Chapter 17

Identification of Trifluralin Metabolites in Soil Using Ion-Trap LC/MS/MS

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Trifluralin degradation in soils is complex, potentially resulting in the formation of 28 metabolites. The objective of this research was to develop an approach for the identification of trifluralin metabolites in soils using ion-trap liquid chromatography/mass spectrometry (LC/MS/MS). Authentic standards of the parent and six metabolites were used to establish appropriate instrument conditions and precursor ion (PI) fragmentation patterns to confirm their identity, as well as to facilitate confirmation of metabolites for which authentic standards were not available. Two soils from herbicide spill sites known to be contaminated with trifluralin were selected for study because of their high potential for metabolite detection. Soils were extracted with 70% aqueous acetonitrile, filtered, and directly injected into the ion-trap LC/MS/MS. Trifluralin metabolites were then identified as follows: 1) screen for PI masses; 2) compare retention time of the PI peak to standards; and 3) obtain PI spectra and compare fragmentation with standards. The validity of this approach was confirmed for the identification of metabolite, TR-20. Preliminary results identified the presence of up to eight metabolites in the soils, and the array of metabolites present were indicative of aerobic trifluralin degradation.

Introduction

Trifluralin is a dinitroaniline herbicide primarily used in soybean and cotton production, but it is also registered for use with numerous crops, vegetables, turf, and ornamentals. Trifluralin was introduced for agricultural use in the early 1960's, and it still ranks as one of the five top-selling herbicides in the U.S. It is currently registered in more than 50 countries for use on over 80 crops (*1*). Although its use has been declining since the late 1980's, 2.85 X 10^6 kg of trifluralin were used on soybeans and cotton in 2001 (*2*). It is currently the 3rd and 4th most commonly used herbicide on cotton and soybeans, respectively.

Extensive reviews of the environmental fate and chemistry of trifluralin have been reported over the last 35 years (*1,3,4*). Trifluralin is a very hydrophobic compound that strongly sorbs to soils, and therefore, its transport to surface or ground waters in the dissolved-phase is very limited. Off-site transport mainly occurs by soil erosion and subsequent sediment deposition in streams and lakes or by volatilization losses following field application.

Trifluralin degradation in soils is complex (Fig. 1), with up to 28 metabolites extracted and identified from a three year field degradation study (*5*). Predominant degradation pathways include dealkylation, reduction, oxidation, and cyclization reactions (*4-6*). Additional reactions include hydrolysis, hydroxylation, and condensation (*5*). Separate aerobic and anaerobic soil degradation pathways have been proposed for trifluralin (*3,4,7*). Aerobic degradation proceeds via the following reactions: dealkylation and partial reduction (TR-2 to TR-6); hydrolysis (TR-20); cyclization (TR-11 to TR-19); and dimeric condensation via formation of the partially reduced TR-39 to form azoxy (TR-28, TR-29, TR-31) and azo (TR-32) metabolites (*4,5*). Anaerobic degradation occurs via the following reactions: reduction and dealkylation (TR-4 to TR-9); cyclization (TR-13 and TR-14); and dimeric condensation (TR-28). Degradation rates of trifluralin have been shown to be faster under anaerobic than aerobic conditions (*4,7,8*).

To correct apparent oversights and to incorporate the findings of other studies, the degradation pathways presented in Figure 1 differ slightly from that published by Golab et al. (*5*). The commonly reported metabolites formed via dealkylation and reduction (TR-2 to TR-8) are shown as having interacting pathways. For instance, TR-4 can degrade to TR-5 via dealkylation, and TR-5 can degrade to TR-8 via reduction. Also, TR-15 could be dealkylated to form TR-18.

In addition to forming multiple metabolites, trifluralin also shows a strong propensity to form bound residues in soil. Studies reviewed by Grover et al. (*1*), reported that 10 to 72% of the applied trifluralin formed non-extractable bound

