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Degradation of 1,3-Dichloropropene (1,3-D) in Soils with Different Histories of Field Applications of 1,3-D[1]

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Abstract: Laboratory experiments were conducted to determine the mineralization rates of 1,3-dichloropropene (1,3-D) in surface and subsurface soil samples collected from three sites in Florida with different histories of 1,3-D exposure. Mineralization rates of uniformly labeled ^{14}C-1,3-D in surface and subsurface samples collected from two of the three sites, one of which was treated with 1,3-D only once and the other which had not been treated with the chemical for 5 years, were similar to the corresponding samples collected from untreated plots, and the rates generally decreased with soil depth. Initial mineralization rates in surface and subsurface samples collected from the site that had repeatedly been treated with 1,3-D at least 6 of the past 12 years were more rapid than those in either the corresponding untreated samples or in samples collected from the two other sites. Not only were the initial mineralization rates in soil samples collected from this site greater, but also the disappearance rates of cis- and trans-1,3-D were greater than in the corresponding untreated samples. Trans-1,3-D was degraded much more rapidly in the enhanced soil than was the cis- form. In addition, no or little trans-3-chloroallyl alcohol (CAA), the hydrolysis product of trans-1,3-D, was formed; large amounts of cis-3-CAA, the hydrolysis product of cis-1,3-D, were detected. This suggest that biological hydrolysis is responsible for the hydrolysis of trans-1,3-D to trans-3-CAA in enhanced soil and chemical hydrolysis is responsible for the hydrolysis of cis- and trans-1,3-D to 3-CAA in nonenhanced soil.

Key words: biodegradation, cis- and trans-1,3-D, cis- and trans-3-CAA, 1,3-D, 1,3-dichloropropene, degradation, differential enhanced degradation, Florida, fumigant, mineralization, nematicide, nematode, pesticide degradation, soil, Telone II.

The active ingredient of the soil fumigant 1,3-D is composed of equal ratios of the cis- and trans-1,3-dichloropropene (1,3-D). They comprise 94% of Telone II (DowElanco, Indianapolis, IN) (3,19). Both cis- and trans-1,3-D are clear liquids with vapor pressures of 43 and 34 mm Hg at 25 C, and water solubilities of 2,700 and 2,800 μg/ml; respectively (18).

In soil, both cis- and trans-1,3-D are initially hydrolyzed to respective cis- and trans-3-chloroallyl alcohol (CAA), which are in turn rapidly oxidized to corresponding 3-chloroacrylic acid (11). Most of the information on the disappearance rates of the two isomers in soils indicate that they are generally similar kinetically (4,13,15, 16). Half-life values for cis-1,3-D in soils under laboratory conditions at 10, 15, and 20 C ranged from 16–46, 7–33, and 3–19 days, respectively (4,12,15,16). Half-life values for trans-1,3-D in soils under laboratory conditions at 10, 15, and 20 C ranged from 17–47, 4–32, and 3–15 days, respectively (4,12,15,16). Unlike cis- and trans-1,3-D, cis- and trans-3-CAA appeared to have different degradation rates in soils, with the trans-isomer being more rapidly degraded than the cis-isomer (4,15).

Enhanced degradation of 1,3-D in some loamy soils, with or without histories of previous field applications of 1,3-D has been reported (12), however such reports are rare. There was no attempt to determine if enhanced degradation was isomer

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specific, but microorganisms were considered capable of degrading 1,3-D in those soils that exhibited enhanced degradation of 1,3-D. Enhanced degradation has been observed in soils for a number of different classes of pesticides, including phenoxy compounds, organophosphorus chemicals, carbamates, and acetamide compounds (10).

As a consequence of the scheduled phaseout of methyl bromide use within the United States by the year 2001 (7), a renewal of interest in 1,3-D as a soil fumigant has recently been reported (1). This investigation was initiated to determine the mineralization rates of 1,3-D in three sandy soils collected from three sites in Florida having different histories of 1,3-D application. Also, the differential disappearance of cis- and trans-1,3-D, as well as formation of cis- and trans-3-CAA, was determined in surface and subsurface soil samples collected from one of the sites that had been treated with 1,3-D at least 6 of the past 12 years.

