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Microbial Degradation of Fluometuron Is Influenced by Roundup WeatherMAX

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Laboratory experiments were conducted to describe the influence of glyphosate and fluometuron on soil microbial activity and to determine the effect of glyphosate on fluometuron degradation in soil and by *Rhizoctonia solani*. Soil and liquid medium were amended with formulated fluometuron alone or with two rates of formulated glyphosate. The soil carbon mineralization was measured hourly for 33 days. The fluometuron remaining in the soil was quantified following 3, 6, 10, 15, 20, 30, and 40 days of incubation. The fluometuron remaining in medium and fungal biomass was measured after 1, 3, 6, 10, 15, and 20 days of incubation. The addition of glyphosate with fluometuron increased C-mineralization and increased the rate of fluometuron degradation relative to fluometuron applied alone. However, more fluometuron remained in the media and less fungal biomass was produced when glyphosate was included.

KEYWORDS: Cotton; degradation; glyphosate; *Gossypium hirsutum*; microbial activity; fluometuron; *Rhizoctonia solani*

INTRODUCTION

The introduction of glyphosate-tolerant crops in 1996 (*1*) has substantially impacted herbicide use (*2*). The popularity of glyphosate-tolerant varieties has been driven by broad-spectrum weed control (*1*), low herbicide cost (*3*), ease of application (*1*), and low environmental and toxicological risk of the herbicide (*4*). However, glyphosate-based weed management systems have resulted in extensive use of the herbicide. In 2006, 91% of soybean [*Glycine max* (L.) Merr], 74% of cotton (*Gossypium hirsutum* L.), and 33% of corn (*Zea maize* L.) planted in the United States were glyphosate-tolerant varieties (*5, 6*).

Throughout the growing season, glyphosate-tolerant cotton is sprayed with multiple postemergence applications of glyphosate and is also treated with additional pesticides that may include the herbicide fluometuron. Fluometuron is used both pre- and postemergence to control grass and broadleaf weeds in cotton. The potential for injury to subsequent crops (*7*) or groundwater contamination (*8*) caused by excessive residues or weed control failure due to excessive degradation makes knowledge of fluometuron soil persistence necessary. Glyphosate may also arrive in the soil by exudation from the roots of treated plants (*9*).

Fluometuron in soil is dissipated by cometabolic microbial degradation, creating the primary metabolites desmethyl-fluometuron and trifluoromethylphenylurea (*10*). The fungus *Rhizoctonia solani* is capable of cometabolizing fluometuron; however, complete degradation of the herbicide was not observed in pure culture (*11*). When soil was amended with glucose and yeast extract, the degradation of fluometuron was enhanced (*10*).

It is unknown if the addition of other pesticides, such as glyphosate, affects the degradation of fluometuron either in soil or in pure culture. Previously published research suggests that the response of soil microbial communities to pesticides is altered by the presence of glyphosate in the soil (*12*−*14*). In addition, simultaneous application of glyphosate and atrazine resulted in enhanced microbial activity (*15*) and varied atrazine degradation (*16*). The objectives of this research were to describe (i) the influence of glyphosate and fluometuron on microbial activity and fluometuron degradation in bulk soil and (ii) the effect of glyphosate on fluometuron degradation and growth of *R. solani* in liquid culture.

MATERIALS AND METHODS

Soil. The soil used in this study was a Weswood silty clay loam (fine-silty, mixed superactive, thermic Udifluventic Haplustept) with pH 8.0 (measured in water solution) with 32% clay and 1.4% organic