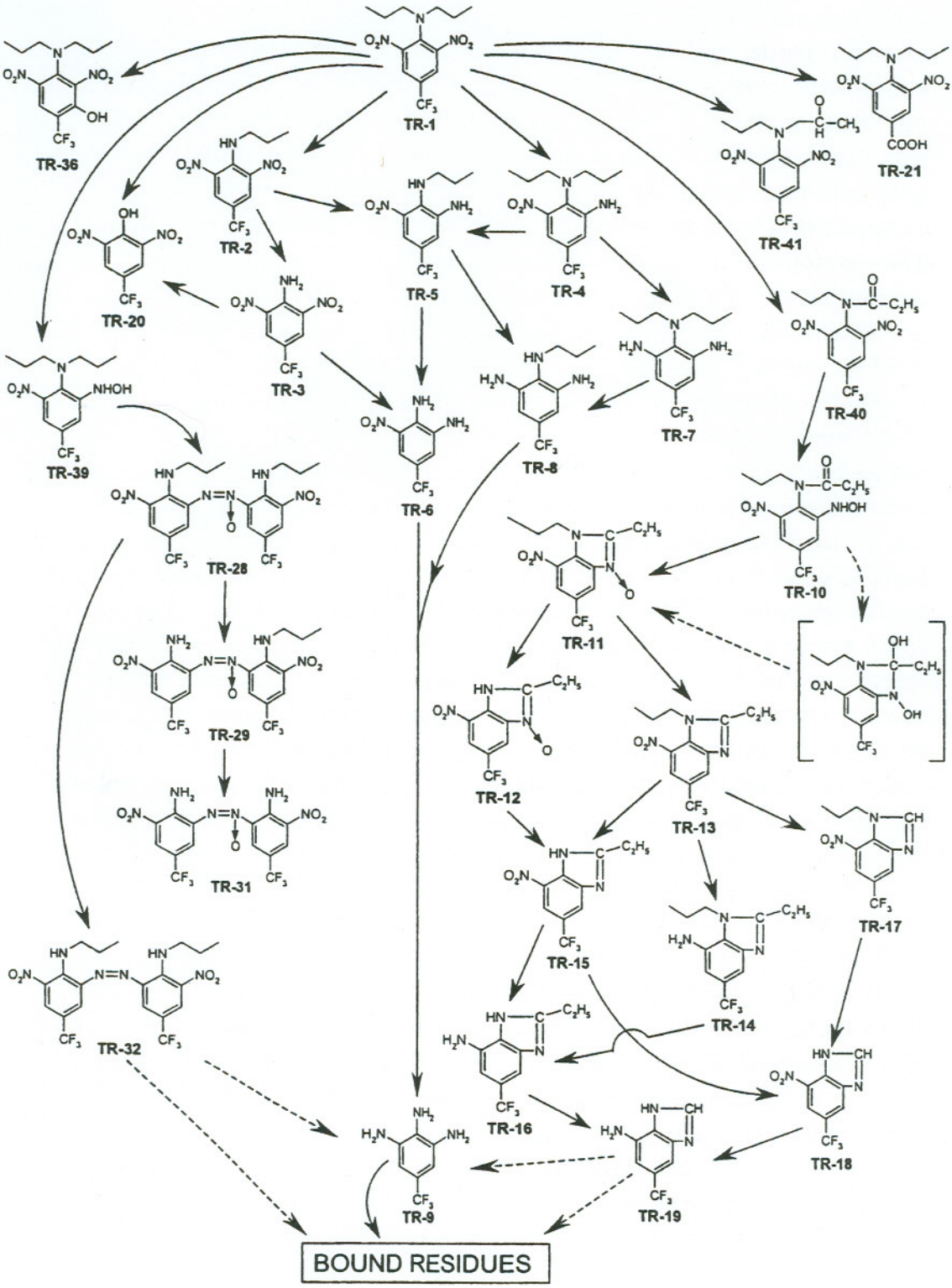

Figure 1. Proposed soil degradation pathways of trifluralin (adapted from Golab et al., 1979).

residues. Major portions of the bound residues were shown to be associated with humin, humic acid, and fulvic acid fractions of soil organic matter (*1,5,9*). Soil microbes also play an important role in bound residue formation (*9,10*). For instance, model aniline compounds have been shown to form oligomers with soil organic matter constituents when incubated in the presence of extracellular microbial enzymes (*10*). In an effort to better understand the nature of bound trifluralin residues, Golab et al. (*5*) performed adsorption-desorption studies with trifluralin and 15 of the metabolites identified in Figure 1. After 3 days, various extractants recovered at least 38% of the parent and metabolites, except TR-9 which could not be recovered by any of the extractants used. This result, combined with the trace amounts of extractable TR-9 from their field study, implicated TR-9 as the key metabolite leading to formation of trifluralin bound residues in soil.

Although a great deal of work has been conducted on the soil degradation of trifluralin, most of this work was conducted 20 to 35 years ago using radiolabeled compounds and thin-layer chromatography for separation and identification of metabolites. The study by Golab et al. (*5*) is still considered as the most authoritative work on the subject, yet only seven of the metabolites presented in Figure 1 were extracted in sufficient quantity to facilitate confirmation by GC/MS or direct probe MS. Thus, there is a compelling need to apply modern mass spectrometry to the identification and confirmation of the major trifluralin metabolites. Ion-trap liquid chromatography/ mass spectrometry (LC/MS/MS) offers great potential for this application because of its ability to facilitate separation, identification, and confirmation of compounds with a wide range of polarities. Ion-trap LC/MS/MS improves the identification and confirmation of unknowns compared to quadrupole LC/MS/MS systems by providing more structural information via MS^n capability.

The work by Golab et al. (*5*) defines the scope of possible soil degradation pathways for trifluralin, but there is no clear indication which pathways predominate. Determination of the major degradation pathways of trifluralin under field conditions needs to be achieved in order to discern the existence of stable metabolites that may contaminate soils, sediments, and waters. Given the heavy usage of trifluralin over the last 40 years, such contamination seems likely. While a comprehensive understanding of the fate of triazine and acetanilide metabolites has largely been achieved, the fate of trifluralin metabolites in the environment remains elusive. Thus, the objective of this research was to develop an approach for the identification of trifluralin metabolites in soils using ion-trap LC/MS/MS.

Materials and Methods

Trifluralin and Metabolite Standards

The analytical grade standard of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, TR-1), with a purity of 99.7%, was obtained from Axact Standards, Inc. (Commack, NY). Metabolite standards of α,α,α -trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine (TR-2), α,α,α -trifluoro-2,6-dinitro-*p*-toluidine (TR-3), α,α,α -trifluoro-5-nitro-N^4-propyl-toluene-3,4-diamine (TR-5), 2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)-benzimidazole (TR-13), and 2-ethyl-7-nitro-5-(trifluoromethyl)-benzimidazole (TR-15) were originally synthesized by Lilly Research Laboratories (currently Dow-Elanco, Indianapolis, IN) or the USDA-ARS Pesticide Degradation Laboratory (Beltsville, MD). The metabolite standards were approximately 20 years old, and their original purity was at least 95% (*11*). For this study, each metabolite standard was obtained as an ~2000 mg/L solution in ethyl acetate from the USDA-ARS Southern Weed Science Laboratory (Stoneville, MS). The exact concentration of each standard was unknown because of solvent evaporation that occurred during long-term storage. However, analysis by ion-trap LC/MS indicated that the standards had remained intact. Mass spectra obtained by ion-trap LC/MS/MS and LC/MS^3 also indicated that standards were acceptable for qualitative confirmation. Synthesis of α,α,α -trifluoro-2,6-dinitro-*p*-cresol (TR-20) was accomplished by mixing 10 mL of a 1 mg/mL solution of trifluralin in 50% aqueous methanol with 1 mL of 1 N NaOH. The mixture was heated to 80°C for 30 minutes in a sealed tube.