Materials and Methods

Soils and soil sampling: Surface and subsurface soil samples were collected from three sites: Green Acres, Gainesville, Florida; Hastings, Hastings, Florida; and Immokalee, Immokalee, Florida. Soil samples at 15-cm increments were collected using a 10-cm-d bucket auger. A composite of four cores was collected from each layer. Subsurface samples were collected to a depth near the tops of the watertables at Hastings and Immokalee and to a 90 cm depth at Green Acres. At the time of sampling, the tops of the watertables at Hastings and Immokalee were around 90 and 75 cm deep, respectively, and the watertable at Green Acres was more than 3 m from the soil surface. Green Acres had been treated with 1,3-D at least 6 of the past 12 years at a rate of 55–110 liters/ha, and at the time of sampling, 21 days after 1,3-D application in 1994, this site was planted with peanut. The Hastings site was planted with potato and had been treated annually with 1,3-D for 10 years; however, at the time of sampling, this site had not been treated with 1,3-D for 5 years. The Immokalee site was treated with 1,3-D-chloropicrin (Telone C17, DowElanco, Indianapolis, IN) once for tomato production, and soil samples were collected 9 months after the field application. At the time of sampling, this site was covered with weeds. Surface and subsurface soil samples were also collected from untreated plots at the three sites. Soils collected from Green Acres, Hastings, and Immokalee were all sandy in nature and classified as Arredondo fine sand, Ellzey fine sand, and Holopaw sand, respectively.

Groundwater samples were also collected from two wells, C1 and C3, at the Immokalee site. The two wells, located at the northwest corner of the treated plot, were about 3 m apart. Water samples were collected 1 m deep at the time of soil sampling. All soil and water samples were transported to Gainesville the same day of sampling and were stored in the dark at 4 C.

Chemicals: Uniformly labeled ^{14}C-1,3-D with a specific activity of 207 MBq/mmol and a radio purity of 98% was purchased from Sigma Chemical Co. (St. Louis, MO). Analytical grade cis- and trans-1,3-D and cis- and trans-3-chloroallyl alcohol (CAA) were gifts from DowElanco (Indianapolis, IN). All other chemicals were analytical grade, scintillation grade, or the highest grade commercially available and were used without further purification.

Mineralization studies: The procedures for determining mineralization of ^{14}C-1,3-D in soil were similar to those reported by Ou (8). One hundred grams of soil (oven dry-weight basis) were placed in a 250-ml glass Erlenmeyer flask with a Teflon-line screw cap, and the soil was then treated with 1,3-D (10 μ/g) and ^{14}C-1,3-D (0.5 kBq/g). After mixing, the flask was immediately closed tightly with a Teflon-lined screw cap under which a stainless steel vial (3-ml capacity) containing 0.5 ml of 8 M KOH was hung. These flasks were incubated in the dark at 28 C. At predetermined intervals, all the vials were re-

moved, and after the weights of the flasks were checked and compensated for water loss, fresh KOH traps were hung immediately. The KOH in the removed vials was transferred to small beakers and diluted with deionized water to 5 ml. Radioactivity in the diluted KOH (0.5 ml) was determined by liquid scintillation spectroscopy (LSC). To differentiate ^{14}C-carbonate from volatile ^{14}C-organics trapped in the KOH, an excess amount of 20% $BaCl_2$ was added to 2 ml of the diluted KOH solutions to precipitate carbonates, including ^{14}C-carbonate. After centrifugation, 0.5 ml each of the supernatants was removed for ^{14}C determination by LSC. The ^{14}C activity in the supernatant is considered to be associated with volatile ^{14}C-organics, such as ^{14}C-1,3-D and its volatile ^{14}C-metabolites. The difference between ^{14}C in the KOH and ^{14}C in the supernatant was considered to be associated with ^{14}C-carbonate. Each treatment was duplicated. Similar procedures used to determine the mineralization rates of ^{14}C-1,3-D in soils were also employed to determine the mineralization rates of ^{14}C-1,3-D in groundwater samples. Data were subjected to analysis by t-tests (6).

Metabolite studies: A series of 40-ml glass centrifuge tubes, each containing 10 g of soil (oven dry-weight basis), were wrapped over the threads with Teflon tape. Equal amounts of analytical grade cis-1,3-D and trans-1,3-D were added to each tube, which was immediately closed with a Teflon-lined cap. The tubes were shaken on a reciprocal shaker at 500 strokes per minute for 5 minutes and incubated in the dark at 28 C. At intervals of 1 hour, 3, 7, 14, and 21 or 28 days, two tubes from each treatment were removed for solvent extraction.

