Effects of pH on Chemical Stability and De-esterification of Fenoxaprop-ethyl by Purified Enzymes, Bacterial Extracts, and Soils

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De-esterification is an initial step in the metabolism of certain herbicides, for example, fenoxaprop-ethyl [(±)-ethyl 2-[4-[(6-chloro-2-benzoxaolyl)oxy]phenoxy]propanoate] (FE). The ethyl−ester bond cleavage of FE to fenoxaprop acid (FA) by purified enzymes, crude bacterial enzyme preparations, and soils was investigated. In similar experiments fluorescein diacetate (FDA) was used as an alternative substrate. FE stability was pH sensitive in acidic buffered solutions; that is, below pH 4.6, rapid nonenzymatic hydrolysis of the benzoxazolyl−oxy−phenoxy ether linkage occurred, forming 6-chloro-2,3-dihydro-benzoxazol-2-one (CDHB) and ethyl 4-hydroxyphenoxypropanoate or 4-hydroxy-phenoxypropanoate. With porcine esterase and cell-free *Pseudomonas fluorescens* extracts, activity on FE and FDA was most rapid at pH 7.6−8.6 but decreased 80−90% at pH 5.6. Yeast (*Candida cylindrica*) lipase-mediated de-esterification of FE and FDA was not as sensitive to pH; that is, activity at pH 4.6 was 70% of that at pH 7.6. Short-term incubations (20 h) were conducted in eight soils (pH 4.5−6.9) treated with ^{14}C-chlorophenyl ring-labeled FE (2 mg kg^{-1}). In the most acidic soils (pH 4.4−4.5) 25% of the ^{14}C was recovered as FA, versus 30−40% in moderately acid soils (pH 5.0−5.6) and 55% in neutral soils (pH 6.8−6.9). There was a similar correlation between soil pH and FDA de-esterification. CDHB was formed in all acidic soils with levels 4-fold greater in pH 4.4−4.5 soils than in pH 5.0−5.6 soils. CDHB was not formed in neutral soils. Results demonstrate some chemical hydrolysis (benzoxazolyl−oxy−phenoxy ether linkage) of FE in acid soils, the sensitivity of enzymatic de-esterification of FE to pH, and the potential of FDA as a colorimetric indicator for esterase hydrolysis of FE.

Keywords: *Herbicide; esterase; biodegradation; lipase; pseudomonads; microbial metabolism*

INTRODUCTION

Fenoxaprop-ethyl [(±)-2-[4-[(6-chloro-2-benzoxazolyl)-oxy]phenoxy]propanoic acid] is used a postemergence herbicide to control various annual grass weeds in soybeans, rice, wheat, and turf (WSSA, 1994). The fate of fenoxaprop-ethyl (FE) has been examined in soils from several geographical areas (Köcher et al., 1982; Smith and Aubin, 1990). These authors demonstrated that FE is rapidly hydrolyzed (de-esterified) to fenoxaprop acid (FA). This hydrolysis occurred rapidly in moist nonsterile soils, suggesting microbial degradation (Köcher et al., 1982; Smith and Aubin, 1990). The de-esterified metabolite FA is phytotoxic and has been shown to inhibit the germination of certain monocot weeds (Bieringer et al., 1982). FA undergoes further degradation in soil forming 6-chlorobenoxazolone, 4-[(6-chloro-2-benzoxazolyl)oxy]phenetole, and 4-[(6-chloro-2-benzoxazolyl)oxy]phenol (Smith, 1985; Smith and Aubin, 1990). In studies using ^{14}C-labeled FE, several researchers found that some metabolites are incorporated into microbial biomass and soil organic matter and that some components of FE can be mineralized to CO_2 (Köcher et al., 1982; Smith and Aubin, 1990).

