Transformation of the Glucosinolate-Derived Allelochemicals Allyl Isothiocyanate and Allylnitrile in Soil

Vladimir Borek, Matthew J. Morra,* Paul D. Brown, and Joseph P. McCaffrey

Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, Idaho 83844-2339

Hydrolysis of glucosinolates in *Brassica* tissues results in formation of allelochemicals potentially useful in controlling soil-borne plant pests. Major degradation products of glucosinolates in soil are organic isothiocyanates and nitriles; however, a clear understanding of allelochemical persistence in soil is lacking. Half-lives of allyl isothiocyanate (AI) and allylnitrile (AN) in six soils were determined using gas chromatographic analysis of ethyl acetate extracts. The half-lives for AI ranged from 20 to 60 h, whereas AN had longer half-lives of 80–120 h. AI transformation increased with reduced soil moisture and higher temperatures and occurred more rapidly in soils containing greater concentrations of organic carbon. AN transformation increased under wetter conditions and lower temperatures and occurred more rapidly in soils having higher inorganic carbon concentrations. Although different mechanisms contribute to substrate disappearance, the relatively rapid dissipation of AI and AN in soil has important implications in the control of soil-borne plant pests with *Brassica* tissues.

Keywords: *Glucosinolates; isothiocyanates; nitriles; allelochemicals; Brassica spp.*

INTRODUCTION

Glucosinolates are organic anions containing a β-D-thioglucose moiety, a sulfonated oxime, and an aliphatic, aromatic, or heterocyclic side chain. They are distributed predominantly in plants of the order Capparales (*census* Cronquist or Taktajan) and, in the context of cultivated plants, mainly in the family Cruciferae (Kjaer, 1974; Crisp, 1976). Incorporation of plant material containing glucosinolates into soil shows promise for reducing soil-borne pathogens and other plant pests (Lichtenstein et al., 1964; Papavizas, 1966; Röbbelen and Thies, 1980; Winkler and Otto, 1980; Chan and Close, 1987; Brown et al., 1991; Mojtahedi et al., 1991). Glucosinolates themselves possess limited biological activity until they are hydrolyzed by the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1). Enzymatic hydrolysis of glucosinolates produces D-glucose, SO_4^{2-}, and a variety of potential allelochemicals dependent on the specific aglycon chain structure and reaction conditions. The latter products of enzymatic glucosinolate hydrolysis generally show toxic, antinutritional, and allelochemical effects (Chew, 1988).

Formation and proportions of isothiocyanates, nitriles, thiocyanates, epithionitriles, oxazolidinethiones, amines, and other reaction products depend on the aglycon chain structure of the particular glucosinolate and on reaction conditions (Uda et al., 1986). Isothiocyanates and nitriles are two groups of compounds that are produced in high yield by enzymatic decomposition of glucosinolates. Isothiocyanates are linked with the majority of phytotoxic, nematocidal, fungicidal, insecticidal, and other allelochemical effects of glucosinolate degradation products. More than 50 organic isothiocyanates have been identified as natural products of glucosinolate breakdown (Ettlinger, 1956; Ettlinger and Lundeen, 1957; Kjaer, 1960). Nitriles are the most abundant degradation products when enzymatic degradation of glucosinolates occurs in a medium with a low pH (Fenwick et al., 1989) or low buffering capacity (Borek et al., 1994).

For unknown reasons the inhibitory effect of glucosinolate-containing plant material on soil-borne plant pests is inconsistent (Papavizas and Lewis, 1971; Waddington, 1978; Parke and Rand, 1989; Choesin and Boerner, 1991). It is plausible that transformation of isothiocyanates and nitriles into less biologically active products is important. Inconsistencies in nematocidal effects were observed with metham-sodium, a soil fumigant whose active component is methyl isothiocyanate. The kinetics of transformation and the movement of methyl isothiocyanate in the soil environment have been described (Ashley and Leigh, 1963a,b; Leistra et al., 1974; Smelt and Leistra, 1974; Smelt et al., 1974, 1987, 1989; Vandenberg et al., 1992). However, the dominant isothiocyanates in *Brassica* species are mostly unsaturated aliphatics or aromatics with 3–10 carbon units (Fenwick et al., 1989). Soil persistence, stability, and toxicity of glucosinolate-derived isothiocyanates are different from those of methyl isothiocyanate. There is a similar lack of information concerning nitrile transformations in soil (Deraadt et al., 1992; Zhang et al., 1990).

The major factors controlling the allelochemical impact of glucosinolate degradation products are biological activities and transformation rates in the soil environment. It is already well established that isothiocyanates and nitriles are produced from glucosinolates and can inhibit a wide range of soil organisms; we focused our efforts on defining the transformation rates of these compounds. Transformation in this context is used to describe a situation wherein the primary compound can no longer express its biological activity as a result of chemical conversion or irreversible sorption to soil constituents. That portion of the primary compound applied but unrecoverable from soil samples by ethyl acetate/water extraction was in the current situation considered transformed.

We used commercially available allyl isothiocyanate

* Author to whom correspondence should be addressed [telephone (208) 885-6315; fax (208) 885-7760; e-mail mmorra@marvin.ag.uidaho.edu].