Extraction and analytical procedures: To minimize volatilization loss, the centrifuge tubes, organic solvent (acetone), glass pipettes, pipette tips, glass GC vials, and other glassware were kept in an ultralow-temperature freezer at −30 C for 1 hour before organic solvent extraction. One tube at a time was removed from the freezer, 20 ml of cold acetone was added, and the tube was immediately closed tightly with a cap. These centrifuge tubes were then shaken for 1 hour on a reciprocal shaker. After centrifugation to precipitate the soil particles, 0.5 ml of the acetone extracts was transferred to glass vials for gas chromatography (GC) analysis.

Cis- and trans-1,3-D and their corresponding 3-CAA were quantified by a Perkin Elmer Autosystem GC equipped with an autosampler, ^{63}Ni electron capture detector, split-splitless injector, Turbochrom 4 software, and a 486 computer. The GC parameters and operational conditions were as follows: column, 30 m × 0.25 mm i.d., RTX-624 coated with 3 μm film thickness; flow rates for carrier gas (He) and make-up gas (95% N_2 and 5% CH_4), 5 ml/minute and 30 ml/minute, respectively; injector temperature, 150 C; detector temperature, 375 C; oven temperature, 50 C for the first minute, ramp at 40 C/minutes, and hold at 120 C for 16 minutes; split valve, off for the first 1 (1,3-D) or the first 1.5 (3-CAA) minutes; and injection volumes of 3 and 5 μl for 1,3-D and 3-CAA, respectively. Under these conditions, the retention times for cis- and trans-1,3-D and cis- and trans-3-CAA were 7.32, 7.96, 9.10, and 10.45 minutes, respectively. Data were subjected to analysis by t-tests (6).

Results

Mineralization of ^{14}C-1,3-D in soils: Mineralization rates of ^{14}C-1,3-D in surface and subsurface soil samples collected from the treated and untreated plots at Immokalee generally decreased progressively with soil depth (Fig. 1). In soil taken from 0–15, 15–30, 30–45, and 60–75 cm deep, 31.4, 29.0, 22.5, and 12.7% of the applied ^{14}C, respectively, was mineralized at the end of 42 days of incubation. In the corresponding surface and subsurface samples collected from the untreated plot, 29.2, 23.3, 14.2, and 9.1% of the applied ^{14}C, respectively, was mineralized in 42 days. Mineralization rates in the treated and untreated surface samples were similar throughout the entire 42 days of incu-

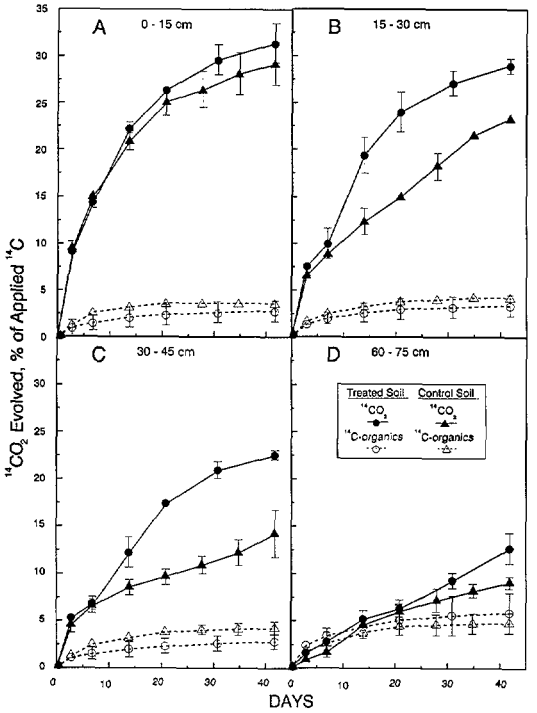

FIG. 1. Evolution of $^{14}CO_2$ and trapped volatile ^{14}C-organics from surface and subsurface soil samples collected from 1,3-D treated and untreated plots at Immokalee, Florida. These samples were treated with ^{14}C-1,3-D and 1,3-D.

bation; mineralization rates in the three subsurface layers collected from the treated plot were initially similar to the corresponding layers collected from the untreated plot. However, after 7 to 21 days, mineralization rates in the treated subsurface samples were greater than in the corresponding untreated subsurface samples. After 42 days, 2.8–5.9% of the applied ^{14}C not associated with $^{14}CO_2$ was also trapped in the KOH traps. The ^{14}C was associated with volatile ^{14}C-organics, possibly with ^{14}C-1,3-D. In general, more volatile ^{14}C-organics were trapped from the subsurface samples than from the surface samples, especially from the deepest subsurface layer (60–75 cm deep). Mineralization patterns of ^{14}C-1,3-D in surface and subsurface samples collected from the treated and untreated plots of the Hastings site were similar to the surface and subsurface samples collected from the Immokalee site (data not shown).