Ion-Trap LC/MS/MS Conditions

Ion-trap LC/MS/MS was used in positive and negative ion modes of operation to identify trifluralin metabolites. The analytes were separated using an HPLC (series 1100, Agilent Technologies, Palo Alto, CA) equipped with a C_{18} analytical column (Phenomenex RP18, Torrance, CA) having dimensions of 250 mm by 3 mm and 5-μm particle diameter. Column temperature was maintained at 25°C. The HPLC mobile phase consisted of acetonitrile and 10 mM ammonium formate buffer (pH 3.7), at a flow rate of 0.6 mL/min. A gradient elution was performed as follows: from 15% A (acetonitrile) and 85% B (10 mM ammonium formate) to 100 percent A and 0 percent B in 40 minutes; then 100% A was held isocratically for 5 minutes; and back to initial conditions

in 10 minutes. The HPLC system was connected to an ion trap mass spectrometer (Esquire LC, Bruker Daltonics, Bellerica, MA) system equipped with an electrospray ionization (ESI) probe. The operating conditions of the MS system were optimized in full-scan mode (*m/z* scan range: 50–400) by flow injection analysis (i.e., HPLC column excluded) of each standard at ~20 µg/mL concentration. The source temperature was held at 350°C. Desolvation was enhanced by a countercurrent flow of nitrogen gas at 12 L/min. The capillary exit voltage was of 70 V. For each scan, 50,000 ions were accumulated, and every 5 scans were summed. Ions were accumulated in the trap for up to 0.2 s. Helium was introduced into the ion trap at 6 x 10^{-6} mbar for the purpose of ion cooling within the trap and to facilitate fragmentation of ions during MS/MS or MS^3 experiments. MS/MS and MS^3 experiments were carried out by isolating each target ion of interest and then fragmenting it. The width of the m/z window for the isolated ion was set at two. Terminology for the various mass spectral experiments are defined as follows: precursor ion (PI) - the ion fragmented for MS/MS experiments, in this work all PIs were either $[M + H]^+$ or $[M - H]^-$; PI spectra - the LC/MS/MS spectra generated from fragmentation of the PI; product ion - an ion generated by fragmentation of the PI via LC/MS/MS; and product/product ion - an ion generated by fragmentation of a product ion via LC/MS^3.

Soils

Soils collected from two herbicide spill sites in Illinois were used because of their high potential for detection of trifluralin metabolites without concentration of the soil extracts (see details below). The intent was to use samples that would provide a straightforward opportunity to test the efficacy of the ion-trap LC/MS/MS system. One sample was collected from a spill site in Piatt County, IL (Piatt soil) in 1989 (*12*). The sample was passed through a 3-mm sieve and stored at 2-4°C. This sample was contaminated with alachlor, metolachlor, atrazine, and trifluralin. The other sample was collected from a herbicide spill site near Lexington, IL (Lexington soil) in 1990 (*13*). It was processed and stored in the same manner as the Piatt soil, and it was also contaminated with the same four herbicides. Prior to collection, the Lexington site was the scene of a fire, and the soils were briefly flooded in the process of extinguishing the blaze. Although the trifluralin concentration of these soils were unusually high, trifluralin degradation occurred under natural environmental conditions.

Soil Extraction of Trifluralin Metabolites

An Accelerated Solvent Extraction system (ASE 200, Dionex Co., Sunnyville, CA) was used for the soil extractions. Ten grams of soil were packed into an 11-mL stainless steel vessel. The packed vessels were sealed at both ends with circular cellulose filters of 2.1 cm diameter (Whatman, Springfield Mill, Maidstone, Kent, UK). Extraction conditions were as follows: 20 mL of acetonitrile: water (7:3) as extraction solvent; temperature of 120°C; pressure of 1500 psi; heating time of 6 minutes; and three cycles of 5 minute static extraction. At the end of each extraction, nitrogen gas was used to expel the extract into glass collection vials (60 second purge). The total volume of extract was ~18 mL. A 1-mL volume of the extract was passed through a syringe filter for cleanup, and 50 µL were injected into the ion-trap LC/MS/MS for analysis.

Results and Discussion

Mass Spectra of Trifluralin and Metabolites

The choice of negative or positive ion mode for a particular compound was based on the intensity of the PI formed, and the signal to noise ratio of the baseline. Compounds with a completely substituted aniline group (TR-1 and TR-13) produced high intensity PIs in positive ion mode, but they were unresponsive in negative ion mode. Compounds with partially substituted or un-substituted aniline groups, or the hydrolysis product TR-20, produced PIs of high intensity in negative ion mode (Table I). Some compounds, particularly TR-15, produced high intensity PIs in both modes. While the positive ion mode resulted in higher intensity PIs for TR-15, the baseline signal to noise ratio in negative ion mode was much lower, making it the preferred mode (Table I).

PI spectra obtained by ion-trap LC/MS/MS, equipped with an electrospray interface, provided ample fragmentation of trifluralin and its metabolites (Table I). From one to four diagnostic ions were produced, providing the needed fragmentation for confirmatory analyses. The term diagnostic ions is used to indicate product ions whose tentative identification was indicative of the PI, and they were of sufficient relative abundance (>25%) in the spectra. Only TR-13 contained the PI (m/z 302) in the PI spectra, indicating the considerable stability

Table I. Tentative identification of the diagnostic product ions in the mass spectra of trifluralin and selected trifluralin metabolites.