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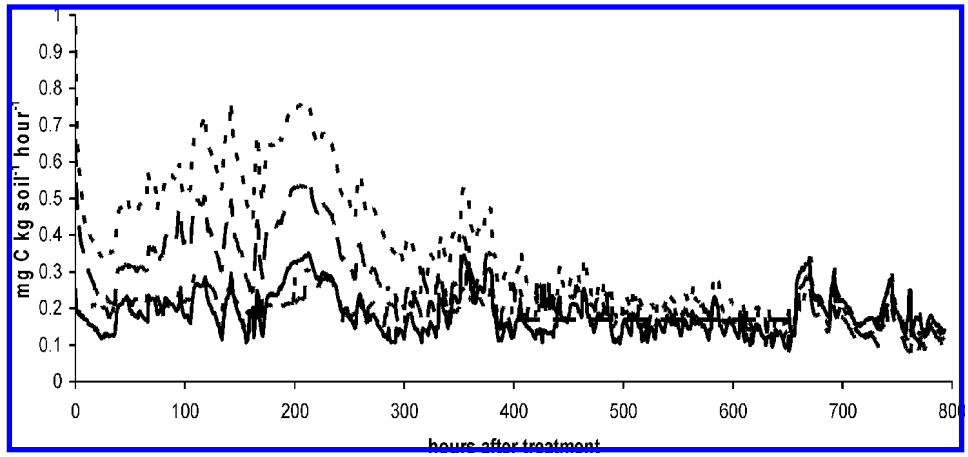

Figure 1. Carbon mineralized for 793 h (33 days) following the addition of 2.25 kg ai/ha fluometuron (—), 2.25 kg fluometuron + 1.25 kg ae/ha glyphosate (— — —), 2.25 kg fluometuron + 2.5 kg ae/ha glyphosate (- - -), or no herbicide (— - —). Data are means of four replicates.

matter. Bulk soil was collected from the upper 5 cm of a fallow field that was previously planted to cotton. Soil was air-dried, passed through a 2 mm sieve, and stored at ambient temperature prior to the beginning of the experiment. No fluometuron was detected in this soil in previous experiments (*12*).

Herbicides. Commercial formulations of glyphosate (Roundup WeatherMAX, Monsanto Co., St. Louis, MO) and fluometuron (Cotoran 4 L, Griffin L.L.C., Valdosta, GA) were used in all experiments. Pesticides will be referred to by their common names throughout this paper. Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (3.5 μg ai g $soil^{-1}$ or 11.7 μg mL $medium^{-1}$), and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (49.7 and 99.5 μg ae g $soil^{-1}$ or 146 and 292 μg mL $medium^{-1}$ representing a 1× and 2× rate, respectively). Herbicide rates were based on recommended application rates and adjusted by an effective interaction depth of 2 mm for glyphosate (*13*) or 50 mm for fluometuron (*17*). The effective soil interaction depth represents the depth in the soil profile to which the herbicide is expected to be found following a field application. Adjusting the herbicide rate by this depth results in a more realistic concentration of the herbicide in soil.

Soil Microbial Respiration. Thirty grams of dry weight equivalent soil was placed into 50 mL plastic beakers with holes for drainage and water absorption that were covered with filter paper. Fifteen milliliters of deionized H_2O was added to gastight chambers, and samples were placed in the chambers. Soil was incubated at 23 ± 0.62 °C to allow stabilization of the soil microbial biomass following the initial flush of activity after rewetting (*18*). After 10 days, soils were fortified with 1.5 mL of herbicide solution described previously, chambers were flushed with N_2 to remove CO_2, and ambient air was reintroduced. The carbon dioxide concentration was determined every hour for 33 days using an infrared gas analyzer (ADC 225MK3, BioScientific Ltd., Great Amwell, England).

Fluometuron Degradation in soil. Ten grams of dry weight equivalent soil was placed into 50 mL plastic beakers, and 2.5 mL of deionized H_2O was added (60% water-filled pore space). The soil was incubated at 25 ± 2 °C with a container of 10 mL of 1 M KOH for 5 days in 1 L gastight containers to allow stabilization of the soil microbial biomass (*18*). Potassium hydroxide traps were then removed, and soil samples were fortified with 0.5 mL of herbicide solution described previously. Samples were returned to gastight containers with 15 mL of fresh KOH solution, which were replaced at 10 day intervals during the experiment to prevent excessive accumulation of CO_2 in the containers. Fluometuron was extracted from soil samples after 1, 3, 6, 10, 15, 20, 30, or 40 days of incubation at 25 ± 2 °C.