In contrast to the soil samples collected from the Immokalee and Hastings sites, mineralization rates of ^{14}C-1,3-D in surface and subsurface samples collected from the Green Acres site in the first 3 days were greater ($P \leq 0.05$) than those in the untreated plot (Fig. 2). After 7 days, however, the trend was reversed, especially, in the two deeper subsurface layers, 45–60 and 75–90 cm deep. In fact, after 3 days, ^{14}C-1,3-D in the two deeper subsurface samples (45–60 and 75–90 cm deep) collected from the untreated plot was mineralized at near-constant rates (Fig. 2), 0.70 and 0.72%/day, respectively. As a result, after 28 days of incubation, total amounts of ^{14}C-1,3-D mineralized in these samples were about the same. The ^{14}C-1,3-D in the subsurface samples (15–30, 30–45, and 60–75 cm deep) collected from the Immokalee site was also mineralized at near-constant rates (see Fig. 1). Small amounts of ^{14}C-organics from the two surface samples were volatilized and trapped in KOH in 28 days (Fig. 2). Larger

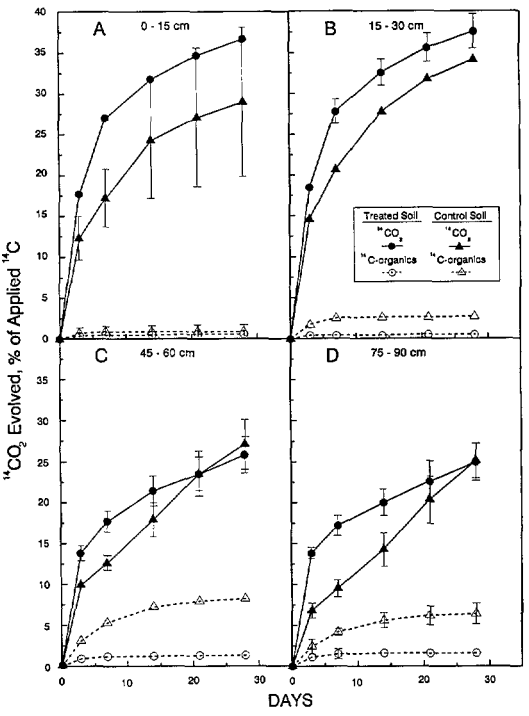

FIG. 2. Evolution of $^{14}CO_2$ and trapped volatile ^{14}C-organics from surface and subsurface soil samples collected from 1,3-D treated and untreated plots at Green Acres, Gainesville, Florida. These samples were treated with ^{14}C-1,3-D and 1,3-D.

amounts of volatile ^{14}C-organics from the subsurface samples collected from the untreated plot were trapped than from samples collected from the treated plot.

Although wells C1 and C3 were about 3 m apart, mineralization of ^{14}C-1,3-D in the groundwater sample collected from well C3 was more rapid than in the groundwater sample collected from well C1 (Fig. 3). Furthermore, the mineralization rate of ^{14}C-1,3-D in the deepest layer (60–75 cm deep) of soil collected from the treated plot at Immokalee was intermediate to the rates of the two groundwater samples (Fig. 1). These soil samples were collected 3–5 m away from the two wells.

Disappearance of cis- and trans-1,3-D: Both cis- and trans-1,3-D in the surface (0–15 cm deep) or the shallow subsurface (15–30 cm deep) soil collected from the untreated plot at Green Acres disappeared at the same rate ($P \leq 0.05$) during 28 days of incubation, although, on average, trans-1,3-D disappeared somewhat faster than cis-1,3-D (Fig. 4). Both cis- and trans-1,3-D in the treated surface and subsurface samples disappeared more rapidly ($P \leq 0.05$) than in the corresponding untreated samples, with the exception of the subsurface sample collected from the treated plot. In this sample, during the first 7 days of incubation, cis-1,3-D disappeared at the same rate as cis- and trans-1,3-D in the surface and subsurface samples collected from the untreated plot. However, after 14 days, cis-1,3-D in the treated subsurface sample disappeared faster than in the two untreated samples. Furthermore, trans-1,3-D in the treated surface and subsurface samples disappeared more rapidly ($P \leq 0.05$) than did cis-1,3-D. After 14 days, the two isomers in the treated surface and subsurface samples were either not detected or detected in trace amounts, with the exception of the treated surface sample that was incubated for 21 days. In this sample, trans-1,3-D had completely disappeared in one of the duplicates, but a considerable amount of the chemical was detected in the other duplicate. Excessive trans-1,3-D might have been accidentally added to this duplicate. The degradation rate of 1,3-D in soil was inhibited when the application rate was 50 μg/g or larger (8). Trans-1,3-D in the treated surface and subsurface samples disappeared much faster than cis-1,3-D did in the same samples, especially in the treated subsurface sample. These degradation patterns were reflected in the half-life values of cis- and trans-1,3-D in these soils (Table 1).