0021-8561/95/1443-1935$09.00/0

Table 1. Characteristics of Soils Used in Allyl Isothiocyanate and Allylnitrile Transformation Experiments

soil series	soil subgroup	pH	organic C, g/kg	total C, g/kg	inorganic C, g/kg	total N, g/kg
Portneuf sil	Durixerollic Calciorthid	7.55	11.6	15.6	4.0	1.7
Latahco sil	Argiaquic Xeric Argialboll	6.10	41.0	41.0	ND[a]	3.9
Avonville sil	Andic Xerumbrept	5.65	39.0	39.0	ND	3.9
Chard sandy loam	Calcic Haploxeroll	9.10	0.5	6.0	5.5	0.1
Porrett silt loam	Typic Ochraqualf	7.25	1.1	3.0	1.9	0
ID 1151-6 Spodosol	Andic Haplocryod	4.35	8.3	8.3	ND	0.25

[a] ND indicates no inorganic carbon detected.

(AI) and allylnitrile (AN) as model compounds to study transformation kinetics in the soil environment. Allyl glucosinolate is contained in *B. carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, and *Crambe abyssinica*, and the corresponding isothiocyanates and nitriles are expected from its hydrolysis. Our objective was to determine the soil variables controlling transformation rates of AI and AN in soil.

MATERIALS AND METHODS

Chemicals and Solutions. Solutions (20 mg/mL) of AI (Aldrich, Milwaukee, WI; 97%; density 1.013) and AN (Aldrich; 98%; density 0.834) were prepared by dissolving pure compounds in acetone. Acetone solutions of AI and AN are stable for at least 1 month when stored at laboratory temperatures. Between 1 and 50 μL of the acetone solution was pipetted into 1 g of soil to amend it with AI and AN in the concentration range of 20−1000 μg/g of soil. A stock solution of the internal standard was prepared by dissolving 10 μL of phenyl isothiocyanate (J. T. Baker, Phillipsburg, NJ; 99%; density 1.130) in 990 μL of ethyl acetate.

Soils. Surface samples (0−20 cm) were collected, air-dried, crushed, and sieved (2 mm) (Table 1). Soil samples were analyzed for pH by combination glass electrode (1:1 soil to water), for organic C by a modified Walkley−Black method (Nelson and Sommers, 1982), for total C and N by Dumas combustion (LECO CHN-600 determinator, St. Joseph, MI), for particle size distribution by the hydrometer or pipet method (Gee and Bauder, 1986), and for water content at a suction of 0.033 MPa using a ceramic pressure plate (Klute, 1986).

Transformation Assay. Air-dried soil was weighed into 5-mL glass vials, deionized water was added to reach a particular soil moisture, and the samples were allowed to equilibrate at 25 °C for at least 72 h. Unless otherwise noted, all soils were incubated at moisture contents equivalent to 75% of the amount held at soil suctions of 0.033 MPa. The acetone solution containing AI and AN was then added to each soil sample. The samples were homogenized with a stainless steel spatula, and the vials were closed with gastight caps and stored during the test period in a temperature-controlled incubator. To minimize losses due to volatilization and interferences with materials other than soil, all assays were conducted using glass vials closed with Teflon-lined lids.

The transformation rates of AI and AN at an initial concentration of 500 μg/g of wet soil were determined in six soils (Table 1) during a period of 10 days at a temperature of 20 °C. Residual amounts of AI and AN were extracted after 3, 6, 12, 24, 48, 120, and 240 h.

The effect of soil moisture was determined using Latahco soil samples with 30, 50, 75, 100, and 300% of the amount of water relative to that held at soil suctions of 0.033 MPa. The starting concentration of the two test chemicals was 500 μg/g of wet soil, and the temperature was maintained at 20 °C. AI and AN were extracted after 3, 6, 12, 24, and 48 h.

The effect of substrate concentration on transformation rates was determined by amending Latahco soil with 50, 100, 250, 500, and 1000 μg of AI and AN/g of soil. The temperature was maintained at 20 °C, and samples were extracted after 3, 6, 12, 24, and 48 h.

The effect of temperature was determined in Latahco soil amended with 500 μg/g of AI and AN. Transformation rates were determined during a period of 48 h at temperatures of 10, 15, 20, and 25 °C with sample extraction after 3, 6, 12, 24, and 48 h.

The role of biological activity in mediating AI and AN disappearance in Latahco soil was determined using soil samples sterilized by autoclaving or ethylene oxide treatment. Sample vials were plugged with cotton and autoclaved for 30 min at a temperature of 120 °C and a pressure of 1.4 MPa, and the procedure was repeated after 24 h. The soil samples were allowed to dry for 24 h at 50 °C, rewetted with sterilized deionized water, and equilibrated for 48 h. Soil samples prepared the same way as for autoclaving were treated with ethylene oxide for 2 h at 40 °C and a pressure of 50 mmHg (Block and Lawrence, 1968). Treated sample vials were plugged with sterile cotton and left to stand for 24 h in the hood, amended with sterilized deionized water, and allowed to equilibrate for 48 h. Samples amended with 100 μg of AI and AN/g of wet soil were incubated at 20 °C and extracted after 3, 6, 12, 24, and 48 h.

