Comparisons	GR_{50} (SE) estimate	P value[b]	GR_{90} (SE) estimate	P value[b]
		Dry weight (% control)		Dry weight (% control)	
Giant ragweed	SS vs. SU	6.6 (3.97)	0.1607	—	—
	SS vs. RS	0.9 (0.74)	0.9319	—	—
	SS vs. RU	0.9 (0.54)	0.8633	—	—
	SU vs. RS	**0.1 (0.11)**	**<0.0001**	—	—
	SU vs. RU	**0.1 (0.08)**	**<0.0001**	—	—
	RS vs. RU	1.0 (0.78)	0.9674	—	—
Horseweed	SS vs. SU	0.6 (0.4)	0.3137	0.7 (0.9)	0.7522
	SS vs. RS	**0.1 (0.1)**	**<0.0001**	**0.1 (0.1)**	**<0.0001**
	SS vs. RU	**0.1 (0.0)**	**<0.0001**	0.4 (0.5)	0.2117
	SU vs. RS	**0.2 (0.2)**	**<0.0001**	**0.1 (0.2)**	**<0.0001**
	SU vs. RU	**0.1 (0.1)**	**<0.0001**	0.6 (0.6)	0.5007
	RS vs. RU	0.4 (0.3)	0.0535	4.4 (5.1)	0.5085
Common lambsquarters	SS vs. SU	1.3 (0.6)	0.6828	10.9 (7.1)	0.1639
	SS vs. TS	**0.3 (0.2)**	**0.0001**	1.0 (0.7)	0.9660
	SS vs. TU	**0.5 (0.2)**	**0.0374**	2.2 (1.4)	0.3896
	SU vs. TS	**0.3 (0.1)**	**<0.0001**	**0.1 (0.0)**	**<0.0001**
	SU vs. TU	**0.4 (0.1)**	**<0.0001**	**0.2 (0.1)**	**<0.0001**
	TS vs. TU	1.5 (0.5)	0.3502	2.3 (1.2)	0.2527

[a] Abbreviations: GR_{50}, glyphosate dose to reduce dry weight by 50%; GR_{90}, glyphosate dose to reduce dry weight by 90%; SE, standard error; SS, susceptible biotype grown in sterile soil; SU, susceptible biotype grown in unsterile soil; RS, resistant biotype grown in sterile soil; RU, resistant biotype grown in unsterile soil; TS, tolerant biotype grown in sterile soil; TU, tolerant biotype grown in unsterile soil.

[b] Values of significance within each weed species are in bold at $\alpha = 0.05$.

while losing the lower roots to glyphosate damage, which possibly contributed to plant survival. Root survival of the SS may have contributed to the lack of reduction in dry weight, allowing the SS to respond to glyphosate similar to both the TS and TU.

When comparing the response of giant ragweed, horseweed, and common lambsquarters biotypes to glyphosate when grown in sterile and unsterile soils, we find that the three weed species responded very differently. In previous research, inconsistency in glyphosate efficacy between weed species has been attributed to various environmental conditions and plant parameters at the time of glyphosate application. Plant height and growth stage at the time of glyphosate application was shown to affect the susceptibility and tolerance of common lambsquarters (Schuster et al. 2007; Sivesind et al. 2011). Differences in soil moisture affected glyphosate absorption and translocation (Moosavi-Nia and Dore 1979; Waldecker and Wyse 1985), and temperature and relative humidity influenced uptake and translocation of glyphosate (Sharma and Singh 2001). Throughout this study, greenhouse environmental conditions, daily watering, weekly fertilizing, plant height, and growth stage at the time of glyphosate application were all

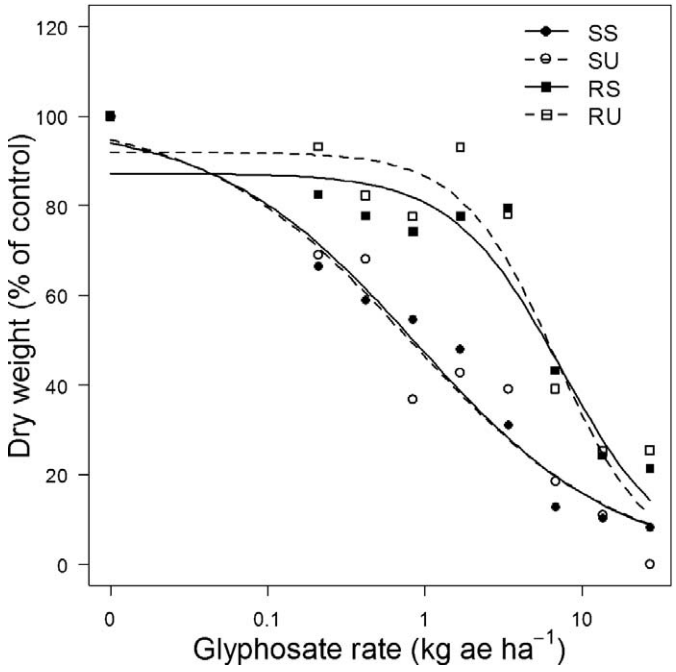

Figure 5. Response (14 d after glyphosate treatment [DAT]) of glyphosate-resistant and glyphosate-susceptible biotypes of horseweed grown in sterile or unsterile field soil. Model fit P = 0.9458 using Equation 1. SS, susceptible biotype grown in sterile soil; SU, susceptible biotype grown in unsterile soil; RS, resistant biotype grown in sterile soil; RU, resistant biotype grown in unsterile soil.