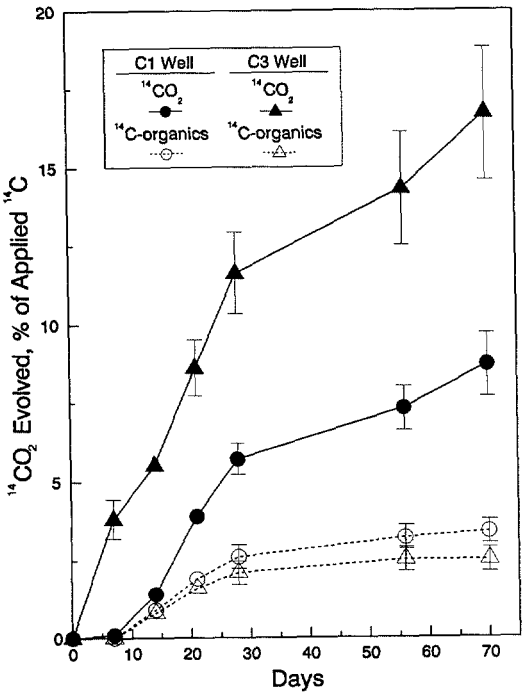

FIG. 3. Evolution of $^{14}CO_2$ and trapped volatile ^{14}C-organics from two groundwater samples collected from C1 and C3 wells at Immokalee, Florida. These samples were treated with ^{14}C-1,3-D and 1,3-D.

Half-life values for the two isomers in the surface and shallow subsurface samples collected from the untreated plot were similar, ranging from 17–20 days, with the half-life values for the trans-isomer being slightly smaller than the cis-isomer. Half-life values for the two isomers in the surface and shallow subsurface samples collected from the treated plot were smaller than in the samples collected from the untreated plot, especially for the trans-isomer. Half-life values for trans-1,3-D in the treated samples were four to six times smaller than in the untreated samples. Also, half-life values for trans-1,3-D in

