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ORIGINAL PAPER

Glufosinate and ammonium sulfate inhibit atrazine degradation in adapted soils

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Abstract The co-application of glufosinate with nitrogen fertilizers may alter atrazine cometabolism, thereby extending the herbicide's residual weed control in adapted soils. The objective of this study was to assess the effects of glufosinate, ammonium sulfate, and the combination of glufosinate and ammonium sulfate on atrazine mineralization in a Dundee silt loam exhibiting enhanced atrazine degradation. Application of glufosinate at rates of 10 to 40 mg kg^{-1} soil extended the lag phase 1 to 2 days and reduced the maximum degradation rate by 15% to 30%. However, cumulative atrazine mineralization averaged 85% 21 days after treatment and was independent of treatment. Maximum daily rates of atrazine mineralization were reduced from 41% to 55% by application of 1 to 8 g kg^{-1} of ammonium sulfate. Similarly, cumulative atrazine mineralization was inversely correlated with ammonium sulfate rates ranging from 1.0 to 8 g kg^{-1} soil. Under the conditions of this laboratory study, atrazine degradation was relatively insensitive to exogenous mineral nitrogen, in that 8 g $(NH_4)_2SO_4$ per kilogram soil repressed but did not completely inhibit atrazine mineralization. Moreover, an additive effect on reducing atrazine mineralization was observed when glufosinate was co-applied with ammonium sulfate. In addition, ammonium fertilization alters the partitioning of ^{14}C-atrazine metabolite accumulation and nonextractable residues, indicating that ammonium represses cleavage of the triazine ring. Consequently, results indicate that the co-application of glufosinate with N may increase atrazine persistence under field conditions thereby extending atrazine residual weed control in adapted soils.

Keywords Accelerated degradation · Atrazine · Herbicide interaction · Nitrogen metabolism

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Introduction

Atrazine [6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine] is a soil-applied, triazine herbicide that provides residual weed control in corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), sugarcane (*Saccharum officinarum* L.), and turf. With application rates ranging from 29 to 34 million kg $year^{-1}$, atrazine is the second most frequently applied pesticide in the USA (USEPA 2003). Widespread use of atrazine, even in herbicide-resistant cropping systems, illustrates the compound's importance to US agriculture. Consequently, factors that limit atrazine's efficacy need to be evaluated. In addition, atrazine is one of the most commonly observed herbicides found in groundwater (Koplin et al. 1996) and surface water (Lerch et al. 1998; Zablotowicz et al. 2006a, b) and thus is an environmental concern as a potential pollutant.

Enhanced degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of microorganisms that has developed the ability to use the pesticide as a carbon, energy, and (or) nutrient source because of previous exposure to the pesticide or an analogue. Enhanced atrazine degradation has been demonstrated in agricultural soils from Europe, and North and South America (Barriuso and Houot 1996; Hang et al. 2003; Houot et al. 2000; Shaner et al. 2007; Zablotowicz et al. 2006a). Moreover, the efficacy of atrazine and other *s*-triazines in agricultural soils exhibiting enhanced degrada-

tion is reduced (Krutz et al. 2007, 2008a, b). Thus, a means to extend atrazine's residual activity in adapted soils is required.

Prior research (Krutz et al. 2003) has indicated that co-application of the herbicide glyphosate [*N*-(phosphonomethyl)glycine] with atrazine can delay atrazine degradation. Genetically modified maize hybrids have been developed with genes conferring resistance to glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] for control of a broad spectrum of weeds in maize (Zuver et al. 2007). Glufosinate has some similarity to glyphosate as both herbicides contain a C–P bond and can give rise to an amino acid upon degradation (Hoagland and Zablotowicz 2001). Glufosinate inhibits ammonium assimilation in plants and microorganisms (Logusch et al. 1990), while glyphosate interferes with biosynthesis of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Hoagland and Zablotowicz 2001). The effect of glufosinate on soil microbiological properties has been described in several studies (Fließbach and Mäder 2004; Pampulha et al. 2007). The co-application of glufosinate and (or) nitrogen fertilizer with atrazine may increase the herbicide's persistence in adapted soils. Similarly, reports indicate that nitrogen fertilization can repress atrazine degradation (Abdelhafid et al. 2000; Assaf and Turco 1994; Sims 2006). Thus, the objective of this study was to assess the effects of glufosinate, ammonium sulfate, and the combination of glufosinate and ammonium sulfate on the persistence of atrazine in a Dundee silt loam exhibiting enhanced atrazine degradation.