Compound[a]	*Molecular Weight*	*Ion Mode*[b]	t_R[c]	*LC/MS Precurson*	*LC/MS/MS Product r Ions*	*LC/MS*^{3} *Product/product Ions*
			Min.	*m/z; identification*	*m/z; identification (RA)*[d]	*m/z; identification (RA)*[d]
TR-1	335	+	34.6	336 $[M + H]^+$	294* $[M - C_3H_6 + H]^+$ (100)	252 $[294 - C_3H_6]^+$ (42)
					276 $[M - C_3H_6 - H_2O + H]^+$ (34)	248 $[294 - NO_2]^+$ (34)
					252 $[M - 2(C_3H_6) + H]^+$ (36)	236 $[294 - C_3H_7NH]^+$ (100)
					248 $[M - C_3H_6 - NO - H_2O + 3H]^+$ (47)	
					236 $[M - (C_3H_7)_2N + H]^+$ (95)	
TR-2	293	-	29.9	292 $[M - H]^-$	204 $[M - C_3H_6 - NO_2 - H]^-$ (100)	ND
TR-3	251	-	23.5	250 $[M - H]^-$	220 $[M - NO - H]^-$ (100)	ND
					203 $[M - HNO_2 - H]^-$ (27)	
TR-5	263	-	28.3	262 $[M - H]^-$	226 Not identified (100)	ND
					215 $[M - HNO_2 - H]^-$ (52)	
					204 $[M - C_3H_7NH - H]^-$ (73)	
					200 $[M - NO_2 - NH_2 - H]^-$ (83)	

TR-13	301	+	28.1	302 $[M + H]^+$	302 $[M + H]^+$ (53)	
					260* $[M - C_3H_6 + H]^+$ (100)	214 $[260 - NO_2]^+$ (100)
TR-15	259	-	20.1	258 $[M - H]^-$	228 $[M - NO - H]^-$ (100)	ND
TR-20	252	-	17.1	251 $[M - H]^-$	221 $[M - NO - H]^-$ (100)	ND

[a]See Figure 1 for compound codes. [b]+ = positive mode; - = negative mode. [c]t_R = Retention time. [d]Tentative identification; RA = relative abundance in percent. * Indicates product ion fragmented for LC/MS^3. ND = not determined.

of its molecular structure. Ion-trap LC/MS^3 provided an additional diagnostic ion for TR-13 by fragmentation of m/z 260 to form product/product ion, m/z 214 (Table I).

Fragmentation patterns in the PI spectra were typically characterized by losses of propyl, NO, NO_2, or propylamine groups (Table I). For those compounds with one or more propyl groups (TR-1, TR-2, TR-5, and TR-13), loss of the propyl group was key to formation of the base peaks, except TR-5. Base peak formation in the spectrum of TR-5 has not yet been resolved. For compounds with no propyl groups (TR-3, TR-15, and TR-20), base peak formation typically occurred by loss of an NO group.

Identification and Confirmation of Trifluralin Metabolites

Trifluralin metabolites were identified and confirmed by the following procedure: 1) perform selective ion monitoring to screen for PI masses; 2) compare HPLC retention time of the PI peak to standards; and 3) obtain PI spectra and compare fragmentation with standards. Hence, the procedure provided three criteria for confirmation. Tentative metabolite identifications presented in Table II were based on the first two confirmation criteria. Because this work is preliminary, PI spectra have not yet been obtained on all metabolites. The presence of a metabolite was considered confirmed if all three confirmation criteria were met. To ascertain the presence of metabolites for which no standard was available, retention times and PI fragmentation were compared to the standard representing the closest structural analogue. Overall, TR-1 and the 28 metabolites identified by Golab et al. (*5*) were screened for their presence in the soil extracts.

In the Lexington soil, trifluralin and eight metabolites were identified, and in the Piatt soil, trifluralin and three metabolites were identified (Table II). Confirmation analyses are pending for TR-6, TR-9, and TR-41 in the Lexington soil, and for TR-13, TR-15, and TR-20 in the Piatt soil. In the Lexington soil, relative intensities of the PIs for all metabolites, except TR-2 and TR-5, ranged from 10^4 to 10^6 ion counts and were comparable to the relative intensity of trifluralin in this soil. Intensity of the TR-5 PI (~900 ion counts) was insufficient to facilitate detectable product ion formation (Table II). The PI intensity of 5300 ion counts for TR-2 was, however, sufficient for detectable product ion formation. In the Piatt soil, PI intensities of TR-13, TR-15, and TR-20 were

Table II. Identification and Confirmation of Trifluralin Metabolites

Soil	Metabolite	Tentative Identification		Confirmation[a]	
		t_R[b]	LC/MS[c]	LC/MS/MS	LC/MS^3
		min.	m/z	m/z	m/z
Lexington	TR-2	30.0	292 $[M - H]^-$	204[d]	ND
	TR-5	28.3	262 $[M - H]^-$	*	
	TR-6	20.3	222 $[M + H]^+$	ND	
	TR-9	15.3	192 $[M + H]^+$	ND	
	TR-13	28.1	302 $[M + H]^+$	260[d]	214[d]
	TR-15	20.1	258 $[M - H]^-$	228[d]	
	TR-20	17.2	251 $[M - H]^-$	221[d]	
	TR-41	36.9	352 $[M + H]^+$	ND	
Piatt	TR-13	28.1	302 $[M + H]^+$	ND	
	TR-15	20.1	258 $[M - H]^-$	ND	
	TR-20	17.1	251 $[M - H]^-$	ND	

[a]Observed diagnostic product ions (see Table I). [b] t_R = Retention time. [c]Observed precursor ion. [d]Base peak. ND = not determined. * = insufficient precursor ion intensity to perform MS/MS.

about an order of magnitude lower than the Lexington soil. This was likely an indication of its lower initial trifluralin levels compared to the Lexington soil.

Four of the eight metabolites identified in the Lexington soil were confirmed by LC/MS/MS or LC/MS^3. For all confirmed metabolites, diagnostic product ions were observed (Table II). Example precursor and product ion spectra for TR-13 and TR-15 from the Lexington soil are given in Figure 2. For TR-13 confirmation, the PI (m/z 302) was isolated in the spectrum from the soil extract and the diagnostic product ion (m/z 260) was observed. Furthermore, the product ion spectra of m/z 260 yielded the diagnostic m/z 214 ion, providing definitive confirmation of this metabolite.