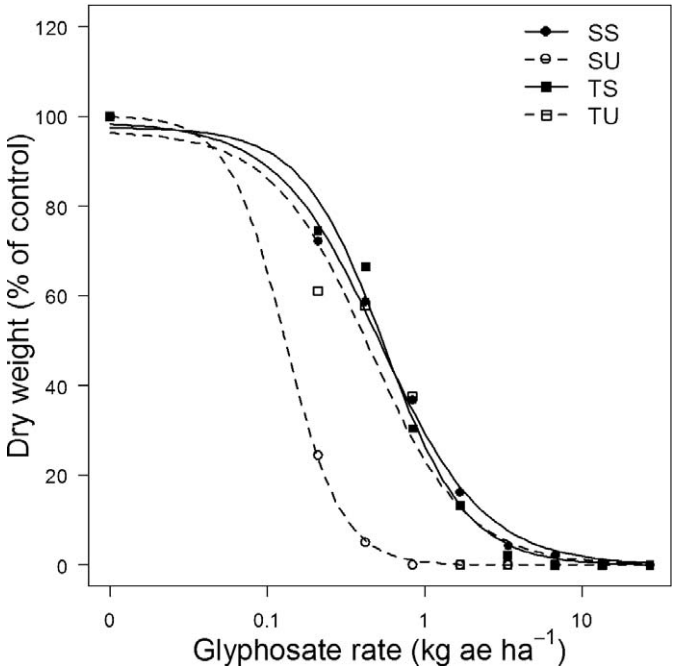

Figure 6. Response (21 d after glyphosate treatment [DAT]) of glyphosate-tolerant and glyphosate-susceptible biotypes of common lambsquarters grown in sterile or unsterile field soil. Model fit 0.9862 using Equation 1. SS, susceptible biotype grown in sterile soil; SU, susceptible biotype grown in unsterile soil; TS, tolerant biotype grown in sterile soil; TU, tolerant biotype grown in unsterile soil.

monitored and kept constant across experiments. The soil in which the plants were grown, specifically the presence or absence of soil microorganisms, appeared to play a vital role in glyphosate efficacy on both biotypes of giant ragweed and susceptible biotype of common lambsquarters.

Survival of plants treated with glyphosate when grown in sterile soil was first demonstrated by Johal and Rahe (1984) in the survival of bean seedlings, and later described by Rahe et al. (1990) as a synergistic relationship between soil-borne fungi and glyphosate. Control of bean seedlings with low rates of glyphosate required synergistic fungi; however, without synergistic fungi, glyphosate could still kill the plants with a higher dose. Smith and Hallett (2006) also demonstrated that the lethal rate of glyphosate on common waterhemp (*Amaranthus rudis* Sauer) was reduced by half when conidia spores of the fungal plant pathogen *Microsphaeropsis amaranthi* was applied 1 to 3 DAT. The glyphosate application predisposed common waterhemp to fungal infection, therefore increasing weed control by glyphosate at low rates. Glyphosate was shown to predispose plants to *Pythium* sp. infection by causing a decrease in lignin defense mechanisms in bean plants (Liu et al. 1995, 1997). In non–glyphosate-treated bean plants lignin content in root exudates increased in response to *Pythium* sp. inoculation, when glyphosate was applied 2 d before exposure to *Pythium* sp. lignin was not produced by the roots, therefore allowing *Pythium* sp. root colonization to be greater in the glyphosate-treated plants. A sublethal dose of glyphosate also suppressed phytoalexin synthesis 12 h after application in sicklepod [*Senna obtusifolia* (L.) H. S. Irwin and Barneby], rendering the plant susceptible to the fungal pathogen *Alternaria cassia* (Sharon et al. 1992). Increased glyphosate efficacy for specific weed species when grown in unsterile soil compared to sterile soil may be due to soil microbial root infection aiding in the herbicidal activity of glyphosate.

In conclusion, based on the data and observations on the specific soil type and biotypes used in this study, soil media used in dose–response screenings to identify susceptible and resistant weeds is very important. Unsterile field soil should be used when conducting dose–response screenings for glyphosate resistance. Continuing research will investigate if giant ragweed and common lambsquarters have a unique relationship with soil microbes, and how these microbes have a synergistic role with glyphosate.

Plant rhizosphere relationships with soil microbes are extremely complex and differ between plant species. This may explain variations in glyphosate efficacy between weed species and biotypes within a species. We hypothesize that the complex dynamics of rhizosphere relationships may have greater importance to the weed–glyphosate interaction than previously acknowledged. Reductions in the response of weed species to glyphosate, the evolution of resistance, may be subject to evolution in soil microbes. Changes in microbial root infection or elevated levels of tolerance to soil microbes may play a role in resistance to glyphosate. Understanding the relationship between soil microbes and the herbicidal activity of glyphosate may provide insight to the evolution of resistance to glyphosate in weed species.

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