Influence of Glyphosate on Fluometuron Disappearance and *R. solani* Growth. *R. solani* (anastomosis group AG4) collected in Brazos County, TX, was grown in a liquid medium containing 20 g glucose, 1 g NH_4NO_3, 0.9 g K_2HPO_4, 0.2 g KCl, 0.2 g $MgSO_4{-}7H_2O$, 0.002 g $FeSO_4{-}7H_2O$, 0.002 $ZnSO_4{-}H_2O$, and 0.002 g $MnCl_2$ per L (*19*). One hundred milliliters of this medium was supplemented with fluometuron alone or in combination with glyphosate at the concentrations described previously. Herbicide concentrations represented the concentration of herbicide in the amount of Weswood silty clay loam needed to hold 100 mL of solution at 33% gravimetric water content. Control treatments of (i) noninoculated media, (ii) nonsupplemented media, and (iii) noninoculated, nonsupplemented media were included.

Cultures were maintained at 37 °C on an orbital shaker (G24, New Brunswick Scientific, Edison, NJ) for 1, 3, 6, 10, 15, or 20 days. Following incubation, cultures were dried, accumulated biomass was quantified, and the fluometuron concentration in the biomass was determined. Prior to desiccation, a 1 mL aliquot of the liquid media was removed to determine the fluometuron content of the media.

Fluometuron Extraction and Analysis. Fluometuron was extracted from soil and fungal biomass samples using accelerated solvent extraction (ASE) methodology (*20*). For soil samples, 1 g of diatomaceous earth (Hydromatrix, Varian, Palo Alto, CA) was thoroughly mixed with each 10 g soil sample volumetric water holding capacity immediately prior to extraction, and samples were transferred to ASE cells assembled with a filter at the bottom. Samples were extracted by the ASE with methanol at 50 °C during three 5 min static cycles. The dessicated fungal biomass was extracted similarly, except that no Hydromatrix was added and only two static cycles were used. Concentrations of fluometuron were quantified as previously published (*20*) using high-performance liquid chromatography−photodiode array detection and a 3.5 μm C8 2.1 mm × 150 mm column (Waters Inc., Milford, MA). The mobile phase was 50:50 acetonitrile:water, the injection volume was 10 μL, and the flow rate was 0.2 mL min^{-1}. Samples were analyzed at 243 nm.

Data Analysis. In all experiments, treatments were arranged in a randomized complete block design. Treatments were repeated in time with four replications in the respiration and soil degradation experiments and three replications in the *R. solani* experiment. All data were analyzed using Statistical Analysis Systems v. 9.1 (SAS Institute, Inc., Cary, NC). Mixed models (*21*) were used to determine and separate treatment means and slope parameters by pairwise comparisons ($\alpha = 0.05$). Time was considered a repeated factor in the mineralization experiments (*21*).

RESULTS AND DISCUSSION

Soil Microbial Respiration. Fluometuron alone had little effect on hourly C mineralization. Carbon mineralization was generally increased when fluometuron was added with glyphosate at the 1× rate, and an additional increase was observed when fluometuron was applied with the 2× rate of glyphosate (**Figure 1**). Maximum hourly mineralization for all treatments occurred approximately 210 h (9 days) after herbicide addition. However, there were considerable fluctuations in the rate of CO_2 production during the first 400 h (16 days) of the experiment. This fluctuation has been observed in other experiments and is likely due to inherent cycles of the microbial community;