Materials and methods

Soil

The Dundee silt loam soil used in this study was collected from plots that were under continuous corn production and had been treated with 2 to 3 kg of atrazine per hectare for six consecutive years (Reddy et al. 2006; Zablotowicz et al. 2007). The soil was collected from the surface 0 to 5 cm in early September, passed through a 2-mm sieve to remove coarser material, and stored at 5°C until used for experimentation. Chemical and physical properties of the soil were determined according to Zablotowicz et al. (2007). This soil is slightly acidic, pH 6.2, with a textural composition of 331, 418, and 252 g kg^{-1} of sand, silt, and clay, respectively. The total carbon and nitrogen content was 13.1 and 1.4 g kg^{-1}, respectively, and water extractable ammonium and nitrate nitrogen were less than 10 mg kg^{-1}. A radiological most-probable number assay characterized populations capable of mineralizing the atrazine ring (Zablotowicz et al. 2006a, b) with approximately 10^5 atrazine-mineralizing bacteria per gram of soil. Based on polymerase chain reaction amplification, both atrazine amidohydrolases, *trzN* and *atzA*, are associated with the atrazine-degrading bacteria from this site (Krutz et al. 2008a, b).

Effect of glufosinate concentration and ammonium sulfate on atrazine mineralization/degradation

Soil (25.0 g dry weight equivalents) was placed in biometer flasks (Bartha and Pramer 1965) and treated with a 1-ml aqueous atrazine stock solution containing a mixture of technical grade atrazine (98% chemical purity, Chem Service, Lancaster, PA) and uniformly ^{14}C-ring-labeled atrazine (9.3 mCi mmol, radiological purity ≥95%, Sigma Chemicals, St. Louis, MO, USA) to attain a final concentration of 2.5 mg kg^{-1} and 150 bq g^{-1} of ^{14}C-atrazine. Subsequently, soils were treated with ammonium glufosinate (98% chemical purity, Chem Service) solution to achieve 10, 20, or 40 mg kg^{-1}.(0.83, 1.66, and 3.22 kg ha^{-1}, respectively). The controls received only distilled water, and the final moisture content was 28% (field capacity 0.33 kPa=25% moisture). Biometer flasks were incubated at 28°C in the dark. At 0, 5, and 21 days after treatment, the soil was extracted twice with 80% methanol, and extracts were recovered by centrifugation (8,000×*g* for 10 min). The first methanol extracts were concentrated to one third initial volume for high-pressure liquid chromatography (HPLC) analysis of ^{14}C-atrazine and metabolites using radiological detection HPLC (RAD-HPLC).

RAD-HPLC method

^{14}C-analytes were quantified on a Waters 2695 HPLC separation module (Waters, Milford, MA) equipped with a 4.6×150 mm C_{18} SunFire™ column (Waters) and an in-line 1,000 µl liquid flow cell detector (β-ram, IN/US, Tampa, FL, USA). The injection volume was 100 µl, and the flow rate was 0.75 ml min^{-1}. Two mobile phases were used in a gradient program. After 1 min, the initial mobile phase consisting of acetonitrile/water (10:90 *v*/*v*) was changed during the next 35 min to the final mobile phase consisting of acetonitrile/water (90:10 *v*/*v*). In-Flow™, 2:1 was used as scintillation fluid at a flow rate of 1.5 ml min^{-1}. Using this methodology, the retention time for atrazine was 26 min, hydroxyatrazine 12.4 min, and cyanuric acid was associated with the solvent peak (2.4 min).