There are relatively few studies on specific microorganisms that are capable of enzymatic transformation of FE. Studies using a mixed microbial consortium showed that FE was utilized as a sole carbon and nitrogen source (Gennari et al., 1995) and that FA and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) were identified metabolites. Our recent studies indicated that certain fluorescent pseudomonads (*Pseudomonas fluorescens* and *Pseudomonas putida*) transform fenoxaprop-ethyl; however, the products of transformation are dependent upon nutritional status (Hoagland and Zablotowicz, 1998). When these *P. fluorescens* strains were grown on a rich medium (tryptic soy broth), FE transformation occurred by an initial de-esterification to FA with subsequent cleavage of the ether bond mediated via glutathione conjugation. When these same strains were grown on minimal media with propionate as sole carbon source, transformation was limited to de-esterification. However, if grown on minimal media with glucose, minimal de-esterification was observed and ∼50% of the FE was transformed to CDHB.

The present studies were conducted to further evaluate the microbial cometabolism of FE focusing on de-esterification, especially the interactions of pH on enzymatic transformations and chemical hydrolysis. The role of soil pH on chemical stability and de-esterification of FE was also assessed. Fluorescein diacetate (FDA) has been shown to be a useful substrate for assessing hydrolytic activity, especially esterases, lipases, and certain proteases in bacteria (Schnürer and Roswall, 1982; Lundgren, 1981), eukaryotic cells (Guilbault and Kramer, 1964), and soils and plant residues (Schnürer

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10.1021/jf991062f
Published on Web 09/13/2000

and Roswall, 1982; Zablotowicz et al., 1998). The relative hydrolysis of FDA compared to FE was studied to assess the feasability of using FDA hydrolytic activity as an indicator for herbicide de-esterification potential in soil.

MATERIALS AND METHODS

Bacterial Strains. *P. fluorescens* strains RA-2 and UA5-40 and *Bacillus thuringiensis* UZ404 have been previously described elsewhere (Zablotowicz et al., 1995; Hoagland and Zablotowicz, 1995). Cultures were maintained as frozen glycerol and tryptic soy agar stocks.

Chemicals. All chemicals and solvents were of analytical grade or higher purity. FE, ^{14}C-labeled chlorophenyl (specific activity = 1107 MBq g^{-1}), and high-purity standard unlabeled metabolites FA (HOE 088406), 6-chloro-2,3-dihydrobenzoxazone (HOE 054014), and hydroxyphenylpropionate (HOE 096918) were supplied by AgroEvo Frankfurt, Germany. Porcine esterase (170 units mg of protein^{-1}) and yeast (*Candida cylindrica*) lipase (300000 units mg of protein^{-1}) were purchased from Sigma Chemical Co. (St. Louis, MO).

Analysis. Thin-layer chromatography (TLC) was used for the analysis of FE and degradation products, as described elsewhere (Hoagland and Zablotowicz, 1998). Aliquots (50 μL) were mechanically spotted (40 °C) with a multispotter (Analytical Instruments Inc., Baltimore, MD) on silica gel plates with fluorecsent indicator (20 × 20 cm, 250 μm; Whatman, Clifton, NJ). Plates were developed at 10 cm in toluene/ethyl acetate/acetic acid/water, 50:50:1.0:0.5 (v/v/v/v). Typical R_f values observed were 0.94 for FE, 0.14 for FA, and 0.55 for CDHB.

Effect of Buffer pH on FE Chemical Stability. The stability of FE was assessed at pH 2.6, 3.6, and 4.6 (phthalate buffer), pH 5.6, 6.6, and 7.6 (potassium phosphate), and pH 8.6 (Tris-HCl) prepared according to the method of Robinson (1979). Triplicate tubes of each buffer were treated with ethanolic solutions of chlorophenyl-labeled FE to attain a final concentration of 6 μM in 3% ethanol. Solutions were incubated at 30 °C and 125 rpm on a orbital shaker. Aliquots were removed after incubation for 3 and 22 h and extracted with 2 volumes of acetone; 50 μL was spotted on silica gel plates, and RAD-TLC was performed as described above.