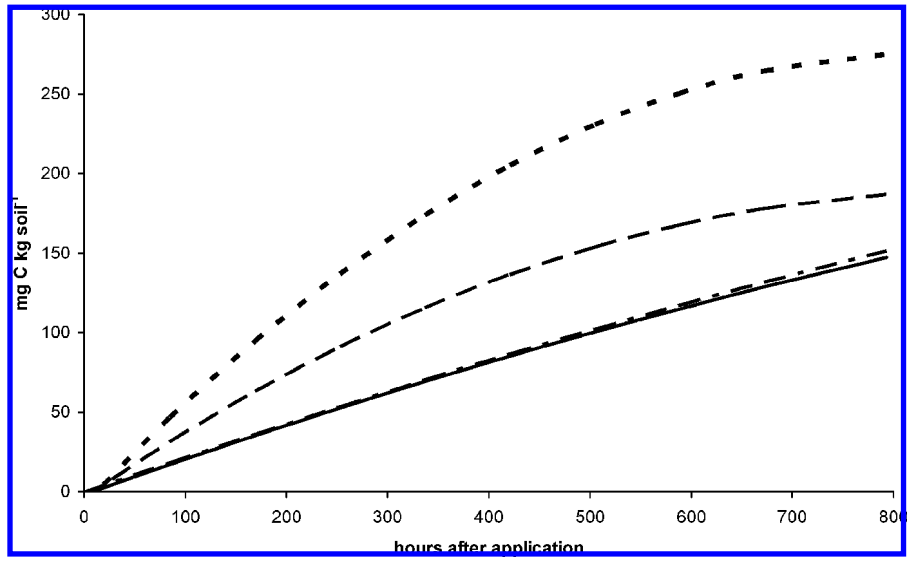

Figure 2. Fitted equations representing cumulative C mineralization 793 h (33 days) following the addition of 2.25 kg ai/ha fluometuron (—), 2.25 kg fluometuron + 1.25 kg ae/ha glyphosate (— — —), 2.25 kg fluometuron + 2.5 kg ae/ha glyphosate (- - -), or no herbicide (— - —). Equation parameters are listed in **Table 2**.

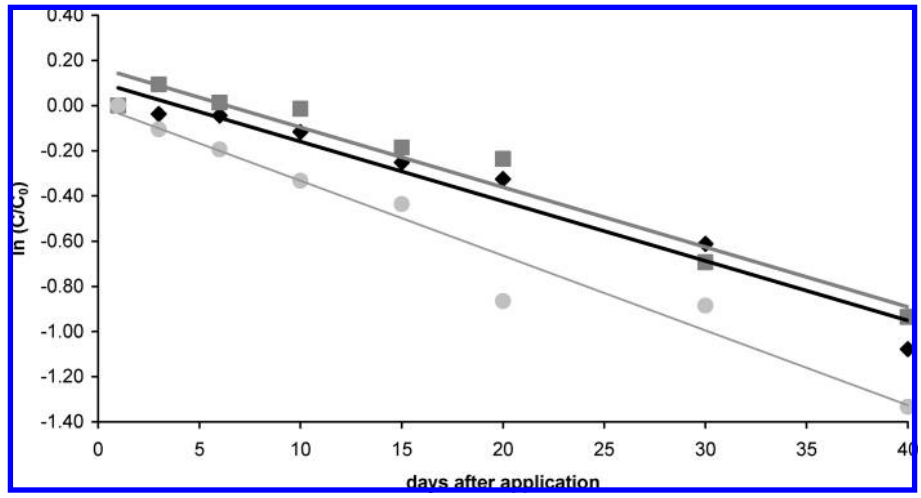

Figure 3. First-order rate plots for degradation of fluometuron applied alone (2.25 kg ai/ha; black diamond) and with 1.25 kg ae/ha glyphosate (gray square) or 2.5 kg ae/ha glyphosate (gray circle). Fitted equations are as follows: $y = -0.03x + 0.13$, fluometuron alone; $y = -0.03x + 0.17$, fluometuron + 1× rate of glyphosate; and $y = -0.03x - 0.001$, 2× rate of glyphosate.

however, the cause has not yet been elucidated (R. Haney, personal communication). The frequent sampling in this experiment allowed these observations, which are in contrast to some previous reports of C mineralization following glyphosate application, which indicate a single maxima in daily respiration 2 days after application (*13, 14*). By approximately 450 h (19 days), C mineralization in all treatments had returned to approximately basal levels of respiration, indicating that the herbicides applied were no longer influencing soil microbial activity. Haney et al. (*13*) reported that C mineralization following the application of glyphosate alone returned to background levels after 14 days.

Rates of C mineralization were analyzed by comparing the linear and quadratic functions of the lines representing CO_2 accumulated during the experiment (**Table 1**). Trends observed in cumulative C mineralization were similar to those in hourly mineralization. Soil treated with fluometuron and 2.5 kg ha^{-1} glyphosate produced the greatest amount of CO_2 over the course of the experiment (**Figure 2**). When fluometuron was applied with 1.25 kg ha^{-1} glyphosate, the amount of CO_2 produced was less than in soil treated with fluometuron and 2.5 kg ha^{-1} glyphosate but greater than in soil treated with only fluometuron. The total amount of C-mineralized in soil treated with fluometuron was similar to the amount of C-mineralized in untreated soil.