Effect of glufosinate on respiration and fluorescein diacetate hydrolysis

To assess the effects of glufosinate on soil respiration, soil (5 g oven-dry equivalent) was added to 100 mL serum

bottles. Bottles received either 200 μl of water or glufosinate to attain the three concentrations of glufosinate (10, 20 or and 40 mg kg^{-1}) with each treatment replicated with four bottles. Soil moisture was adjusted to 28%, the bottles sealed with a rubber septum stopper and incubated at 28°C. The air in the bottles was sampled after 24, 48, 96, and 168 h and carbon dioxide concentration analyzed using a gas chromatograph equipped with a thermal conductivity detector as already described by Zablotowicz and Reddy (2007). Fluorescein diacetate (FDA) hydrolysis was determined according to Zablotowicz et al. (2000). Soil (2 g oven-dry weight) was added to 50 ml polypropylene centrifuge tubes, and the tubes received either 80 μl of water or glufosinate to attain the three concentrations of glufosinate (10, 20, and 40 mg kg^{-1}) with each treatment replicated four times. The final moisture content was adjusted to 28% and incubated at 25°C in the dark. Following 24, 48, 96, and 168 h after treatment with glufosinate, FDA assays were conducted on four tubes with one tube used as a no-substrate blank.

Ammonium sulfate effects on atrazine mineralization

The effect of ammonium sulfate on atrazine degradation was initially evaluated at 4 g kg^{-1} as reported for the glufosinate study. A second study evaluated the effects of ammonium sulfate rate (0, 1, 2, 4, and 8 g kg^{-1}) on atrazine mineralization, using the same atrazine concentration, temperature, and moisture as previously described above except the study was conducted for 28 days. Separate soil incubations were also conducted to assess recovery of ammonium nitrogen. Polypropylene centrifuge tubes were filled with soil (10 g) and aqueous atrazine and ammonium sulfate added to attain the four ammonium concentrations described above. At 0, 7, and 14 days after treatment, four tubes were extracted with 20 ml of 0.1 KCl for 30 min and the supernatant recovered following centrifugation (8,000×*g* for 10 min). Concentration of extracted ammonium was determined using a Thermo specific ion electrode and meter (Thermo-Orion, Beverly, MA, USA).

Glufosinate and ammonium sulfate interactions

The fourth study assessed the interaction of glufosinate and ammonium sulfate on atrazine mineralization. Biometer flask assays were established as previously described with four replicates of six treatments: (1) no amendments; (2) glufosinate (40 mg kg^{-1}); (3) 0.25 g kg^{-1} $(NH_4)_2SO_4$; (4) 1.0 g kg^{-1} $(NH_4)_2SO_4$; (5) 0.25 g kg^{-1} $(NH_4)_2SO_4$ + glufosinate (10 mg kg^{-1}); (6) 1.0 g kg^{-1} $(NH_4)_2SO_4$ + glufosinate (10 mg kg^{-1}). The incubation temperature was reduced to 24°C, lower than previous studies to slow atrazine degradation rates (Krutz et al. 2008a, b). Soils were also extracted with 80% methanol at 0, 4, and 21 days after treatment and evaluated for extractable and non-extractable radioactivity. Atrazine and metabolites were quantified using RAD-HPLC on concentrated extracts as previously described.

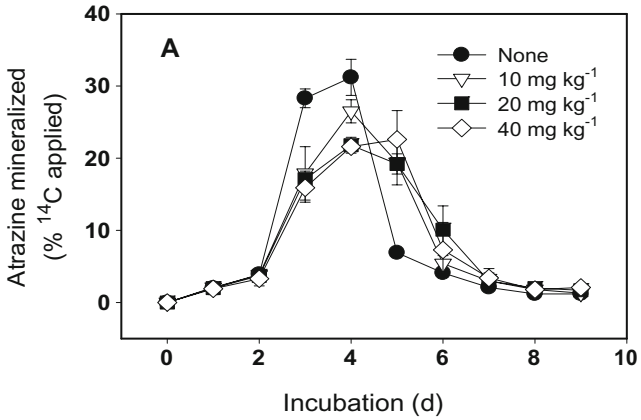

Fig. 1 Daily atrazine mineralization rates as affected by various glufosinate rates (*A*); means and standard deviation of four replicates

Results and discussion

Glufosinate effects on atrazine mineralization/persistence

Glufosinate transiently delayed atrazine mineralization (Pr≥ 95%), with the greatest degree of inhibition at the 20 and 40 mg kg^{-1} glufosinate rate (Fig. 1). Atrazine mineralization was only temporarily inhibited by glufosinate in that a similar final cumulative mineralization was observed after 21 days incubation (Table 1). Fitting the cumulative mineralization data to the Gompertz model indicated that a lower mineralization constant (*k*) and longer lag phase (ti) were observed at 20 and 40 mg kg^{-1} glufosinate, while maximum mineralization (*a*) was similar for all treatments (Table 1).