FE and FDA Purified Enzyme De-esterification Activty. Two purified enzymes, porcine esterase and yeast esterase, were evaluated for de-esterification activity with FDA and FE in buffers ranging from pH 3.6 to 8.6 as described above. FDA hydrolysis was determined spectrophotometrically using a Shimadzu UV160 spectrophotmeter (Shimadzu Instruments, Baltimore, MD) equipped with a temperature-controlled CPS240A cell positioner to facilitate kinetic reading of optical density. Reactions consisted of 1500 μL of buffer, 100 μL of enzyme (3.2 ng of protein esterase and 3 mg of protein for lipase), and 20 μL of FDA substrate (5 ng in acetone). Each assay consisted of 10 determinations at 490 nm taken at intervals every 20−60 s, with four replicates per pH value. FE de-esterification was determined radiologically. Reaction mixtures consisted of 180 μL of buffer, 20 μL of enzyme (3.2 ng of protein esterase and 3 mg of protein for lipase), and 10 μL of FDA substrate (1.2 nmol in ethanol) in 2.0 mL of polypropylene microcentrifuge tubes. Each pH level was replicated four times and incubated for 1 and 3 h. Reactions were terminated by extraction with 2 volumes of acetone, spotting 50 μL on silica gel TLC plates with analysis as described above.

FE and FDA Hydrolysis by Bacterial Cell-Free Extracts. Bacterial cultures (48 h tryptic soy broth cultures) were washed twice in 12.5 mM phosphate buffer (pH 6.8) and resuspended in the same buffer at a ratio of 1:4 (cells to buffer). The cell suspension was sonicated four times (20 s burst) at 0−4 °C and then centrifuged for 20 min at 15000*g*. Protein contents of cell-free extracts (cfe) were determined using the Bradford reagent (Bradford, 1976). FDA assays were conducted spectrometrically (490 nm) in a kinetics mode [read every 30 s for 5 min or every 1 min for 10 min (depending on activity), at 30 °C], as described for purified enzymes, using 150 μL of cfe (~4 mg of protein), 50 ng of FDA in 20 μL of acetone, and 1350 μL of buffer (pH 5.6, 6.6, 7.6, and 8.6). Tests on each pH level were replicated four times. FE de-esterification assays were conducted in a similar fashion as described elsewhere (Hoagland and Zablotowicz, 1998). Briefly, cfe (150 μL) and 1350 μL of buffer (pH 5.6, 6.6, 7.6, or 8.6) were placed in polypropylene microfuge tubes. Controls with buffer in lieu of cfe were included. An ethanolic stock of chlorophenyl-labeled FE (9 nmol) was added, and the tubes were vortexed and incubated at 30 °C and 100 rpm on a orbital shaker. Subsamples were removed at 2.5 and 5 h, extracted with 2 volumes of acetone by sonication (20 min), and clarified by centrifugation (10 min, 15000*g*). Extracted solutions (50 μL) were spotted on silica gel TLC plates and analyzed as described above. The effect of phenylmethanesulfonyl fluoride (PMSF) and the insecticide carbaryl (1-naphthyl-*N*-methylcarbamate) on the inhibition of FDA and FE hydrolysis was determined using methodology described above with inhibitor concentrations of 50−500 μM for FDA hydrolysis and only 500 μM for FE hydrolysis by cfes from the two *P. fluorecens* strains.

Soils. Several soils of various pH values were selected for study. These soils were chosen from samples collected across a transect of a grower's field from a study evaluating the spatial variability of soil properties in Leflore County, Mississippi (Staddon et al., 1999). Soils were collected from the surface 5 cm, sieved through a 2.44 mm sieve, and stored at 5 °C at field moisture levels (8−15% moisture) until required. Selected biological, chemical, and physical properties of these soils are presented in Table 4. Soil pH was determined with a pH meter using a 1:2 soil/0.01 M $CaCl_2$ slurry. Soil textural analysis was conducted using a hydrometer. Soil organic matter was determined by dichromate digestion (Nelson and Sommers, 1982). Dehydrogenase activity was determined using triphenyltetrazolium chloride as substrate using a modification of the procedure of Casida et al. (1964) as described elsewhere (Wagner and Zablotowicz, 1997).