The increase in total C mineralized from each treatment was approximately equal to the relative amounts of glyphosate added in each treatment (**Table 2**). This indicates that the addition of glyphosate is directly related to increased C mineralization due to either degradation of the herbicide (*13*) or a priming effect of glyphosate (*22*).

Fluometuron Degradation in Soil. No fluometuron residues were detected in untreated soil samples (data not shown). The concentration of fluometuron remaining in the soil was similar among all treatments 1, 3, 6, and 30 days after application (**Table 3**). Ten, 15, 20, and 40 days after herbicide application, less fluometuron was present in soil treated with fluometuron plus the 2× rate of glyphosate relative to soil treated with fluometuron alone. Ten, 20, and 40 days after application, soils treated with fluometuron plus the 2× rate of glyphosate also contained less fluometuron than soils treated with fluometuron plus the 1× rate of glyphosate. Forty days after treatment, the

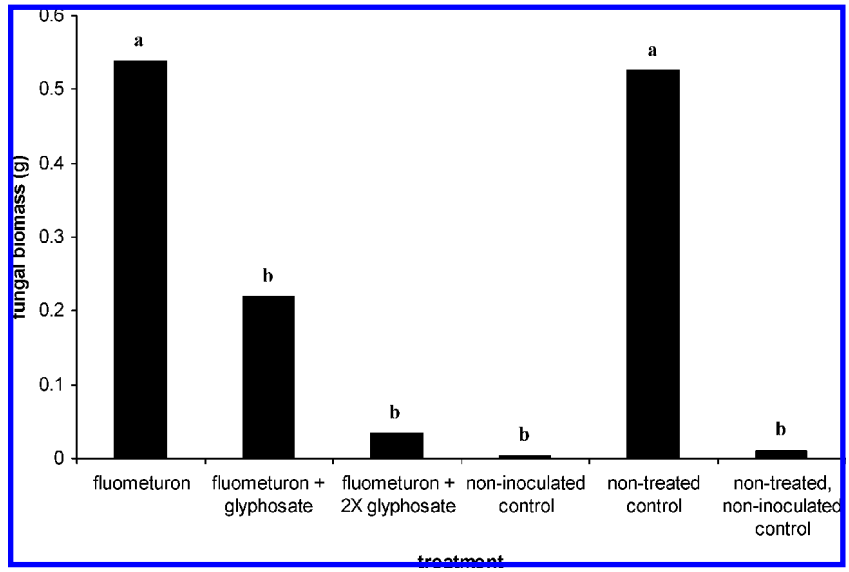

Figure 4. *R. solani* biomass accumulated following 20 days of growth in minimal media containing 20 g glucose, 1 g NH_4NO_3, 0.9 g K_2HPO_4, 0.2 g KCl, 0.2 g $MgSO_4-7H_2O$, 0.002 g $FeSO_4-7H_2O$, 0.002 g $ZnSO_4-H_2O$, and 0.002 g $MnCl_2$ per L amended with 11.7 μg mL^{-1} fluometuron alone and with 146 or 292 μg glyphosate mL^{-1}. Bars labeled with similar letters are similar according to pairwise *t* tests ($\alpha = 0.05$).

Table 1. Slope Parameters of Modeled Cumulative C Mineralization Data[a,b]

treatment[b]	intercept parameter[c]	linear parameter	quadratic parameter
fluometuron	−1.89 a	0.22 a	−0.0001 a
fluometuron + 1× glyphosate	−4.04 b	0.44 b	−0.0003 b
fluometuron + 2× glyphosate	−7.93 c	0.68 c	−0.0004 c
untreated	−1.15 a	0.24 a	−0.0001 a
P value	0.008	<0.0001	<0.0001

[a] Values within a column followed by the same letter are not significantly different at ($P < 0.05$) according to Tukey's multiple pairwise comparisons. [b] Parameters for polynomial equation: $y = \beta_0 + \beta_1 x + \beta_2 x^2$; β_0 = intercept, β_1 = linear, and β_2 = quadratic. [c] Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (3.5 μg ai g $soil^{-1}$), and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (49.7 and 99.5 μg ae g $soil^{-1}$).