Table 1 Effect of various rates of glufosinate on cumulative atrazine mineralization at 21 days after treatment and mineralization parameters derived from the Gompertz growth model

Treatment	a^a (%)	*k* (day^{-1})	ti (days)
None	82.6±0.9	1.08±0.08	2.9±0.1
Glufosinate, 10 mg kg^{-1}	83.8±0.7	0.84±0.06	3.2±0.1
Glufosinate, 20 mg kg^{-1}	85.0±0.7	0.73±0.07	3.4±0.1
Glufosinate, 40 mg kg^{-1}	83.8±0.7	0.74±0.07	3.4±0.1

Mean of four replicates, standard deviation in parentheses
[a] Gompertz parameters: *a* the plateau representing maximum percent mineralization; *ti* the abscissa of the inflection point; *k* the mineralization rate constant

Table 2 Recovery of radio-labeled atrazine as affected by glufosinate concentration 5 and 18 days after treatment

Treatment	Extractable	Atrazine	Hydroxy atrazine	Non-extractable	Mineralized
5 days					
None	8.6±0.4	5.0±2.4	3.3±1.8	6.3±0.5	72.3±1.5
Glufosinate (10 mg kg^{-1})	18.9±1.2	16.7±2.3	1.7±1.9	6.1±0.5	69.7±1.5
Glufosinate (20 mg kg^{-1})	27.7±0.7	24.3±2.1	3.3±1.8	6.2±0.9	63.8±3.3
Glufosinate (40 mg kg^{-1})	28.6±1.1	18.2±4.2	8.7±3.0	5.9±0.2	65.6±1.4
Least significant difference (LSD) Pr≤0.05	1.5	4.5	3.1	1.0	3.5
21 days					
None	1.6±0.1	nd	nd	5.4±0.4	85.5±2.2
Glufosinate (10 mg kg^{-1})	2.8±0.5	nd	nd	5.9±0.3	85.7±1.8
Glufosinate (20 mg kg^{-1})	2.5±1.2	nd	nd	6.4±0.4	86.9±4.3
Glufosinate (40 mg kg^{-1})	3.0±0.3	nd	nd	7.3±0.4	85.5±2.7
LSD Pr≤0.05	0.9			0.6	5.1

Mean and standard deviation of four replicates
nd Not determined, below detection limits of HPLC assay

Extraction of soil 5 days after treatment (DAT) recovered from 8.6% to 40.4% of the ^{14}C applied. The extractable radioactivity was inversely related to the amount of ^{14}C-atrazine mineralized to CO_2 (Table 2). In non-treated control soils, only 5% of the atrazine applied was recovered as atrazine, while significantly greater (Pr≥95%) atrazine (17% to 24%) was recovered in glufosinate-treated soil, thereby confirming glufosinate inhibition of atrazine dissipation. A similar amount of ^{14}C was partitioned into non-extractable atrazine residues in non-treated and glufosinate-treated soil, with a greater amount of hydroxyatrazine accumulated in soil treated with the highest glufosinate rate. Minimal differences in the partitioning of the ^{14}C label was found among treatments at 21 days after treatment, with low levels of extractable ^{14}C-atrazine residues in glufosinate and untreated soil (1.6% to 3.0%); however, slightly greater ^{14}C residues were observed in non-extractable fraction in soil treated with the two higher rates of glufosinate.

These data indicate that glufosinate slowed atrazine mineralization and increased atrazine persistence and metabolite accumulation. The transient effects on atrazine degradation can be limited by glufosinate persistence. Glufosinate persistence was not determined in this study; however, other studies indicate that glufosinate degrades fairly rapidly in soil with half lives of 3 to 7 days (Smith 1989; Gallina and Stephenson 1992). Although glufosinate can interfere with ammonium assimilation in vitro, inhibition of specific bacterial populations, e.g., actinomycetes, has been shown in soil studies using viable counts (Pampulha et al. 2007). These studies do indicate that glufosinate can inhibit biotransformation of atrazine.