In a separate set of experiments, repeated applications of the two test chemicals were used in an attempt to promote enhanced biodegradation. Weighed soil samples were wetted, allowed to equilibrate for 48 h, and amended five times during 10 days with solutions of AI and AN in acetone (20 μL of solution/g of wet soil at each application).

Extraction from Soil. Soil samples were amended with 2.0 mL of ethyl acetate, 25 μL of the phenyl isothiocyanate internal standard solution, and 1.0 mL of a 0.1 M calcium chloride solution. The samples were vigorously shaken for 1−2 min to create a suspension of soil, water, and ethyl acetate. Vials were then shaken for an additional 30 min on a reciprocal shaker and centrifuged for 5 min at 322*g*. Ethyl acetate extracts (top layers) were removed and dried with anhydrous sodium sulfate, filtered through 0.45 μm disk filters (PTFE, Gelman), and stored at −20 °C before GC analysis.

GC Analysis of Ethyl Acetate Extracts. Concentrations of AI and AN in ethyl acetate extracts were determined by GC (HP 5890A, Hewlett-Packard, Avondale, PA) using a DB-5 capillary column (30 m × 320 μm, 0.25 μm film, J&W Scientific, Folsom, CA) and a temperature program from 65 to 270 °C. Compounds were detected using a flame ionization detector (FID) and quantified using phenyl isothiocyanate as an internal standard. Response linearity of the FID was tested by analysis of ethyl acetate solutions of AI and AN at concentrations from 2 to 1000 μg/mL. Extraction efficiency and reproducibility of the method were verified by analysis of soil samples amended with known amounts of AI and AN in the concentration range of 2−1000 μg/mL. Extraction was performed as previously described 15 min after soil amendment.

Data Analysis. All treatments were replicated three times. Concentration−time data were analyzed using regression analysis to obtain kinetic parameters of AI and AN transformation rates in soil. Attempts were made to fit the data with linear equations, kinetic equations from zero to second order, and empirical kinetic relationships (Zar, 1984; Laidler, 1987; Sparks, 1989; Stone and Morgan, 1990). The following integral equation describing the first-order decrease in concentration of the reactant was used for regression analysis:

$$\ln(c_0 - c_a) = \ln(c_0) - k_a t \quad (1)$$

c_a is the actual concentration of AI or AN at time t, c_0 the initial concentration of each respective substrate, and k_a the reaction rate constant. The relationship between the reaction rate constant and the reaction half-life, $t_{1/2}$ (time at which concentration of the reactant decreases to 50% of the starting value), is expressed by the following relationship (Laidler, 1987):

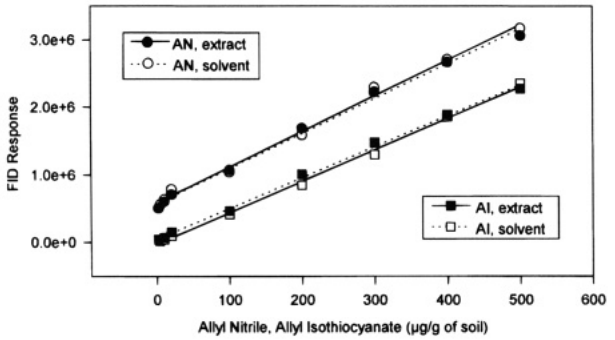

Figure 1. Comparison of gas chromatographic detector response to AI and AN in calibration solutions and soil extracts. Each point represents the mean of three replications. Variability of replications is smaller than can be displayed. All four data sets fit a linear regression model: FID response = concentration; AI solvent, F = 2290, Pr > F = 0.0001; AI extract, F = 2857, Pr > F = 0.0001; AN solvent; F = 1125, Pr > F = 0.0001; AN extract, F = 1178, Pr > F = 0.0001. Solvent and extract curves are not significantly different as determined using a coincidence test of regression lines: AI, F = 3.24, Pr > F = 0.0749; AN, F = 0.40, Pr > F = 0.6758.

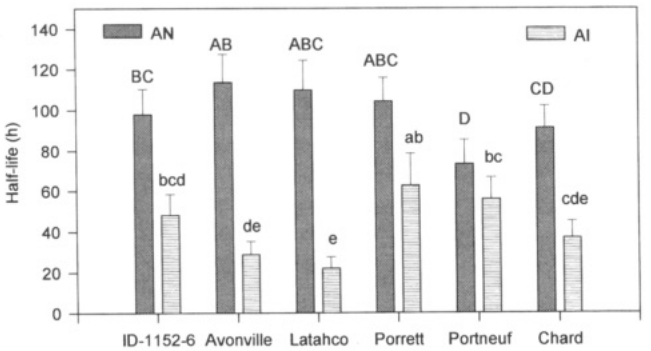

Figure 2. Means ± SE of three replications of AI and AN half-lives in six soils. Multiple comparison tests showed that means in the AI and AN data sets are significantly different (Fisher's least significant difference test; AI, small letters; d_f = 6, 14; F = 8.51, Pr > F = 0.0005; AN, capital letters; d_f = 6, 14; F = 5.52, Pr > F = 0.0041).

$$t_{1/2} = \ln(2)/k_a \quad (2)$$

Half-lives calculated from the kinetic data were used to evaluate transformation rates of AI and AN in soils. SAS software (SAS Institute Inc., 1985) was used for all statistical and numerical calculations, and all statistical inferences were determined at a 95% level of probability.