FDA Hydrolysis in Soil. To select an appropriate solvent for the termination of FE hydrolysis in soil, initial studies were conducted to evaluate the effectiveness of several solvents for the inhibition of FDA de-esterification in soils. Soils (1.0 g of fresh weight) were placed in 25.0 mL Corex centrifuge tubes, and 5 mL of potassium phosphate buffer was added. Tubes received 10 mL of acetone, acetonitrile, and methanol and 5 or 10 mL of buffer (eight tubes per solvent). Six of the eight tubes per treatment received 0.1 mg of FDA in 100 μL of acetone, and two tubes per solvent remained untreated (control). All tubes were placed on a shaking incubator (30 °C and 125 rpm) and incubated for 2 and 4 h before termination. The treatment that was incubated without solvent received 10 mL of acetone and was vortexed prior to centrifugation (10000*g* for 10 min). All others were centrifuged at 10000*g* for 10 min before the optical density (490 nm) of the supernatant was determined.

Assays conducted to assess FDA hydrolysis in soil were modifications of the protocol described by Schnürer and Rosswall (1982). Briefly, soil (2.0 g of fresh weight) was placed in 50 mL polypropylene centrifuge tubes. Either 20 mL of buffer (50 mM potassium phosphate, pH 7.6) or distilled water was added, triplicate tubes were treated with 250 μL of an acetone solution of FDA (2 mg mL^{-1}, 0.5 mg per tube), and the tubes were capped, vortexed, and inubated on an incubated shaker for 1 h (30 °C, 100 rpm). Controls receiving no FDA to correct for soil were included. The assay was terminated by extraction with 20 mL of acetone and centrifugation (10000*g* for 10 min), before the optical density (490 nm) of the supernatant was determined.

FE Degradation in Soil. Incubation studies to ascertain FE degradation were conducted in 25 mL screw-cap Corex tubes. Soil (1.5 g, dry weight basis) was placed in tubes, and 100 μL of water was added dropwise to moisten soil. An ethanolic solution (45 μL) of ^{14}C-labeled FE containing 9 nmol of FE was added. Water was added to attain a final moisture content of 30% and to aid in the distribution of FE in the soil

Table 1. Buffer pH Effects on Nonenzymatic Transformations of FE

	^{14}C recovered (%)					
	3 h incubation			22 h incubation		
pH	FE	FA	CDHB	FE	FA	CDHB
2.6	55.7 ± 4.7[a]	nd[b]	44.2 ± 4.7	10.5 ± 2.3	nd	83.3 ± 2.3
3.6	93.6 ± 2.8	nd	6.4 ± 2.8	49.4 ± 6.4	nd	51.6 ± 6.4
4.6	100	nd	nd	100	nd	nd
5.6	100	nd	nd	100	nd	nd
6.6	100	nd	nd	100	nd	nd
7.6	100	nd	nd	100	nd	nd
8.6	100	nd	nd	87.4 ± 3.1	13.6 ± 3.1	nd

[a] Mean and standard deviation of three replicates. [b] nd, none detected.

matrix. Triplicate soils were extracted at initiation and 20 h after incubation. Soils were initially extracted with 12 mL of acetonitrile for 2 h and centrifuged for 10 min at 10000*g*, and the supernatant was removed. Soils were re-extracted with 8 mL of acetonitrile for 1 h, the supernatants were combined, and recovery of radioactivity was determined via liquid scintillation counting. The acetonitrile extracts were reduced to ~2.0 mL under nitrogen, and 75 μL aliquots were spotted on silica gel TLC plates and analyzed as described above. The second study was conducted to assess the effects of soil pH modification using a similar method, except soils were moistened with either phosphate buffer, pH 7.6, or distilled water.