Effect of glufosinate on soil microbiological activity

Soil respiration was increased by addition of glufosinate with a similar stimulation found at all three glufosinate application rates (Table 3). In contrast, FDA hydrolytic

Table 3 Effect of glufosinate on soil respiration and fluorescein diacetate hydrolytic activity

Treatment	24	48	96	168
Cumulative respiration (nmol CO_2 g^{-1} soil)				
None	83±3	140±5	235±14	681±85
10 mg kg^{-1}	94±5	165±11	282±18	797±36
20 mg kg^{-1}	93±3	161±5	278±7	767±31
40 mg kg^{-1}	97±2	166±11	280±25	776±68
LSD Pr≤0.05	5	12	24	85
Fluorescein diacetate hydrolysis (nmol fluorescein formed g^{-1} soil h^{-1})				
None	257±9	342±28	266±24	232±33
10 mg kg^{-1}	213±12	327±16	257±36	267±61
20 mg kg^{-1}	190±14	326±13	279±47	265±32
40 mg kg^{-1}	156±13	327±12	279±30	251±49
LSD Pr≤0.05	13	23	49	49

Mean and standard deviation of four replicates.

Table 4 Effect of ammonium sulfate 4.0 g kg^{-1} on the recovery of ^{14}C atrazine residues

Treatment	Extractable	Atrazine	Hydroxy atrazine	Non-extractable	Mineralized
% of ^{14}C applied recovered					
5 days					
None	8.6±0.4	5.0±2.6	3.3±1.8	8.3±0.5	73.3±1.5
$(NH_4)_2SO_4$	40.4±3.1	34.1±3.6	4.5±3.8	17.7±0.6	31.4±10.9
LSD Pr≤0.05	5.6	9.4	6.3	1.2	17.7
18 days					
None	1.6±0.1	nd	nd	5.4±0.4	85.5±2.2
$(NH_4)_2SO_4$	7.1±2.9	nd	nd	11.6±0.8	79.4±6.6
LSD Pr≤0.05	4.5			1.9	11.5

Mean of four replicates, means followed by the same letter do not differ at the 95% confidence level.
nd Not determined

activity was only inhibited for 24 h, with the greatest inhibition at highest glufosinate concentrations. Studies by Fließbach and Mader (2004) indicated that spraying potato fields with glufosinate and dinoseb reduced microbial biomass, with a twofold greater reduction from dinoseb compared to glufosinate. Pampulha et al. (2007) found that glufosinate inhibited dehydrogenase activity and inhibited or stimulated specific organisms. The increased respiration in soil treated with glufosinate in this current study is similar to that observed in soils treated with glyphosate (Haney et al. 2002). Both glyphosate and glufosinate can be used by the soil microbial population as carbon substrate or a nitrogen or phosphorous source, and this biostimulation may be a result of increased availability of these nutrients. An additional 1.2 to 4.8 mg kg^{-1} of nitrogen was added by amending with 10 to 40 mg kg^{-1} glufosinate.

Effect of various concentrations of ammonium sulfate on atrazine mineralization

In soil treated with 4 g kg^{-1} $(NH_4)_2SO_4$, atrazine mineralization was reduced by 57% at 5 DAT and did not recover to the level of non-treated soil at 21 DAT (Table 4). Conversely, a greater amount of the ^{14}C applied was recovered as atrazine, and the greatest level of non-extractable ^{14}C residues was observed at 5 DAT (Table 4). In the $(NH_4)_2SO_4$-treated soil, the highest level of extractable and non-extractable residues was found at 5 DAT, indicating a greater degree of *N*-repression of atrazine degradation. HPLC analysis of methanol extracts at 21 DAT was not conducted as radioactivity was too low in most extracts.

All four $(NH_4)_2SO_4$ concentrations delayed atrazine mineralization with inhibition increasing with increased concentrations of $(NH_4)_2SO_4$ (Fig. 2). Atrazine mineralization kinetics was adequately described by the Gompertz growth model ($r^2 \geq 0.99$, Table 5). Based upon analysis of variance, the cumulative atrazine mineralization observed at 28 days after treatment was highest in the non-treated control soil (90%) compared to 83% to 85% for soils receiving ammonium sulfate. However, the growth model predicted a similar end point for atrazine mineralization. Ammonium was fairly persistent in this soil, with approximately 50% applied recovered as ammonium N after a 14-day incubation regardless of initial concentration (Fig. 3). The increased lag (ti) values for the onset of atrazine