RESULTS

Extraction Efficiency. Calibration curves of ethyl acetate solutions of AI and AN were linear in the concentration range 0.1−1000 μg/mL (Figure 1). Calibration curves for AI and AN extracted from spiked soil were also linear in the concentration range 2−1000 μg/mL. Concentrations of both tested chemicals in soil extracts were not different from the respective calibration curves of the same compounds directly dissolved in ethyl acetate solutions.

Transformation Rates in Different Soils. The transformation rate of AI was faster than that of AN in the six soils tested (mean half-life of AI was 47 ± 27 h; mean half-life of AN was 102 ± 24 h) (Figure 2). During the first 24 h, an average of 29.8% of AI and 15.0% of the original AN was transformed. An average of 97.1% of the AI and 80.0% of the AN was transformed during the first 10 days. AI was transformed most rapidly in Latahco soil and most slowly in Porrett soil. AN disappeared most rapidly in Portneuf and most slowly in Avonville soil. Means of the transformation half-lives of AI as well as AN were determined to be different using Fisher's least significant difference test (AI, F = 8.51, Pr > F = 0.005; AN, F = 5.52, Pr > F = 0.0041).

Table 2. Correlation of Soil Characteristics with Transformation Rates of Allyl Isothiocyanate (AI) and Allylnitrile (AN)[a]

compd	pH	organic C	inorganic C	total N	clay	sand
AI	0.2296	−0.6666[a]	0.2129	−0.6292[a]	0.1256	−0.1307
AN	−0.3217	0.4803	−0.5456[a]	0.3801	0.0481	0.0271

[a] Correlation coefficients that are significant at P < 0.05.

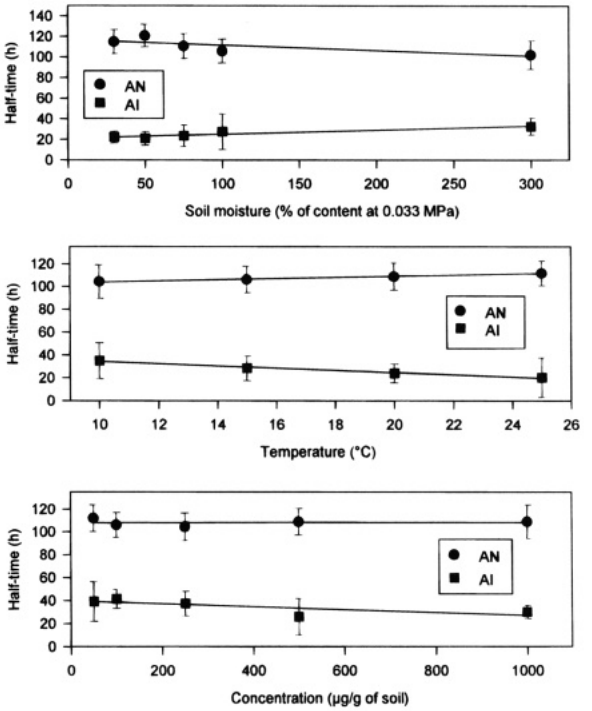

Figure 3. Relationship of AI and AN half-lives in Latahco soil to soil moisture content, incubation temperature, and original substrate concentration.

Influence of Soil Characteristics on Transformation Rates. Half-lives of AI negatively correlated with organic carbon and total nitrogen contents (Table 2). Half-lives of AN negatively correlated with inorganic carbon content. Correlations of the remaining soil characteristics with half-lives of AI and AN are not significant (P < 0.05) (Table 2).

Alteration in Transformation Rates As Related to Soil Moisture. Half-lives of AI positively correlated with increased soil water content in Latahco soil (r = 0.940), but the half-lives of AN negatively correlated with increased soil water content (r = −0.778) (Figure 3). Half-lives for AI in Latahco soil varied from 22 (lowest water content) to 26 h (highest water content) and for AN from 115 h (lowest water content) to 102 h (highest water content).

Alteration in Transformation Rates As Related to Temperature. Increased incubation temperature from 10 to 25 °C negatively correlated with the half-life of AI in Latahco soil (r = −0.996) but positively correlated with the half-life of AN (r = 0.990) (Figure 3). The difference between the half-lives of AI at 10 and 25 °C was 15 h (35 h at 10 °C and 20 h at 25 °C). The difference between the half-lives of AN at 10 and 25 °C was 7 h (104 h at 10 °C and 111 h at 25 °C).