RESULTS AND DISCUSSION

Effect of Buffer pH on FE Chemical Stability. The chemical stability of FE was greatly affected by buffer pH (Table 1). Under the most acidic conditions (pH 2.6 and 3.6), FE underwent a rapid nonenzymatic hydrolysis with hydrolysis of the benzoxazolyl−oxy−phenoxy ether linkage occurring, as CDHB accumulated. We have not ascertained whether ethyl 4-hydroxyphenoxypropanoate or 4-hydroxyphenoxypropanoate accumulated from the other portion of the molecule. Under slightly alkaline pH, a relatively slower hydrolysis of the ester bond was observed and FA accumulated. Nonenzymatic de-esterification of fluazifop-butyl [butyl 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate] occurs in water at pH 9, but was not observed at pH 7 or 4 (Negre et al., 1988). Hydrolysis of FE under acidic conditions has been postulated as Balinova (1996) reported inefficient extraction of FE from water samples acidified to pH 2.0. Treatment with strong acid (1 N HCl, 80 °C) hydrolyzed FA and glutathione and cysteine conjugates to CDHB (Tal et al., 1993). Previously, Hoagland and Zablotowicz (1998) have shown that when *P. fluorescens* strain RA-2 or UA5-40 was grown on glucose minimal medium, CDHB accumulated, but this was not observed on propionate minimal medium. Although the medium was buffered (~pH 7.0), the medium pH became acidic (pH 4.6) during growth on glucose media; however, medium pH remained neutral during growth on propionate. This lower pH most likely was responsible for nonenzymatic cleavage of the benzoxazolyl−oxy−phenoxy ether linkage of FE. In studies by others (Gennari et al., 1995), CDHB was also reported as the major accumulating metabolite in enrichment cultures; the formation of CDHB via nonenzymatic processes in both studies also should be considered.

FE and FDA Purified Enzyme De-esterification Activty. The de-esterification of FDA and FE by two purified enzymes (porcine esterase and yeast esterase) was affected by buffer pH (Table 2). Porcine esterase activity was more sensitive to acidity compared to *Candida* lipase. Porcine FDA and FE de-esterification activity was completely inhibited at pH 3.6. While at pH 3.6, *Candida* lipase activity with FDA and FE was about 5 and 50%, respectively, of the maximum activity (pH 5.6−6.6). Similar results were observed by Gaynor (1992) in that there was minimal effect of pH on *Rhizopus* lipase-mediated de-esterification of dicloflop-methyl; however, porcine esterase-mediated hydrolysis was greatest near pH 7.0, with a lower level of dicloflop-methyl hydrolysis observed under acidic conditions.

Table 2. Buffer pH Effects on Porcine Esterase and *C. cylindrica* Lipase Activity with FDA or FE as Substrate

	μmol of product formed mg^{-1} of protein h^{-1}			
	porcine esterase		*Candida* lipase	
buffer pH	FDA[a]	FE[b]	FDA	FE
3.6	<0.2	<0.1	2 ± 1[c]	1.3 ± 0.2
4.6	<0.5	0.3 ± 0.1	4 ± 2	1.9 ± 0.1
5.6	3 ± 1	0.5 ± 0.1	18 ± 1	2.6 ± 0.2
6.6	15 ± 6	3.0 ± 0.3	37 ± 1	2.5 ± 0.2
7.6	63 ± 4	3.9 ± 0.3	35 ± 0.3	2.3 ± 0.1
8.6	262 ± 8	4.1 ± 0.4	35 ± 0.8	2.1 ± 0.2

[a] Product formed, fluorescein. [b] Product formed, fenoxaprop. [c] Mean and standard deviation of six replicates for FDA and mean and standard deviation of four replicates for FE.

Table 3. Buffer pH Effect on FDA and FE De-esterification Activity by Cell-Free Extracts of *B. thuringinesis* Strain UZ404 and *P. fluorescens* Strains RA-2 and UA5-40

buffer pH	*B. thuringiensis* UZ404	*P. fluorescens* RA-2	*P. fluorescens* UA5-40
FDA (nmol of Fluorescein Formed mg^{-1} of Protein min^{-1})			
5.6	9 ± 2[a]	<0.2	1.2 ± 0.1
6.6	90 ± 1	3.6 ± 0.3	8.2 ± 0.6
7.6	160 ± 6	28.3 ± 2.1	17.6 ± 0.3
8.6	105 ± 3	36.0 ± 1.4	22.3 ± 0.7
FE (nmol of Fenoxaprop Acid Formed mg^{-1} of Protein h^{-1})			
5.6	0.7 ± 0.3[b]	<0.1	<0.1
6.6	1.9 ± 0.1	0.2 ± 0.1	0.6 ± 0.3
7.6	2.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
8.6	2.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.1

[a] FDA hydrolytic activity, mean and standard deviation of four replicates. [b] FE hydrolysis, mean and standard deviation of three replicates.