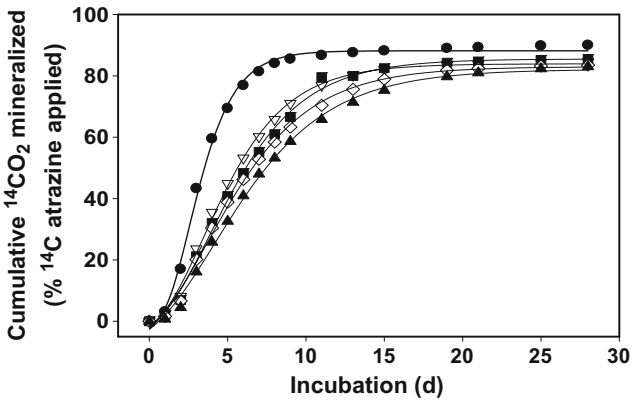

Fig. 2 Cumulative atrazine mineralization as affected by three concentrations of ammonium sulfate; *circles* none, *open triangles* 1 g kg^{-1}, *closed squares* 2 g kg^{-1}, *diamonds* 4 g kg^{-1}, and *closed triangles* 8 g kg^{-1}. Data points are means of four replicates and curve fitted to Gompertz model

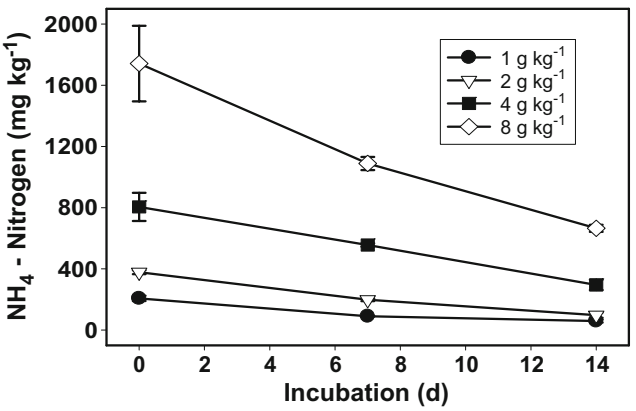

Fig. 3 Recovery of ammonium nitrogen as affected by various application rates of ammonium sulfate; mean and standard deviation of four replicates

Table 5 Effect of various rates of ammonium sulfate on cumulative atrazine mineralization at 28 days after treatment and mineralization parameters derived from the Gompertz growth model

Treatment	Cumulative mineralization at 28 days (%)	a[a] (%)	k (days^{-1})	ti (days)
None	90.0±2.4[b]	90.1±1.5	0.63±0.03	2.5±0.2
1 g kg^{-1}	84.6±1.2	88.8±2.5	0.34±0.02	3.5±0.2
2 g kg^{-1}	85.5±1.1	90.8±3.5	0.30±0.04	3.9±0.3
4 g kg^{-1}	83.3±1.4	91.3±3.8	0.27±0.02	3.7±0.2
8 g kg^{-1}	83.0±1.4	90.3±3.7	0.24±0.02	4.1±0.3

[a] Gompertz parameters: *a* the plateau representing maximum percent mineralization; *ti* the abscissa of the inflection point; *k* the mineralization rate constant and standard error of the parameters

[b] Mean and standard deviation of four replicates of the observed cumulative mineralization

mineralization due to $(NH_4)_2SO_4$ did not correspond to a significant reduction in ammonium N availability.

Nitrogen fertilization has been shown to decrease the diversity of soil prokaryotes (Ruppel et al. 2007) as well as alter the microbial community structure of an atrazine-degrading soil (Rhine et al. 2003). Nitrogen starvation/availability is a factor controlling the biodegradation rates of N-heterocyclic compounds such as atrazine in soil (Sims 2006). Sims (2006), using soils that did not exhibit enhanced atrazine degradation, showed that adding 20 mg kg^{-1} $(NH_4)_2SO_4$ reduced mineralization from 12% to 5% in 40 days. In an adapted soil, Abdelhafid et al. (2000), with 2.5 g kg^{-1} of $(NH_4)_2SO_4$, reduced atrazine mineralization (92%), and residual atrazine after 50 days was 54% of applied compared to 2% residual in non-treated soil. In mixed enrichment cultures, atrazine degradation was depressed in the presence of NH_4NO_3 (Mandelbaum et al. 1995).