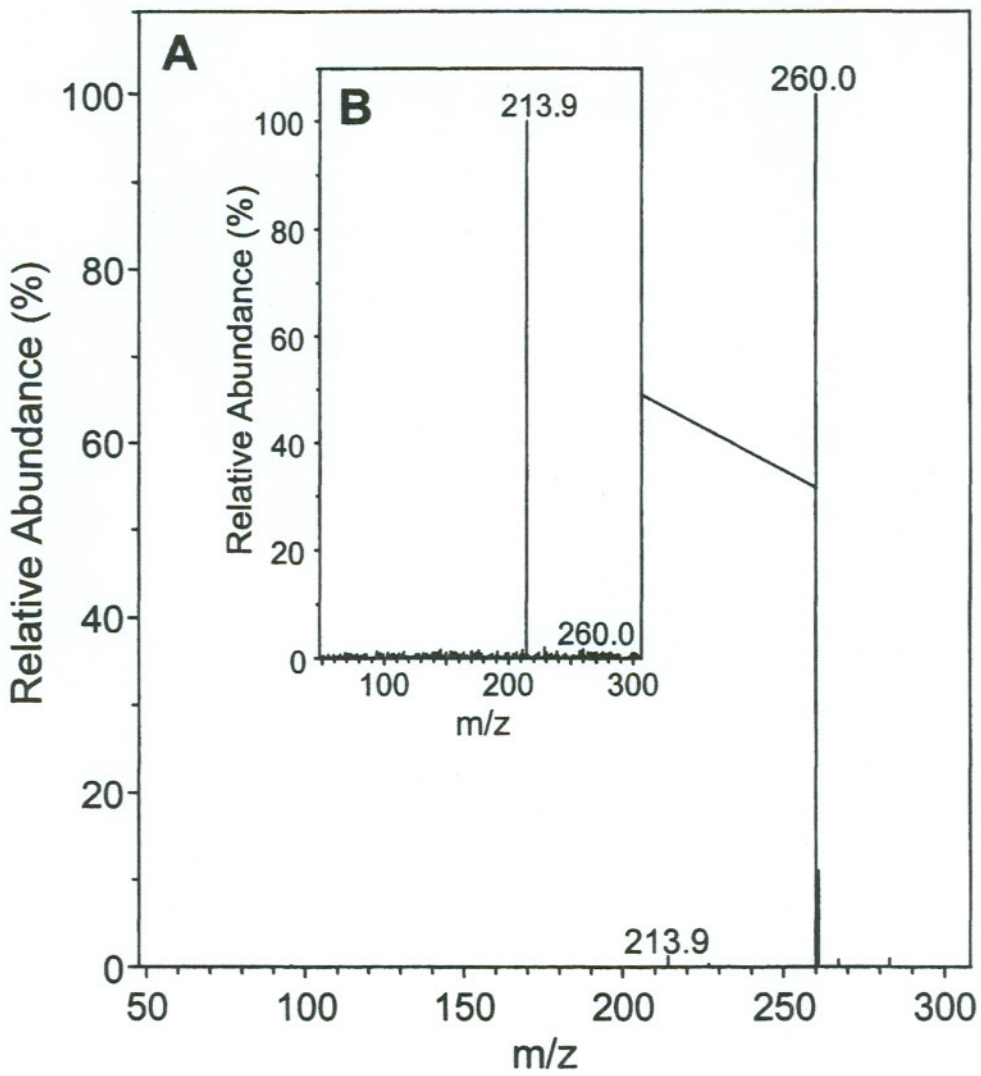

Figure 2. Confirmation of TR-13 and TR-15 in Lexington soil extract. A. Ion-trap LC/MS/MS of TR-13 precursor ion (m/z 302) yields diagnostic m/z 260 product ion. B. Ion-trap LC/MS^3 of TR-13 product ion (m/z 260) yields diagnostic m/z 214 product/product ion. C. Ion-trap LC/MS/MS of TR-15 precursor ion (m/z 258) yields diagnostic m/z 228 product ion.