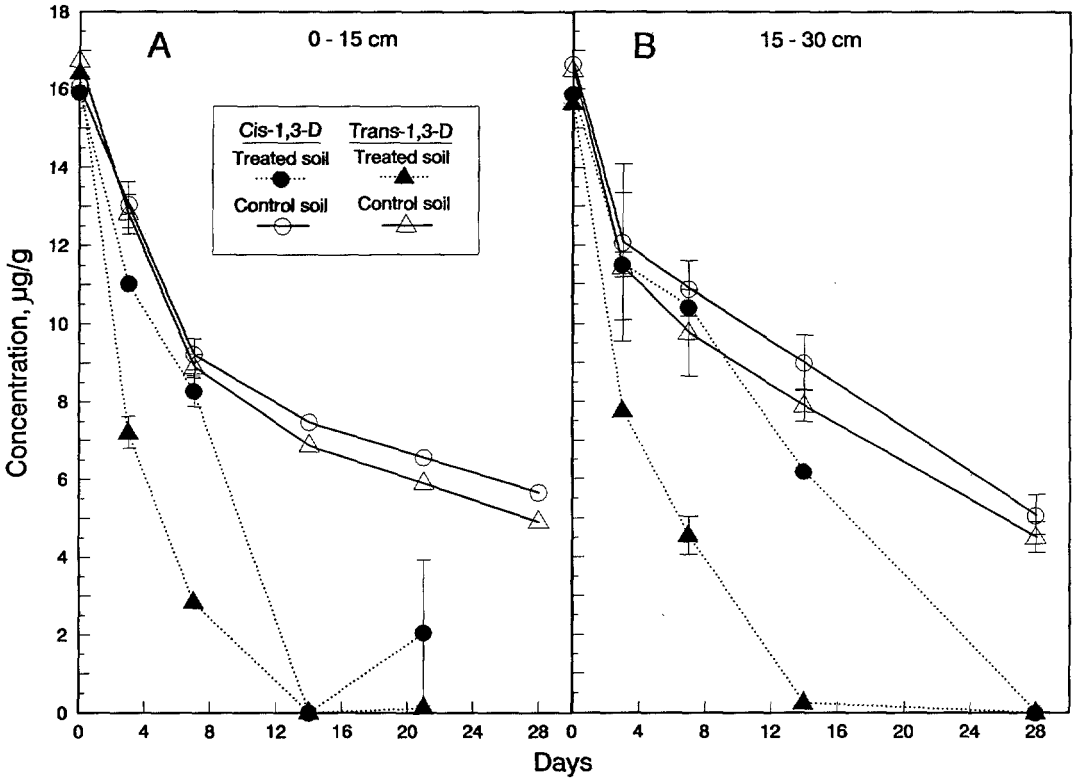

FIG. 4. Disappearance of cis- and trans- 1,3-D in surface and subsurface soil samples collected from treated and untreated plots at Green Acres, Gainesville, Florida. These samples were treated with an equal ratio of analytical grade cis- and trans-1,3-D.

these two soil layers were about three times smaller than for the cis-isomer.

Formation and disappearance of cis- and trans-CAA: No trans-3-CAA was detected in the surface and shallow subsurface samples collected from the treated plot during 21 or 28 days of incubation with an equal mixture of cis- and trans-1,3-D (Fig. 5). Trace amounts of trans-3-CAA were detected in the surface and shallow subsurface samples collected from the untreated plot in the first 3 days of incubation, but no trans-3-CAA was detected in these samples after 7 days. Greater ($P \leq 0.05$) concentrations of cis-3-CAA than trans-3-CAA were detected in the surface and shallow subsurface samples collected from the treated and untreated plots during the entire 28 days of incubation, with more cis-3-CAA being formed in soil samples collected from the untreated plot than those collected from the treated plot. In all soil sam-

TABLE 1. Pseudo-first-order rate coefficients (k_1) and half-life ($t_{1/2}$) values of cis- and trans-1,3-D in surface and subsurface soil samples collected from treated and untreated plots at Green Acres, Gainesville, Florida. These samples were treated with an equal ratio of cis- and trans-1,3-D.

Soil depth (cm)	K_1 (1/days)		$t_{1/2}$ (days)		r^2	
	Cis-1,3-D	Trans-1,3-D	Cis-1,3-D	Trans-1,3-D	Cis-1,3-D	Trans-1,3-D
			Control soil			
0–15	3.51×10^{-2}	4.05×10^{-2}	20	17	0.903	0.919
15–30	3.81×10^{-2}	4.15×10^{-2}	18	17	0.970	0.958
			Treated soil			
0–15	9.08×10^{-2}	2.52×10^{-1}	8	3	0.977	0.999
15–30	6.08×10^{-2}	1.76×10^{-1}	11	4	0.967	0.977