The lowest rate of $(NH_4)_2SO_4$ used in this study (1 g^{-1} kg) is equivalent to ~10% of a typical commercial application rate for high-yielding maize in North America (150 to 200 kg N ha^{-1}), and the maximum rate of 8 g kg^{-1} of $(NH_4)_2SO_4$ is what would be applied in maize production. In the adapted Dundee soil used for these experiments, we typically observe a half-life of 3 to 5 days in laboratory microcosms; however, field half-lives are ~9 days (Krutz et al. 2007) where 175 kg ha^{-1} of N (ammonium nitrate/urea fertilizer) was applied before planting. Thus, field application rates of ammonium sulfate reduce the dissipation of atrazine in laboratory soils similar to that observed under corn management in the field.

Glufosinate and ammonium sulfate interactions

The effects of glufosinate, ammonium sulfate, and combinations of the fertilizer and herbicide on atrazine mineralization are presented in Fig. 4. A slight delay in maximal atrazine mineralization in response to glufosinate was observed, and increased inhibition was observed comparing the 0.25 and 1.0 g^{-1} kg rates of $(NH_4)_2SO_4$ to the non-treated control soil. (Fig. 4). An additive effect of glufosinate and $(NH_4)_2SO_4$ treatment on repression of atrazine mineralization was also observed considering mineralization parameters such as the mineralization constant (k) and lag phase (ti) (Table 6). Nitrogen added from the glufosinate treatment was 1.2 mg kg^{-1}, and 52.5 and 210 mg kg^{-1} were added from the low and higher rates of ammonium sulfate, respectively. Thus the relative contribution of additional nitrogen derived from glufosinate is relatively low compared to the nitrogen derived from ammonium sulfate and would be a minor contributor to the additive effects observed when glufosinate and ammonium sulfate are added.

Extractable ^{14}C was generally inversely related to atrazine mineralization (Table 7). However, analysis of methanol extracts at 4 days after treatment indicated that 4% of the initial atrazine was recovered in non-treated soil, while twofold more atrazine was extracted from soil treated with $(NH_4)_2SO_4$, and four- to fivefold more with co-application of $(NH_4)_2SO_4$ and glufosinate. Although the

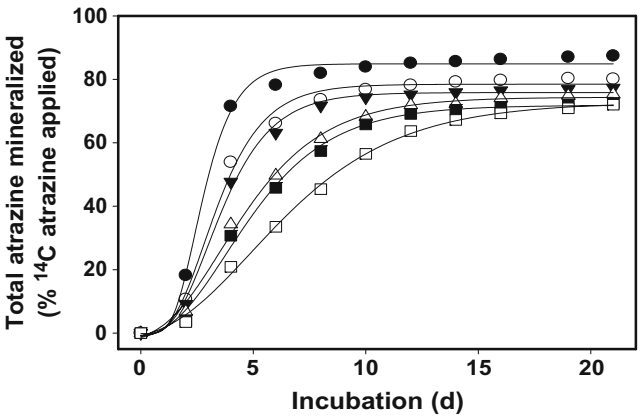

Fig. 4 Atrazine mineralization in a Dundee silt loam soil, interaction of glufosinate and ammonium sulfate; *circles* no ammonium sulfate, *triangles* 0.25 g kg^{-1}, *squares* 1.0 g kg^{-1}, *black symbols* no glufosinate, *white symbols* glufosinate 20 mg kg^{-1}. Data points are means of four replicates and curve fitted to Gompertz model

Table 6 Interaction of glufosinate and ammonium sulfate on atrazine mineralization parameters derived from the Gompertz growth equation

Treatment	a[a] (% cumulative mineralization)	k (day^{-1})	ti (days)
None	85.0±2.7	1.03±0.10	2.4±0.1
Glufosinate	79.3±3.0	0.67±0.07	2.8±0.2
$(NH_4)_2SO_4$ (0.25 g kg^{-1})	76.7±2.0	0.65±0.11	3.0±0.1
Glufosinate + $(NH_4)_2SO_4$ (0.25 g kg^{-1})	76.7±2.0	0.40±0.10	3.5±0.1
$(NH_4)_2SO_4$ (1.00 g kg^{-1})	73.9±2.9	0.40±0.04	3.8±0.2
Glufosinate + $(NH_4)_2SO_4$ (1.00 g kg^{-1})	75.3±2.6	0.27±0.05	4.9±0.2