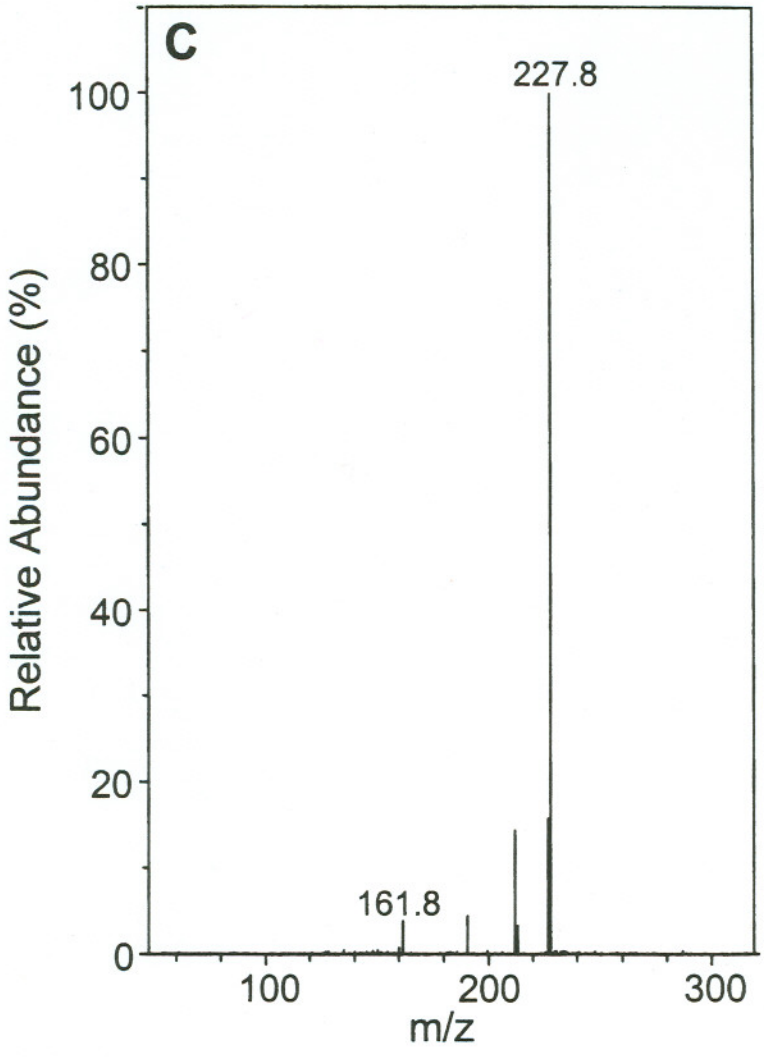

Figure 2. *Continued.*

Validation of the Procedure - Confirmation of TR-20

Upon initiation of these studies, authentic standards were not available for four of the metabolites (TR-6, TR-9, TR-20, and TR-41) tentatively identified in the soils (Table II). Of these four metabolites, TR-20 was detected in both soils with high PI intensities, and it was the most facile to synthesize because of the susceptibility of trifluralin to alkaline hydrolysis at high temperatures (*14*). Thus, the tentative identification of TR-20 in both soils provided an ideal opportunity to test the validity of the ion-trap LC/MS/MS procedure to identify metabolites for which standards were (initially) unavailable.

Flow injection MS of the trifluralin hydrolysis products showed that TR-20 (PI m/z 251) was the major product form, with much smaller amounts of one other product (m/z 287) (Figure 3). Subsequent PI spectra of m/z 251 showed the product ion base peak of m/z 221, formed by the loss an NO group (Figure 3; Table I). The next step was to confirm the tentative identification of TR-20 in the soil extracts against the synthesized TR-20 standard, using the same conditions as employed for the initial metabolite identification. Ion-trap LC/MS of the PI (m/z 251) showed an exact retention time match between the soil extracts and the TR-20 standard (Figure 4). Furthermore, PI spectra confirmed the presence of TR-20 by producing the diagnostic product ion, m/z 221 (Figure 4). These experiments validated the developed procedure for identifying trifluralin metabolites in soils, even for cases in which authentic standards were not available. Furthermore, the efficacy of ion-trap LC/MS/MS as a tool for identifying pesticide metabolites in environmental samples was convincingly demonstrated.

Trifluralin Degradation Pathways

The presence and absence of the 28 metabolites provided insight to the predominant trifluralin degradation pathways in these soils (Figure 1). Because of the high levels of trifluralin and other herbicides present, these soils certainly do not represent typical agronomic settings. Nonetheless, the study by Wheeler et al. (*6*) showed that extremely high trifluralin levels (20,000 mg/kg) did not affect degradation rates or pathways compared to levels that were one- or three-orders of magnitude less. Therefore, the metabolite information obtained from these soils may still contribute relevant information to our understanding of trifluralin degradation.