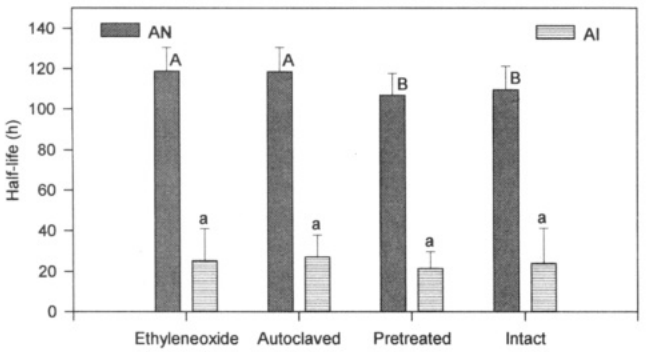

Figure 4. Means ± SE of three replications of AI and AN half-lives in differentially treated Latahco soil. Multiple comparison tests showed that only means within the AN data set are significantly different (Fisher's least significant difference test; AI, small letters; $d_f = 3, 8$; $F = 1.02$, Pr > $F = 0.4343$; AN, capital letters; $d_f = 3, 8$; $F = 8.00$, Pr > $F = 0.0086$).

Influence of Substrate Concentration on Transformation Rates. There was a negative correlation between AI concentration and its respective half-life in Latahco soil ($r = -0.950$). The difference between the half-lives of AI at 50 and 1000 μg/g was 9 h (39 h at 50 μg/g and 30 h at 1000 μg/g). In contrast, AN concentration did not correlate with half-life in Latahco soil ($r = 0.068$), thus indicating substrate-independent transformation rates (Figure 3). Linear regression analysis confirmed that only the slope of the curve for AI half-life with concentration differs significantly from zero.

Altered Transformation Rates as a Result of Soil Pretreatment. No differences occurred in half-lives of AI as a result of Latahco soil pretreatment ($d_f = 3, 8$; $F = 1.02$, Pr > $F = 0.4343$) (Figure 4). Soil sterilization by ethylene oxide treatment or autoclaving increased AN half-life as compared to both untreated Latahco soil or Latahco soil previously treated with AN ($d_f = 3, 8$; $F = 8.00$, Pr > $F = 0.0086$) (Figure 4).

DISCUSSION

Transformation rates of AI and AN in soil were expressed in the form of half-lives calculated using a first-order kinetic model. First-order models have been shown to adequately describe transformation rates of insecticides and other toxicants (Sparks, 1989). This approach is further justified by the fact that higher order kinetic reactions will appear to be first-order, yielding pseudo-first-order kinetics in the presence of low substrate concentrations, a typical situation in the soil environment. Moreover, data sets obtained from short test periods as used here most likely will fit a first-order kinetics regression model.

In 82% of the cases, decreases in AI and AN concentrations with time fit a first-order model with statistical significance at the 95% probability level. Statistical analysis did not indicate that a longer test time was associated with inadequacy of the first-order kinetics model. In fact, the first-order model failed to fit the 48 h test data more frequently than the data collected at 240 h. Additionally, the frequency of outliers occurred most often for the shorter incubation times.

The mean half-life of AI was approximately 2 days and that of AN 4 days in six different soils incubated at 20 °C. The relatively rapid dissipation of both compounds in soil has important implications with respect to controlling soil-borne plant pests by *Brassica* species tissues. Pesticidal activity of glucosinolate-containing plant tissues will be short-lived with little expected residual activity, much like various soil fumigants. Control of an organism from the one-time amendment of plant tissues, therefore, requires the release of a sufficient concentration of AI or AN to inhibit or kill the organism. Avoidance or protection mechanisms that allow the pest species to survive and flourish after AI and AN dissipate will limit control effectiveness of glucosinolate-containing tissue amendments.

By understanding soil variables that impact the residence time of the allelochemicals, it may be possible to optimize the use of glucosinolate-containing tissues in pest control strategies such as crop rotations, green manures, or tissue amendments to soil. The statistical relationship of observed transformation rates of the test chemicals, expressed in units of half-life, was correlated with the soil characteristics listed in Table 2. These correlations demonstrated that faster rates of AI disappearance occurred in soils with greater organic carbon contents and total nitrogen concentrations.

Isothiocyanates are very reactive compounds, although apparently less reactive than isocyanates. The fundamental principles of isothiocyanate reactivity are well established (Bogemann et al., 1955; McKenzie, 1970; Jones, 1979). They react with nucleophilic reagents forming N-monosubstituted thiocarbamic esters with alcohols and phenols, dithiocarbamic esters with thiols and thiophenols, and thiourea derivatives with ammonia, amines, hydroxyamines, hydrazines, or related amine derivatives. Isothiocyanates form carboxylic amides with carboxylic and thiocarboxylic acids, extruding carbon oxysulfide or carbon disulfide (Jones, 1979). Heterocyclic compounds are often produced in reactions between isothiocyanates and a nucleophilic reaction partner, either spontaneously or by additional external influence from α-mercapto acids or α-amino acids. Isothiocyanates with sufficiently acid α-hydrogen atoms react with aldehydes and ketones to produce 1,3-oxazolidine-2-thiones (Daxenbichler and VanEtten, 1974). Thus, the isothiocyanate functional group can react with nucleophilic groups commonly found in organic matter and a negative correlation of AI half-life with soil organic matter concentration is expected. The negative correlation of AI half-life with total soil N content occurs because most soil N exists in organic form as an integral part of soil organic matter or possibly because AI reacts with amino groups. Although the heterogeneity of the soil system precludes exact identification of the reactions, the reduction in extractable AI implies less effective control of soil-borne plant pests in soils having greater organic matter concentrations.