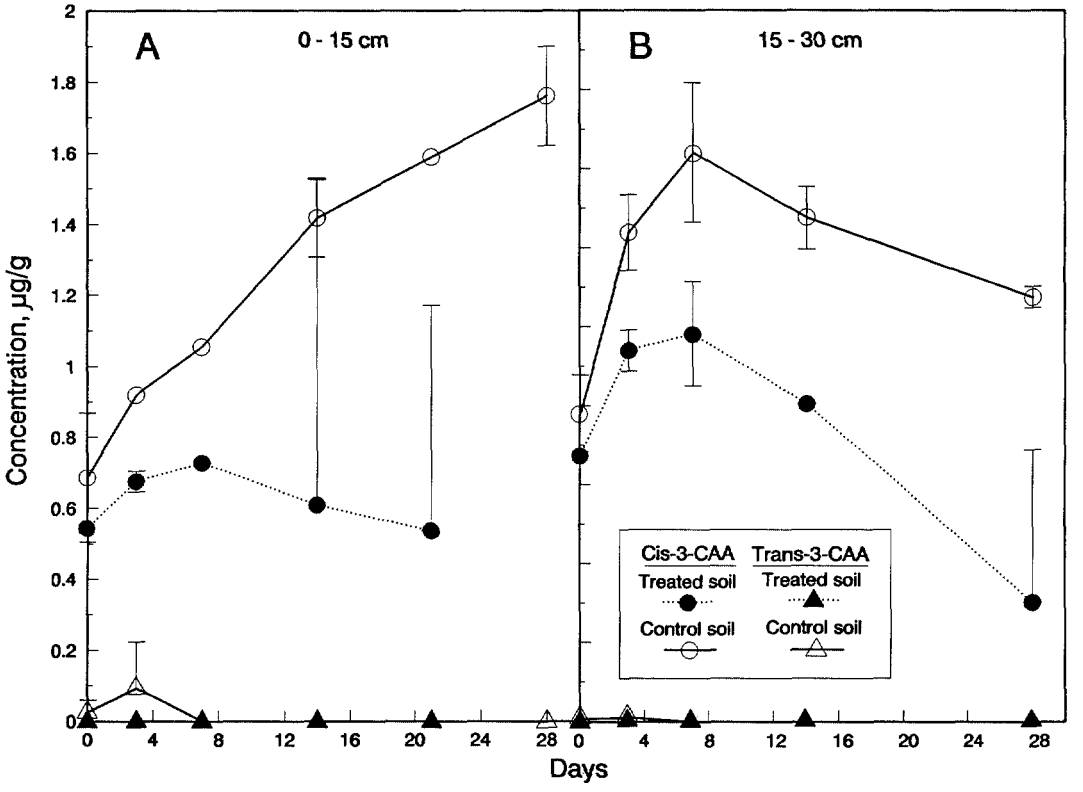

FIG. 5. Formation and disappearance of cis- and trans-3-CAA in surface and subsurface soil samples collected from treated and untreated plots at Green Acres, Gainesville, Florida. These samples were treated with an equal ratio of analytical grade cis- and trans-1,3-D.

ples, cis-3-CAA (0.52 and 0.98 µg/g) was rapidly formed within the first hour of incubation. Formation of cis-3-CAA peaked on Day 7 and levelled off thereafter, with the exception of the surface sample collected from the untreated plot. In this sample, the concentration of cis-3-CAA increased throughout the entire 28 days of incubation.

DISCUSSION

Initial mineralization of ^{14}C-1,3-D in surface and subsurface soils collected from a treated plot at Green Acres was more rapid than in samples collected from an untreated plot, as well as in surface and subsurface samples collected from Hastings and Immokalee. Apparently, a greater microbial population capable of mineralizing 1,3-D was present at Green Acres because of the repeated applications of 1,3-D to this site. Although the Hastings site had a history of 1,3-D treatment, no enhanced degradation was observed. It is possible that in 5 years any enhancement of degradation was lost. The duration of enhanced degradation of the nematicide fenamiphos in the soil at Hastings lasted more than 3 years but less than 4 years (9). Mineralization of ^{14}C-1,3-D in soil samples collected from the treated plot at Immokalee, which was treated only once with 1,3-D, was not enhanced, however the fumigant formulation contained 17% chloropicrin. The effect of chloropicrin on the enhancement process is not known.

Before this study, degradation rates of cis- and trans-1,3-D in soils were considered to be the same (4,8,11–13,15,16). In enhanced degradation studies of 1,3-D in loamy soils, the degradation rates of cis- and trans-1,3-D were not determined individually (12). As a result, it was not determined whether enhanced degradation occurred for the two isomers, or one of the isomers, or with a different degree of enhanced degradation for the two isomers. Furthermore, enhanced degradation of

1,3-D occurred in loamy soils with one or two field applications and in some loamy soils with no previous exposure to the chemical. The enhanced degradation was attributed to the presence of 1,3-D degraders. No enhanced degradation was reported in a sandy soil treated six times with 1,3-D at a rate of 150 liters/ha (12). It was likely that, due to higher field soil temperature and lower application rate (110 liters/ha) of 1,3-D at Green Acres, volatilization of 1,3-D into the atmosphere was more rapid than in the Dutch site. As a result, inhibition of soil microbial activity at Green Acres was not as great as at the Dutch site, and microorganisms capable of degrading 1,3-D developed in the Green Acres soil.