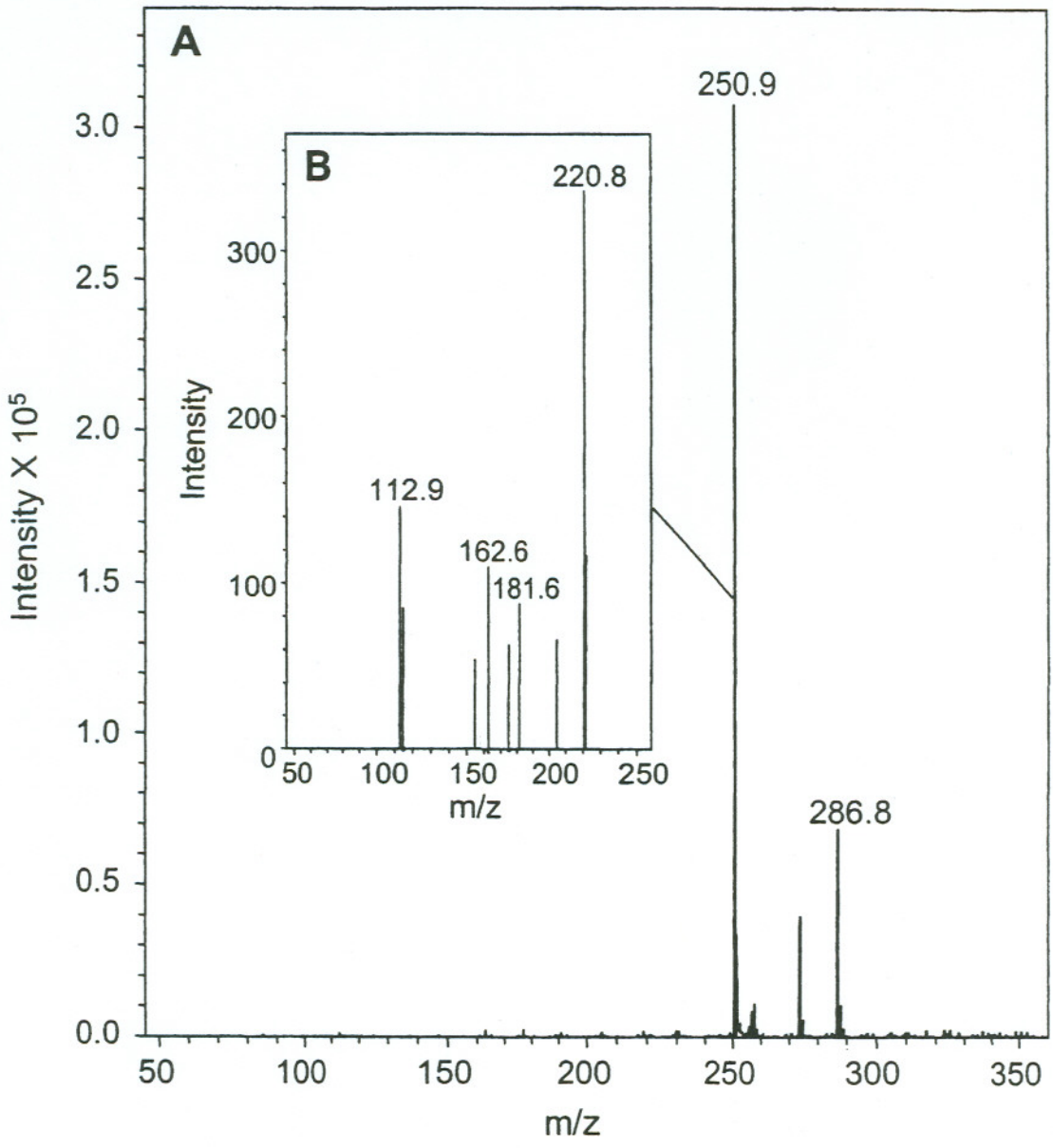

Figure 3. Flow injection ion-trap mass spectra of synthesized TR-20 standard. A. Negative ion mode MS of trifluralin hydrolysis products shows the TR-20 precursor ion (m/z 251). B. Precursor ion (m/z 251) spectra (MS/MS) yields diagnostic product ion, m/z 221.

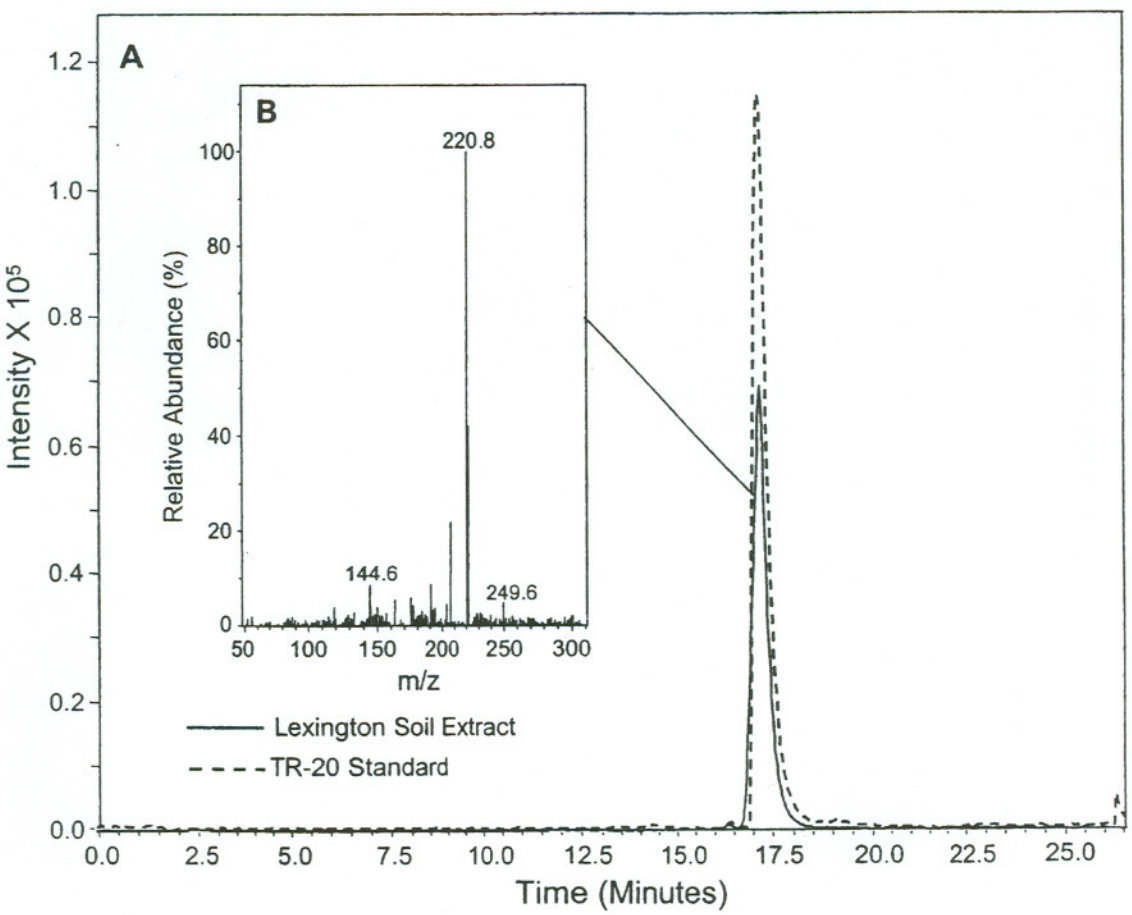

Figure 4. Confirmation of TR-20 in Lexington soil. A. Ion-trap LC/MS chromatograph of the precursor ion (m/z 251) from Lexington soil extract and TR-20 standard. B. Ion-trap LC/MS/MS of precursor ion from Lexington soil extract yields diagnostic m/z 221 product ion.

Dealkylation, Reduction, and Hydrolysis

The presence of TR-2, TR-5, TR-6 and TR-9 demonstrated the combined importance of dealkylation and reduction reactions to trifluralin degradation in the Lexington soil. The presence of these metabolites implied that aerobic degradation conditions prevailed (*4*). This was further supported by the absence of the completely reduced metabolites (TR-7 and TR-8). Furthermore, TR-4 was also absent, although its formation has been noted to occur under aerobic conditions (*5*). It is possible that TR-4 was an unstable intermediate in the pathway leading to TR-9 formation. However, Wheeler et al. (*6*) detected TR-2, but not TR-4, when trifluralin was degraded under aerobic conditions, a finding similar to the results of this study. Therefore, a more likely scenario was initial dealkylation of trifluralin to TR-2 followed by a combination of dealkylation and reduction reactions leading to formation of TR-9. The intensity of the TR-6 PI in the Lexington soil was about an order of magnitude greater than that of TR-9. This finding suggested that TR-6 was present in greater concentration than TR-9. Reduction of the first nitro group of a polynitro-aromatic (e.g., TR-2 or TR-3) can occur rapidly, but reduction of the second nitro group is a much slower reaction (*15*). Therefore, the reaction kinetics of polynitro-aromatic reduction favor the formation and stability of TR-6 over that of TR-9, especially when aerobic conditions prevail. The absence of TR-3, combined with the presence of TR-2, TR-6, and TR-20, suggested that it is either an unstable intermediate or that its formation is excluded in favor of reduction or direct hydrolysis of trifluralin. The presence of TR-20 in both soils showed the importance of its direct formation via trifluralin hydrolysis or its indirect formation via dealkylation and subsequent hydrolysis of TR-3.