A negative correlation as occurred for AI half-life and soil organic C did not occur for AN; instead, the data reflect a possible positive correlation of AN half-life and organic C ($P = 0.059$). The reactivity of nitriles is much lower than that of isothiocyanates and, therefore, reaction with nucleophilic reagents requires catalysis by acids or bases. Hydration of the carbon−nitrogen triple bond is particularly sluggish. It can be accelerated using the more potent nucleophilic hydroperoxy anion, which often smoothly and efficiently converts nitriles to amines in weakly basic conditions. Although enzymes have the ability to hydrate nitriles under neutral conditions (Harper, 1977), the negative correlation of AN half-life with soil inorganic C content indicates that AN half-life is shortened in calcareous soils and that nonenzymatic, base-catalyzed reactions may be more important.

The fact that AN half-life is not shortened by increased amounts of soil organic C implies only that the compound remained extractable. Unlike the conclusions for AI in which half-life reduction indicates a corresponding reduction in biologically available material, it cannot be assumed that all extractable AN retains its potential to participate in pest control. Although the exact mechanism by which nonionic compounds interact with soil constituents remains a controversial area (Chiou, 1989; Chiou and Kile, 1994; Murphy et al., 1990, 1994), our data indicate that interaction of AN with soil constituents is sufficiently weak to allow extraction with ethyl acetate. It cannot be determined if AN exists in a location and form potentially available to interact with soil-borne organisms.

In addition to inherent characteristics of the soils, environmental variables also impact the half-lives of AI and AN. Manipulation of soil moisture in Latahco soil showed that the half-life of AI increased with increased soil water content, whereas the opposite was true for AN (Figure 3). These results indicate that AN is sorbed or reacts more quickly at the water phase than at the gas phase and, perhaps, that the total reaction rate is controlled by the gas/water phase partition coefficient. The fate of AI does not appear to be controlled by the same mechanism in that stability increased with increased soil moisture.

Similarly, temperature was significantly correlated with both AI and AN disappearance in Latahco soil (Figure 3), but in opposite directions. The transformation rate of AI increased with increased incubation temperature. This is in agreement with the already described effect of temperature on the transformation of methyl isothiocyanate (Ashley and Leigh, 1963b; Leistra et al., 1974; Smelt and Leistra, 1974; Smelt et al., 1989). The transformation rate of AN decreased with increased temperature, suggesting an endothermic transformation reaction.

The effect of substrate concentration in Latahco soil was also determined in an attempt to explore the true kinetics of the observed transformation rates. In the case of first-order kinetics, reaction half-life does not change with different initial substrate concentrations, whereas in the case of pseudo-first-order kinetics a reaction rate change is expected. While AN transformation appears to obey first-order kinetic relationships, the transformation of AI appears to be only pseudo-first-order (Figure 3).

Small differences between the transformation rates of the substrates in unsterilized Latahco soil and in soil sterilized with ethylene oxide or by autoclaving occurred only for AN, indicating possible microbial participation in transformation. No such differences occurred for AI, but it is important to consider that disappearance was determined 48 h after substrate addition. Differences may become more obvious if longer assay times are used. Likewise, repeated applications of the substrates to enrich for microorganisms capable of degrading AI and AN failed to demonstrate any modification of half-lives in Latahco soil. Soil microorganisms can accelerate the transformation rate of methyl isothiocyanate in repeatedly treated fields, although results are inconsistent (Smelt et al., 1989). However, no evidence of enhanced degradation from repeated application was observed for either compound in Latahco soil.

The combined results indicate that both AI and AN are short-lived in various soils, with AN having a longer half-life. The mechanisms contributing to substrate disappearance are different for each of the compounds as reflected by correlations with soil characteristics and incubation parameters. AI disappearance is promoted in warm, dry soils having high concentrations of organic carbon. AN disappearance will occur most rapidly in wet, cool soils having higher concentrations of inorganic carbon. Although speculation as to the responsible molecular mechanisms is possible, further research to increase our understanding of the fate of these allelochemicals in soil would be valuable in predicting the efficacy of glucosinolate-containing plant tissues to control soil-borne plant pests.

ABBREVIATIONS USED

AN, allylnitrile; AI, allyl isothiocyanate; GC, gas chromatography; FID, flame ionization detector.

LITERATURE CITED

Ashley, M. G.; Leigh, B. L. The action of metham-sodium in soil. I. Development of an analytical method for the determination of methyl isothiocyanate residues in soil. *J. Sci. Food Agric.* **1963a**, *14*, 148–153.

Ashley, M. G.; Leigh, B. L. The action of metham-sodium in soil. II. Factors affecting the removal of methylisothiocyanate residues. *J. Sci. Food Agric.* **1963b**, *14*, 153–161.