Table 2. Comparison of C Added as Glyphosate in Each Treatment and C Mineralized from Each Treatment

	C-added as glyphosate		C-mineralized	
treatment[a]	mg C kg $soil^{-1}$	ratio	mg C kg $soil^{-1}$	ratio
fluometuron	0	0	147.4	1
fluometuron + 1× glyphosate	10.6	1	193.2	1.3
fluometuron + 2× glyphosate	21.2	2	284.4	1.9
untreated	0	0	151.5	1

[a] Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (3.5 μg ai g $soil^{-1}$), and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (49.7 and 99.5 μg ae g $soil^{-1}$).

concentration of fluometuron remaining was 32% applied in soils treated with fluometuron alone or with 1× glyphosate and 24% in soil treated with fluometuron plus 2.5 kg ha^{-1} glyphosate.

Soil amendments commonly enhance pesticide degradation, often as a result of biostimulation. Wagner and Zablotowicz (*23*) reported that biostimulation resulted in enhanced fluometuron degradation when soils were amended with poultry litter, cornmeal, or ryegrass. In addition, Bozarth and Funderburk (*10*) reported that fluometuron degradation was more rapid in soils amended with glucose than in nonamended soils. However, the influence of amendments such as glucose or glyphosate on pesticide degradation is variable and is influenced by the nutrient status of a soil, particularly the C/N ratio of soil organic matter (*22*).

First-order kinetics were used to describe fluometuron degradation in this experiment, as well as others (*8, 24*−*26*). Linear regression of the natural log of concentration remaining/initial concentration and days after application are presented in **Figure 3**. The calculated degradation rate constant, half-life, and coefficient of determination for each line are presented in **Table 4**. The half-life calculated for fluometuron alone was 28.6 days. This is greater than the half-life of 18 days reported by Mueller et al. (*8*) and substantially shorter than the half-life of 49 days reported by Brown et al. (*24*) but within the range of half-lives reported for fluometuron in eight soils by Willian et al. (*25*). Differences in these reported half-lives may be due to differences in organic matter content, pH, microbial biomass, or microbial activity of the soil used in each experiment (*8, 25, 26*).

When fluometuron was applied with a 1× rate of glyphosate (**Table 4**), the rate of fluometuron degradation was similar to fluometuron applied alone. However, the half-life of fluometuron was shorter in soils that were treated with fluometuron plus the 2× rate of glyphosate relative to fluometuron applied alone. Soils treated with the 2× rate of glyphosate also exhibited greater respiration during the first 15 days of the experiment. Respiration was increased 4−15 days after treatment with glyphosate, while accelerated fluometuron degradation was observed later.

Glyphosate application has been associated with increased microbial activity (*13, 14*) as well as numerically greater microbial populations (*27*). Fluometuron degradation has been positively correlated with microbial respiration, as well as soil microbial biomass in other studies (*8, 26*). Therefore, it is possible that the enhanced fluometuron degradation observed when glyphosate was applied with fluometuron and is related to a glyphosate-induced increase in cometabolism of fluometuron.

Influence of Glyphosate on Fluometuron Disappearance and *R. solani* Growth. There were no differences among herbicide treatments in the fluometuron concentration of *R. solani* biomass at any extraction time (data not shown; limit of

Table 3. Fluometuron Concentration in Soil[a]

	μg fluometuron g soil^{-1}							
treatment[b]	1 day	3 days	6 days	10 days	15 days	20 days	30 days	40 days
fluometuron	3.2	3.1	3.0	2.8 a	2.5 a	2.3 a	1.7	1.1 a
fluometuron + 1× glyphosate	2.9	3.1	2.9	2.8 a	2.4 ab	2.2 a	1.4	1.1 a
fluometuron + 2× glyphosate	3.2	2.9	2.6	2.3 b	2.1 b	1.4 b	1.3	0.84 b
Pr > F	NS	NS	NS	0.0169	0.0206	0.0019	NS	<0.0001

[a] Values within a column followed by the same letter are not significantly different at ($P < 0.05$) according to Tukey's multiple pairwise comparisons. [b] Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (3.5 μg ai g soil^{-1}), and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (49.7 and 99.5 μg ae g soil^{-1}).