Benzimidazole Formation, Condensation, and Other Reactions

The benzimidazole metabolites, TR-13 and TR-15, were two of the most prevalent metabolites detected in both soils, yet neither their antecedent metabolites (TR-40, TR-10 to TR-12) nor their subsequent degradation products (TR-14, TR-16 to TR-19) were detected. This implied that the antecedent metabolites were unstable intermediates and that TR-13 and TR-15 represent potentially stable end-points in the soil environment. This conclusion is supported by the findings of Wheeler et al. (*6*), and by the pathways of aerobic degradation proposed by Probst et al. (*4*). Oxidation of a propyl group to form TR-41 was also observed in the Lexington soil, further supporting the importance of aerobic degradation in this soil. The absence of TR-39, TR-32, and the azoxy metabolites (TR-28, TR-29, and TR-31) in either soil, eliminated condensation reactions as an important pathway for trifluralin degradation in

these soils. The absence of metabolites formed by hydroxylation (TR-36) or trifluoromethyl oxidation (TR-21) indicated that neither of these pathways were significant to trifluralin degradation.

The array of metabolites present in these soils was consistent with aerobic degradation of trifluralin (*4,6*) and suggested that the predominant degradation pathways were as follows: 1) benzimidazole formation, TR-1→TR-40→TR-10→TR-11→TR-13→TR-15; 2) dealkylation and reduction, TR-1→TR-2→TR-5→TR-6→TR-9; 3) direct TR-20 formation via trifluralin hydrolysis, TR-1→TR-20; and 4) indirect TR-20 formation via dealkylation and hydrolysis, TR-1→TR-2→TR-3→TR-20.

Implications for Environmental Contamination

Stable pesticide metabolites represent potential environmental contaminants. Based on this study and other studies, the most stable metabolites of trifluralin are TR-6, TR-9, TR-13, TR-15, and TR-20. Previous studies of trifluralin degradation have emphasized that none of the identified trifluralin metabolites represented more than 3 to 4% of the applied trifluralin (*4,5*). Similarly low percentages of the atrazine metabolites, deethylatrazine and deisopropylatrazine, have been shown to form as a result of atrazine degradation in soils (*16,17*). However, these two metabolites are among the most significant contaminants of surface and ground waters in the Midwestern U.S. (*18-20*). Thus, seemingly insignificant formation of metabolites based on soil degradation studies, alone, can lead to the erroneous conclusion that the metabolites are of minimal environmental concern. With regards to off-site hydrologic transport, soil degradation studies do not take into account the importance of metabolite chemical properties, nor the impact of repeated herbicide usage on metabolite accumulation in soils.

Of the trifluralin metabolites identified in this study, TR-20 is the only metabolite that represents a potentially important dissolved-phase contaminant in surface and ground waters due to the likelihood of a low acid dissociation constant. Acid dissociation constants of structurally similar dinitrophenols are about 10^{-4}. Therefore, TR-20 should be present in the environment as an anion under the typical pH range of soils. The other stable trifluralin metabolites, TR-6, TR-9, TR-13, and TR-15, are likely to contaminate soils and sediments in watersheds with high trifluralin usage. These metabolites are likely to have high sorption intensity as a result of their hydrophobicity (TR13 and TR-15) or tendency to form bound residues (TR-6 and TR-9). All four metabolites would also have a strong propensity to form H-bonds with soil colloids. Since these metabolites will primarily be transported from fields sorbed to soil particles,

their impact on the aquatic environment will likely occur via slow desorption from stream or lake sediments.

Summary

Ion-trap LC/MS/MS was successfully applied to the identification and confirmation of eight trifluralin metabolites in soils. The developed analytical procedure provided three points of confirmation based on PI formation, HPLC retention time, and diagnostic product ion formation. The procedure was validated for metabolite identification, even for cases in which standards were unavailable, by confirming the presence of a tentatively identified metabolite (TR-20). To date, four of the eight metabolites tentatively identified have been confirmed. Metabolites present in the soils represented formation via benzimidazole formation (TR-13 and TR-15), hydrolysis (TR-20), dealkylation (TR-2, TR-5, TR-6, TR-9), reduction (TR-5, TR-6, TR-9), and oxidation (TR-41). This array of metabolites was consistent with aerobic degradation of trifluralin and indicated that the predominant degradation pathways occurred via the following reactions: 1) benzimidazole formation; 2) dealkylation and reduction; 3) direct TR-20 formation via trifluralin hydrolysis; and 4) indirect TR-20 formation via dealkylation and hydrolysis. The most stable trifluralin metabolites appear to be TR-6, TR-9, TR-13, TR-15, and TR-20. These stable metabolites represent potential environmental contaminants. TR-20 represents a potentially important dissolved-phase contaminant in surface and ground waters. The other stable trifluralin metabolites are likely to contaminate soils and sediments in watersheds with current or historically high trifluralin usage. Future work will focus on completing the confirmation analyses of trifluralin metabolites in these soils, as well as soils collected from typical agronomic settings. In addition, the presence of these metabolites in soils, sediments, and waters obtained from watersheds with high trifluralin usage will be pursued.

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