Rapid hydrolysis of 1,3-D to 3-CAA occurred in aqueous media (5). The hydrolysis rate depended on temperature but was independent of pH at each temperature. At 20 C and 30 C, the half-life values for 1,3-D in water were 11.3 days and 3.1 days, respectively. These values were smaller than for the 17- to 20-day half-life values for cis- and trans-1,3-D in the surface and shallow subsurface Green Acres untreated samples incubated at 28 C. If all the 1,3-D remained in the soil solution, the half-life value for 1,3-D at 28 C would be about 5 days. In a closed system under dark conditions, however, 1,3-D vapor in the headspace and soil pore space must dissolve in soil solution to be hydrolyzed. Thus, at the same temperature the hydrolysis half-life for 1,3-D in soil should be greater than the hydrolysis half-life in water. Differential enhanced degradation of cis- and trans-1,3-D in treated soil at Green Acres was likely due to preferential use by microorganisms of the trans-form over the cis-form. The microorganisms either produced a larger amount of enzyme hydrolyzing the trans-1,3-D than the cis-form, or the enzyme had a higher hydrolytic activity toward the trans-form than the cis-form. A coryneform bacterium capable of using cis- and trans-3-chloroacrylic acid as a sole carbon source for growth produced two dehalogenases, one specific for the cis-isomer and the other for the trans-isomer (17). Biological hydrolysis could be a major factor in the degradation of trans-1,3-D in enhanced soil, with chemical hydrolysis being a minor factor; biological and chemical hydrolysis could be equally important in the degradation of cis-1,3-D in enhanced soil. In nonenhanced soil, chemical hydrolysis appeared to be the main factor in the degradation of cis- and trans-1,3-D, and biological hydrolysis was either negligible or a minor factor. Isolation of microorganisms capable of hydrolyzing 1,3-D from the enhanced soil could shed some light on the microorganisms' preference for the trans-form over the cis-form.

Initial rapid formation of cis-3-CAA in enhanced and nonenhanced soils could be due to soil surface induced hydrolysis of cis-1,3-D rather than aqueous solution induced hydrolysis. Trans-3-CAA could also have formed initially, but apparently was rapidly degraded microbiologically. As a result, little or no trans-3-CAA was formed in these samples throughout the entire 21 or 28 days of incubation. Lower levels and more rapid disappearance of cis-3-CAA after 7 days in the enhanced samples could be attributed to more rapid microbial degradation than in the nonenhanced samples. Similar to results with trans-1,3-D, microorganisms seem to prefer trans-3-CAA over the cis-isomer. Half-life values of trans-3-CAA in three surface soils collected from different fields were about three times smaller than for cis-3-CAA, being 0.8–1.4 vs. 2.3–4.2 days, respectively (4). A strain of *Pseudomonas* sp. was found to have the capacity to degrade 3-CAA (2), and another strain of *Pseudomonas* sp. was able to degrade an analogous chemical, 2-chloroallyl alcohol (14).

Both cis- and trans-1,3-D are volatile, with vapor pressures of 43 and 34 mm Hg (25 C), respectively (18). Either commercial 1,3-D or the individual isomers were used in most of the degradation studies. Without employment of radio-labeled 1,3-D, the mass balance for 1,3-D cannot be determined. Ou (8) reported that even though the screw caps were removed briefly for changing KOH traps, total ^{14}C recoveries in sandy soils treated with ^{14}C-

1,3-D after 28 days were poor, ranging from 50–70%. Even in water, more than 50% of the applied ^{14}C-1,3-D was volatilized in 5 hours (19). Addition of organic solvent (20 ml) to a 40-ml glass centrifuge tube containing 10 g of 1,3-D-treated soil would cause a reduction of more than half of the headspace and all the pore space, resulting in vaporization of 1,3-D into the atmosphere. At ambient temperature (23 ± 2 C), 72–75% of the mixture of cis- and trans-1,3-D from soil samples was recovered as soon as 1,3-D was added and mixed. Similar recovery (73%) for ^{14}C activity in ^{14}C-1,3-D-treated soil was reported (19). Keeping the soil samples treated with the two isomers for 1 hour at −30 C, rather than at 23 C, before extraction greatly reduced vapor pressures. At the lower temperature, evaporation of 1,3-D from headspace and soil pores was minimal, being 4–8% at 0 hour rather than 25–27%.

Because biological hydrolysis of 1,3-D, especially trans-1,3-D, appeared to be involved in the initial step of degradation in enhanced soil, it should be possible to isolate microorganisms capable of hydrolyzing or mineralizing 1,3-D. It is also possible that a microbial consortium may be responsible for complete degradation of 1,3-D, one or more responsible for the hydrolysis of 1,3-D, and the others responsible for subsequent degradation of 3-CAA. Ou (8) isolated a mixed bacterial culture from soil that mineralized ^{14}C-1,3-D.

In conclusion, differential enhanced degradation of cis- and trans-1,3-D occurred in Arredondo fine sand that had been treated with 1,3-D at least 6 times in the past 12 years. Trans-1,3-D in the enhanced soil disappeared more rapidly than did cis-1,3-D. Our results suggest that biological hydrolysis is a major factor in the hydroly-sis of trans-1,3-D in enhanced soil, and chemical hydrolysis is the main factor for the hydrolysis of cis- and trans-1,3-D in nonenhanced soil.

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