Table 4. Properties of Soils Used in This Study

soil pH, tillage	organic C (%)	sand (%)	silt (%)	clay (%)	dehydrogenase activity[a]
4.4, CT[b]	0.9	20.7	55.7	23.6	11.7
4.5, CT	1.0	21.4	60.0	18.6	11.3
5.0, CT	1.6	19.5	62.8	13.6	23.5
5.1, CT	1.3	21.3	65.2	13.5	19.1
5.5, CT	0.8	26.9	59.0	14.1	17.8
5.6, NT[b]	1.2	17.7	56.6	25.7	26.5
6.8, NT	1.0	38.3	49.2	12.5	20.7
6.9, NT	1.1	43.2	44.5	12.3	20.7

[a] Dehydrogenase activity = nmol of triphenylformazan formed g^{-1} soil h^{-1}. [b] NT, no tillage; CT, conventional tillage.

FE and FDA Hydrolysis by Bacterial Cell-Free Extracts. Under acidic pH (<5.6) proteins in bacterial cfes were denatured; thus, studies were conducted only between pH 5.6 and 8.6. Differential inhibition of FDA and FE de-esterification by pH was observed by comparing cfes from the three bacterial strains (Table 3). Maximal FDA and FE de-esterification activites were observed at pH 7.6 in *B. thuringiensis* cfe. FDA and FE activities by *B. thuringiensis* cfe at pH 5.6 were about 6 and 24%, respectively, of those observed at pH 7.6. In

Table 5. FDA Hydrolytic Activity of Soils of Various pH Values As Determined in HPLC Grade Water or pH 7.6 Potassium Phosphate Buffer

soil pH, tillage	nmol of fluorescein formed g^{-1} of soil h^{-1}	
	HPLC grade water	buffer, pH 7.6
4.4, CT[a]	<2	226 ± 28[b]
4.5, CT	<2	313 ± 21
5.0, CT	11 ± 2	625 ± 37
5.1, CT	13 ± 1	707 ± 53
5.5, CT	10 ± 2	420 ± 34
5.6, NT[a]	42 ± 3	413 ± 22
6.8, NT	337 ± 7	877 ± 35
6.9, NT	408 ± 8	927 ± 41

[a] CT, conventional tillage; NT, no tillage. [b] Mean and standard deviation of four replicates.

P. fluorescens strain RA-2 cfe, hydrolysis of both FDA and FE was completely inhibited at pH 5.6. In *P. fluorescens* strain UA5-40 cfe, a low level of FDA but no FE activity was observed at pH 5.6. Our previous studies showed only minimal FE de-esterification when either RA-2 or UA5-40 was grown on glucose minimal media compared to tryptic soy broth or propionate minimal media (Hoagland and Zablotowicz, 1998). Although the medium was buffered (~pH 7.0), the medium pH became acidic (pH 4.6) during growth on glucose media. This lower pH most likely inhibited FE-esterase activity in these bacterial cultures. These data also agree with observations that de-esterification of FE by RA-2 is more readily inhibited than FE de-esterification activity by UA5-40. These results also indicate that pH effects on de-esterification of both substrates by various bacteria can be genus- and even strain-specific. Certain *P. fluorescens* strains have been shown to produce four different esterases that differ in substrate specificity, cellular location, and other properties (Choi et al., 1990). A similar duplicity of enzymes most likely exists in the bacteria studied here with different pH sensitivities and substrate specificities. Lipase activity may not be as prevalent in bacteria but is widely distributed in fungi (Moat, 1979). However, lipases have been purified from several pseudomonads (Sharon et al., 1998; Dharmsthiti and Kukasuntisuk, 1998).