[a] Gompertz parameters: a the plateau representing maximum percent mineralization; ti the abscissa of the inflection point; k the mineralization rate constant

highest recovery of hydroxyatrazine was recovered in non-treated soil, a relatively high amount of highly polar material (retention time of 2.5 min) was found in soil treated with the highest rate of $(NH_4)_2SO_4$ or either rate of $(NH_4)_2SO_4$ and glufosinate. This peak corresponds to cyanuric acid; however, confirmation using mass spectroscopy has not been used. The amount of residues recovered as non-extractable was related to rate of $(NH_4)_2SO_4$ application with the greatest found where 1.0 g kg^{-1} was applied and least where no $(NH_4)_2SO_4$ was applied, regardless of co-application of glufosinate. Generally, genes *atzA*, *atzB*, and *atzC* coding for enzymes in the initial steps of atrazine degradation are constitutively expressed (Shapir et al. 2007). However, the operon that degrades cyanuric acid is highly repressed by ammonium and induced by cyanuric acid (Garcia-Gonzalez et al. 2005). Accumulation of the polar metabolite with the same retention as cyanuric acid indicates that this may be occurring in soil receiving $(NH_4)_2SO_4$ fertilization.

Collectively, these studies demonstrate that the ability of accelerated atrazine degradation in soil can be modulated by nitrogen fertilization or the use or co-application of the herbicide glufosinate especially under N fertilization. The inhibition of atrazine degradation observed with glufosinate is similar to that observed by glyphosate. Glyphosate inhibits aromatic amino acid synthesis, while glufosinate inhibits ammonium assimilation. Combined applications of either glufosinate or glyphosate with atrazine should be evaluated in field studies for prolonging the weed control efficacy of atrazine.

Table 7 Effect of glufosinate (10 mg kg^{-1}) and two rates of ammonium sulfate alone or in combination on recovery of ^{14}C-atrazine and degradation components 4 and 21 days after treatment

Treatment	Mineralized to ^{14}C–CO_2	Total extractable	Atrazine	Polar metabolite	Hydroxy-atrazine	Non-extractable
4 days						
None	71.5±2.7	15.9±0.6	4.3±1.8	2.9±0.8	9.6±1.1	10.8±0.5
Glufosinate	53.8±4.2	18.8±2.7	7.6±2.7	1.0±2.7	8.1±2.7	12.6±2.7
$(NH_4)_2SO_4$ 0.25 g kg^{-1}	47.8±2.8	22.7±3.6	9.8±2.2	4.4±3.1	7.4±1.2	14.5±1.2
Glufosinate + $(NH_4)_2SO_4$ 0.25 g kg^{-1}	34.3±3.7	37.4±5.4	16.5±1.9	10.7±6.7	5.2±0.9	14.4±0.7
$(NH_4)_2SO_4$ 1.00 g kg^{-1}	30.7±1.6	45.3±4.6	9.0±2.7	31.8±7.7	4.4±1.7	16.9±1.1
Glufosinate + $(NH_4)_2SO_4$ 1.00 g kg^{-1}	20.9±3.0	53.6±0.5	19.7± 4.2	26.0±7.3	3.7±0.2	17.0±0.6
LSD	4.9	4.4	4.2	7.6	2.2	1.2
21 days						
None	87.5±1.7	2.9±1.4	nd	nd	nd	5.3±0.3
Glufosinate	80.7±3.9	3.7±0.7	nd	nd	nd	6.5±0.3
$(NH_4)_2SO_4$ 0.25 g kg^{-1}	77.3±1.3	8.2±5.6	nd	nd	nd	8.9±2.0
Glufosinate + $(NH_4)_2SO_4$ 0.25 g kg^{-1}	75.3±4.0	8.7±1.9	nd	nd	nd	9.6±0.9
$(NH_4)_2SO_4$ 1.00 g kg^{-1}	72.5±4.0	8.1±0.6	nd	nd	nd	11.1±0.3
Glufosinate + $(NH_4)_2SO_4$ 1.00 g kg^{-1}	72.0±2.0	8.2±7.7	nd	nd	nd	11.7±0.3
LSD	4.0	5.1				1.4

Mean and standard deviation of four replicates
nd Not determined

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