Table 4. First-Order Rate Constant (k), Half-Life ($t_{1/2}$), and Coefficient of Determination (R^2) of Fluometuron in Soils Treated with Fluometuron Alone or with Glyphosate[a]

treatment[b]	k (days^{-1})	$t_{1/2}$ (days)	R^2
fluometuron	0.025 a	28.6 a	0.81
fluometuron + 1× glyphosate	0.026 a	26.9 ab	0.71
fluometuron + 2× glyphosate	0.033 b	21.2 b	0.92

[a] Values within a column followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's multiple pairwise comparisons. [b] Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (3.5 μg ai g soil^{-1}) and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (49.7 and 99.5 μg ae g soil^{-1}).

Table 5. Percent Fluometuron Remaining in Minimal Medium Inoculated with *R. solani* Following Incubation[a]

treatment[b]	1 day	3 days	6 days	10 days	15 days	20 days
fluometuron	115.0	82.2	90.8	76.7 a	65.0	0.0 a
fluometuron + 1× glyphosate	100.5	95.7	101.8	99.3 b	99.2	94.5 b
fluometuron + 2× glyphosate	109.2	100.0	99.6	97.3 b	96.0	92.1 b
noninoculated control	101.8	100.0	97.6	102.8 b	99.8	91.1 b
Pr > F	NS	NS	NS	0.0159	NS	0.0009

[a] Values within a column followed by the same letter are not significantly different at ($P < 0.05$) according to Tukey's multiple pairwise comparisons. [b] Fluometuron was applied at a concentration of 2.25 kg ai ha^{-1} (11.7 μg mL medium^{-1}), and glyphosate was applied at concentrations of 1.25 and 2.5 kg ae ha^{-1} (146 and 292 μg mL medium^{-1}).

quantitation 0.1 ppm). However, after 10 and 20 days, the concentration of fluometuron detected in the medium was greater when glyphosate was included at either the 1× or 2× rate than when fluometuron was applied alone (**Table 5**). After 20 days, no fluometuron was detected in the medium that did not contain glyphosate. It is important to note that lack of detection does not equal complete degradation of fluometuron, rather, the inability to detect the parent molecule. When glyphosate was included in the media, the amount of fluometuron remaining was 63 and 85% of the amount detected the first day after the herbicides were added in the low concentration and high concentration glyphosate treatments, respectively.

Treatments that included glyphosate also suppressed the growth of *R. solani* biomass (**Figure 4**). After 20 days, medium containing fluometuron alone supported fungal growth similar to media containing no herbicides. Fungal growth in media containing fluometuron and either rate of glyphosate was not statistically greater than the noninoculated samples. Feng et al. (*28*) reported that glyphosate also suppressed diseases caused by fungi *Puccinia striiformis* f. sp. *tritic* and *Phakopsora pachyrhiz* in wheat (*Triticum aestivum* L.) and soybean, respectively.

The reduction in fungal biomass may be related to the presence of proprietary adjuvants in the glyphosate formulation. Lee et al. (*29*) reported reduced growth of *Sclerotinia sclerotiorum* mycelia in the presence of a Roundup formulation blank. In addition, this finding is supported by the fact that *R. solani* grows poorly in the presence of the amino acids alanine and leucine (*30*). Glyphosate is an amino acid analogue with a chemical structure very similar to the amino acid glycine (*4*).

In conclusion, even though the simultaneous addition of glyphosate enhanced the degradation of fluometuron in soil, growth of *R. solani*, an organism known to cometabolize fluometuron, was reduced when grown in media containing glyphosate. This is supportive of other research, which indicates that glyphosate application does not increase the occurrence of soybean diseases caused by *R. solani* (*31*). However, increased growth of other pathogens in response to glyphosate has been reported (*9, 32*).

The different responses noted for microbial degradation of fluometuron when applied with glyphosate to soil and in pure culture demonstrate the complex nature of microbial interactions with regard to pesticide degradation. This complexity is enhanced when pesticide degradation is considered in multipesticide systems, which are more representative of agricultural practices. Additional research is needed to further elucidate the effects of glyphosate-based weed management programs on soil microorganisms.

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