FDA and FE de-esterification activity of cfe by both *P. fluorescens* strains was unaffected by 500 μM carbaryl or PMSF (data not shown). Previous studies indicated that 98% of the aryl acylamidase activty of RA-2 cfe was inhibited by 500 μM carbaryl (Hoagland and Zablotowicz, 1995); thus, the aryl acylamidase enzyme of RA-2 is distinct from its FDA and FE hydrolyzing enzyme(s). Carbaryl inhibits specific esterases such as acetylcholinesterase provided the ester linkage is intact (Benson and Dorough, 1984). PMSF inhibits a wide range of esterases in plants and animals (Turini, 1969).

FDA Hydrolysis in Soil. Soils treated with either acetone or acetonitrile exhibited ~2% of the FDA hydrolysis observed in soil incubated in phosphate buffer alone (data not shown). However, soils incubated in the presence of methanol exhibited twice the FDA hydrolysis of untreated soils (data not shown). Acetone, as recommended by Schnürer and Rosswall (1982), was a suitable terminating solvent for assessing FDA hydrolytic activity in soil.

FDA hydrolytic activity was observed in all soils incubated in pH 7.6 phosphate buffer, whereas little or no activity was observed in the most acidic soils when incubated in distilled water (Table 5). The highest FDA activity was observed in the near-neutral soils (pH 6.8−6.9) and the lowest activity in the two most acidic soils (pH 4.4−4.5), when incubated in phosphate buffer. However, a linear relationship between soil pH and FDA hydrolysis in phosphate buffer was not observed in this set of soils. Other factors such as soil microbial activity and populations as well as soil carbon and nutritional status can affect soil FDA hydrolytic activity (Schnürer and Rosswall, 1982). Furthermore, studies by our group with more extensive samples (W. J. Staddon, unpublished observations) found a positive relation between soil pH and FDA hydrolysis in a transect across the field site from which these samples were collected, thereby emphasizing the importance of soil acidity on this enzymatic activity.

Table 6. Recovery of FE and Degradation Products from Soils of Various pH Values following a 20 h Incubation

soil pH, tillage	% extractable radioactivity		
	FE	FA	CDHB
4.4, CT[a]	60 ± 2[b]	22 ± 6	18 ± 1
4.5, CT	58 ± 1	26 ± 2	16 ± 1
5.0, CT	51 ± 1	43 ± 1	5 ± 1
5.1, CT	56 ± 2	40 ± 3	4 ± 1
5.5, CT	56 ± 1	41 ± 2	3 ± 1
5.6, NT[a]	64 ± 1	30 ± 2	4 ± 1
6.8, NT	44 ± 2	56 ± 2	nd[c]
6.9, NT	42 ± 5	58 ± 5	nd

[a] CT, conventional tillage; NT, no tillage. [b] Mean and standard deviation of three replicates. [d] nd, none detected.

Table 7. Recovery of FE and Degradation Products (20 h Incubation) from Soils of Various pH Values As Influenced by Adjusting the Moisture Content with Potassium Phosphate Buffer (pH 7.6) or HPLC Grade Water

soil, tillage	addition	% extractable radioactivity		
		FE	FA	CDHB
4.4, CT[a]	water	61 ± 4[b]	18 ± 4	21 ± 2
4.4, CT	buffer	62 ± 3	30 ± 4	8 ± 2
4.5, CT	water	58 ± 5	26 ± 3	16 ± 2
4.5, CT	buffer	53 ± 5	41 ± 4	6 ± 1
6.8, NT[a]	water	40 ± 2	60 ± 2	nd[c]
6.8, NT	buffer	35 ± 5	65 ± 5	nd
6.9, NT	water	48 ± 4	52 ± 4	nd
6.9, NT	buffer	39 ± 3	61 ± 3	nd

[a] CT, conventional tillage; NT, no tillage. [b] Mean and standard deviation of three replicates. [c] nd, none detected.