Block, S. S.; Lawrence, C. A. *Disinfection, Sterilization, and Preservation*; Lea & Febiger: Philadelphia, 1968; pp 126–138.

Bogemann, M.; Petersen, S.; Schultz, O.-E.; Soll, H. Sulphur compounds. In *Methoden der Organishen Chemie*; Muller, E., Ed.; Houben-Weyl: Berlin, 1955; Vol. IX, pp 804–809.

Borek, V.; Morra, M. J.; Brown, P. D.; McCaffrey, J. P. Allelochemicals produced during sinigrin decomposition in soil. *J. Agric. Food Chem.* **1994**, *42*, 1030–1034.

Brown, P. D.; Morra, M. J.; McCaffrey, J. P.; Auld, D. L.; Williams, L., III. Allelochemicals produced during glucosinolate degradation in soil. *J. Chem. Ecol.* **1991**, *17*, 2021–2034.

Chan, M. K. Y.; Close, R. C. *Aphanomyces* root rot of peas. 3. Control by use of cruciferous amendments. *N. Z. J. Agric. Res.* **1987**, *30*, 225–233.

Chew, F. S. Biological effects of glucosinolates. In *Biologically Active Natural Products: Potential Use in Agriculture*; Cutler, H. G., Ed.; American Chemical Society: Washington, DC, 1988; pp 155–180.

Chiou, C. T. Theoretical considerations of the partition uptake of nonionic organic compounds by soil organic matter. In *Reactions and Movement of Organic Chemicals in Soils;* Sawhney, B. L., Brown, K., Eds.; SSSA and ASA: Madison, WI, 1989; pp 1–29.

Chiou, C. T.; Kile, D. E. Effects of polar and nonpolar groups on the solubility of organic compounds in soil organic matter. *Environ. Sci. Technol.* **1994**, *28*, 1139–1144.

Choesin, D. N.; Boerner, R. E. J. Allyl isothiocyanate release and the allelopathic potential of *Brassica napus* (Brassicaceae). *Am. J. Bot.* **1991**, *78*, 1083–1090.

Crisp, P. Trends in the breeding and cultivation of cruciferous crops. In *The Biology and Chemistry of the Cruciferae*; Vaughan, J. G., MacLeod, J. G., Jones, B. M. G., Eds.; Academic Press: London, 1976; pp 69–74.

Daxenbichler, M. E.; VanEtten, C. H. 5,5-Dimethyloxazolidine-2-thione formation from glucosinolate in *Limnanthes alba* Benth. seed. *J. Am. Oil Chem. Soc.* **1974**, *51*, 449–450.

Deraadt, A.; Klempier, N.; Faber, K.; Griengl, H. Chemoselective enzymatic-hydrolysis of aliphatic and alicyclic nitriles. *J. Chem. Soc. Perkin Trans. 1* **1992**, 137–140.

Ettlinger, M. G. ; Lundeen, A. J. The structure of sinigrin and sinalbin: an enzymatic rearrangement. *J. Am. Chem. Soc.* **1956**, *78*, 4172.

Ettlinger, M. G.; Lundeen, A. J. First synthesis of a mustard oil glucoside: the enzymatic Lossen rearrangement. *J. Am. Chem. Soc.* **1957**, *79*, 1764.

Fenwick, G. R.; Heaney, R. K.; Mawson, R. Glucosinolates. In *Toxicants of Plant Origin: Glycosides*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. II, pp 1–41.

Gee, G. W.; Bauder, J. W. Particle-size analysis. In *Methods of Soil Analysis*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph 9; ASA and SSSA: Madison, WI, 1986; Part 1, pp 383–411.

Harper, D. B. Fungal *Fusarium solani* degradation of aromatic nitriles. *Biochem. J.* **1977**, *167*, 685–692.

Jones, D. N. Sulphur, selenium, silicon, boron, organometallic compounds. In *Comprehensive Organic Chemistry. The Synthesis and Reactions of Organic Compounds*; Barton, D., Ollis, W. D., Eds.; Pergamon Press: Oxford, U.K., 1979; Vol. 3, pp 461–477.

Kjaer, A. Naturally derived isothiocyanates (mustard oils) and their parent glucosides. *Fortschr. Chem. Org. Naturst.* **1960**, *18*, 122.

Kjaer, A. The natural distribution of glucosinolates: a uniform class of sulphur containing glucosides. In *Chemistry in Botanical Classification*; Bendz, G., Santesson, J., Eds.; Academic Press: London, 1974; pp 229–234.

Klute, A. Water retention: Laboratory methods. In *Methods of Soil Analysis*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph 9; ASA and SSSA: Madison, WI, 1986; Part 1, pp 635–662.

Laidler, K. J. *Chemical Kinetics*, 3rd ed.; Harper & Row: New York, 1987.

Leistra, M.; Smelt, J. H.; Nollen, H. M. Concentration-time relationship for methyl isothiocyanate in soil after injection of metham-sodium. *Pestic. Sci.* **1974**, *5*, 409–417.

Lichtenstein, E. P.; Morgan, D. G.; Mueller, C. H. Naturally occurring insecticides in cruciferous crops. *J. Agric. Food Chem.* **1964**, *12*, 158–161.