FE Degradation in Soil. Short-term incubation studies (20 h) with soils treated with ^{14}C-chlorophenyl-labeled FE indicated a significant interaction between soil pH and metabolite formation (Table 6). The highest level of FA formation was observed in neutral soils, and the lowest formation was observed in the most acidic soils. CDHB was not formed in the near-neutral soils (pH 6.8−6.9), a moderate accumulation was observed in moderately acidic soils (pH 5.0−5.6), and 16−18% of the radioactivity was recovered as CDHB in the most acidic soils. When the acidic soils (pH 4.5) were treated with pH 7.6 buffer, CDHB formation was reduced and FA formation increased compared to soils treated with distilled water (Table 7). A minimal effect of pH 7.6 buffer was observed on near-neutral soils. These results indicate similar effects of pH on FE stability and de-esterification found in the in vitro studies. Namely, chemical hydrolysis of FE cleaving the benzoxazolyl−oxy−phenoxy ether linkage is observed even under moderately acidic conditions, and FE de-esterification is promoted under neutral conditions. A linear relationship between FDA hydrolysis in soil and FA formation

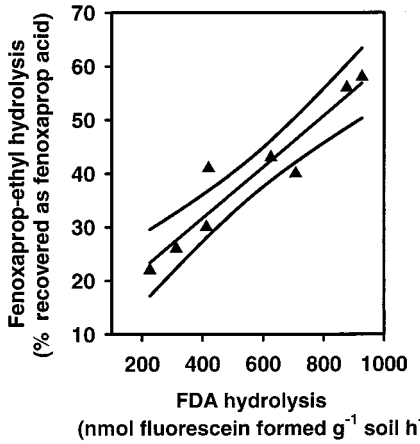

Figure 1. Relationship between FDA hydrolytic activity and de-esterification of FE in soils collected from a Mississippi field.

was observed (FE = 0.04779 × FDA + 12.56, $R^2 = 0.898$, Prob = 0.003, Figure 1), suggesting that simple colorimetric assays can be employed to ascertain the potential for de-esterification of FE and perhaps other herbicides in soil. Other researchers (Reed et al., 1989) suggested that FDA hydrolytic activity can be used to assess the potential for accelerated degradation of thiocarbamate herbicides in soil.

SUMMARY AND CONCLUSIONS

In vitro studies under controlled conditions indicate that both chemical hydrolysis and enzymatic de-esterification of FE are sensitive to pH. FE is rapidly hydrolyzed to CDHB under acidic conditions, as previously noted by Tal et al. (1993). We found nonenzymatic de-esterification of FE only under slightly alkaline conditions (pH 8.6). This phenomenon has also been reported for another herbicide, fluazifop-butyl (Negre et al., 1988). Porcine esterase-mediated de-esterification of both FDA and FE was highly inhibited by acidity. *Candida* lipase-mediated de-esterification of FDA was strongly inhibited and FE de-esterification only moderately inhibited at pH 3.6. Typically, bacterial de-esterification of both FDA and FE is favored by neutral to slightly alkaline pH; however, pH sensitivity varies among bacterial genera and in some strains of the same species.

The effect of soil pH on FE degradation is similar to that observed in vitro. However, a significant amount of FE is hydrolyzed to CDHB in moderately acidic soils, with greater formation associated with the more acidic soils. Likewise, de-esterification of FE is favored as soil pH becomes more neutral. Thus, there is a significant interaction between chemical hydrolysis and enzymatic activity (de-esterification) that can influence the fate of FE in soil. Cleavage of the benzoxazolyl−oxy−phenoxy ether linkage of FE under acidic conditions is a substantial degradation step compared to de-esterification. Under certain circumstances, for example, highly acidic soils, chemical degradation of FE may be a significant determinant in the environmental fate of this herbicide.

FDA hydrolysis is a fairly simple enzyme assay for assessing soil microbial activity (Schnürer and Rosswall, 1982; Zablotowicz et al., 1998). The present studies suggest that FDA hydrolysis may be used as a rapid indicator to assess the potential for de-esterification of FE and perhaps other herbicides in soil.

LITERATURE CITED

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Received for review September 27, 1999. Accepted August 1, 2000.

JF991062F