McKenzie, S. Thiocarbonyl compounds. In *Organic Compounds of Sulphur, Selenium, and Tellurium*; Reid, D. H., Ed.; The Royal Chemical Society: London, 1970; Vol. 1, pp 207–210.

Mojtahedi, H.; Santo, G. S.; Hang, A. N.; Wilson, J. H. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *J. Nematol.* **1991**, *23*, 170–174.

Murphy, E. M.; Zachara, J. M.; Smith, S. C. Influence of mineral-bound humic substances on the sorption of hydrophobic organic compounds. *Environ. Sci. Technol.* **1990**, *24*, 1507–1516.

Murphy, E. M.; Zachara, J. M.; Smith, S. C.; Phillips, J. L.; Wietsma, T. W. Interaction of hydrophobic organic compounds with mineral-bound humic substances. *Environ. Sci. Technol.* **1994**, *28*, 1291–1299.

Nelson, D. W.; Sommers, L. E. Total carbon, organic carbon and organic matter. In *Methods of Soil Analysis*, 2nd ed.; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; Agronomy Monograph 9; ASA and SSSA: Madison, WI, 1982; Part 2, pp 539–579.

Papavizas, G. C. Suppression of *Aphanomyces* root rot of peas by cruciferous soil amendments. *Phytopathology* **1966**, *56*, 1071–1075.

Papavizas, G. C.; Lewis, J. A. Effect of amendments and fungicides on *Aphanomyces* root rot of peas. *Phytopathology* **1971**, *61*, 215–220.

Parke, J. L.; Rand, R. E. Incorporation of crucifer green manures to reduce *Aphanomyces* root rot of snap beans. *Annu. Rep. Bean Improv. Coop.* **1989**, *32*, 105–106.

Röbbelen, G.; Thies, W. Variation in rapeseed glucosinolates in breeding for improved meal quality. In *Brassica Crops and Wild Allies*; Tsunoda, S., Hinata, K., Gomez-Campo, C., Eds.; Japan Scientific Press: Tokyo, 1980; pp 285–299.

SAS Institute Inc. *SAS Programmer's Guide*, version 5 ed.; SAS Institute: Cary, NC, 1985.

Smelt, J. H.; Leistra, M. Conversion of metham-sodium to methyl isothiocyanate and basic data on the behaviour of methyl isothiocyanate in soil. *Pestic. Sci.* **1974**, *5*, 401–407.

Smelt, J. H.; Leistra, M.; Nollen, H. M. Concentration-time relationship for methyl isothiocyanate in soil after injection of metham-sodium. *Pestic. Sci.* **1974**, *5*, 409–417.

Smelt, J. H.; Teunissen, W.; Crum, S. J. H.; Leistra, M. Accelerated transformation of aldicarb, oxamyl and ethoprophos after repeated application of metham-sodium. *Crop Prot.* **1987**, *67*, 295–303.

Smelt, J. H.; Crum, S. J. H.; Teunissen, W. Accelerated transformation of the fumigant methyl isothiocyanate in soil after repeated application of metham-sodium. *J. Environ. Sci. Health* **1989**, *24*, 437–455.

Sparks, D. L. *Kinetics of Soil Chemical Processes*; Academic Press: San Diego, CA, 1989.

Stone, A. T.; Morgan, J. J. Kinetics of chemical transformation in the environment. In *Aquatic Chemical Kinetics: Reaction Rates of Processes in Natural Waters*; Stumm, W., Ed.; Wiley: New York, 1990; pp 1–41.

Uda, Y.; Kurata, T.; Arakawa, N. Effects of pH and ferrous ion on the degradation of glucosinolates by myrosinase. *Agric. Biol. Chem.* **1986**, *50*, 2735–2740.

Vandenberg, F.; Leistra, M.; Roos, A. H.; Tuinstra, L. G. M. T. Sampling and analysis of the soil fumigants 1,3-dichloropropene and methyl isothiocyanate in the air. *Water Air Soil Pollut.* **1992**, *61*, 385–396.

Waddington, J. Growth of barley, bromegrass and alfalfa in the greenhouse in soil containing rapeseed and wheat residues. *Can. J. Plant Sci.* **1978**, *58*, 241–248.

Winkler, H.; Otto, G. Replant losses with strawberries and suggestions for their reduction. *Hortic. Abstr.* **1980**, *50*, 344.

Zar, J. H. *Biostatistical Analysis*, 2nd ed.; Prentice-Hall: Englewood Cliffs, NJ, 1984.

Zhang, Z. Z.; Sparks, D. L.; Scrivner, N. C. Acetonitrile and acrylonitrile sorption on montmorillonite from binary and ternary aqueous solutions. *Soil Sci. Soc. Am. J.* **1990**, *54*, 1564–1571.

Received for review December 30, 1994. Accepted May 8, 1995.[⊗] Funding was provided by USDA/CSRS as part of the Water Quality Initiative (Grant 92-01333), Advanced Materials from Renewable Resources program (93-COOP-1-9543), and Solutions to Environmental and Economic Problems program (91-34261-6281).

JF940741H

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1995.