Some Biocidal Properties of 1, 3-D and its Degradation Product

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ABSTRACT

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The toxicity of fumigant residues to sweet orange seedlings planted in a moist sandy loam soil 80 days after treatment with 62.4, 125, 250 μg/g of 1, 3-dichloropropene (1, 3-D) was shown to be due mainly to chloroallyl alcohol, a degradation product of 1, 3-D. When 31.2 μg/g of 1, 3-D was applied in nonsterilized soil, the chloroallyl alcohol formed was biologically degraded and was not toxic to sweet orange seedlings. However, concentrations of 62.4-250 μg/g 1, 3-D in soil interfered with the biological degradation of chloroallyl alcohol, and the fumigant residue was highly toxic to sweet orange seedlings planted 80 days after treatment. Likewise in steam-sterilized soil treated with concentrations of 31.2-250 μg/g of 1, 3-D or 40-320 μg/g of chloroallyl alcohol the residues were highly toxic to sweet orange seedlings 80 days after treatment. The 1, 3-D was more toxic to citrus nematodes and to *Phytophthora parasitica* than to organisms that biodegrade chloroallyl alcohol. Concentrations of 4.6 and 5.6 μg/g of 1, 3-D in a sandy loam soil were lethal to 100% of the second-stage citrus nematode larvae, and 37.2 μg/g was lethal to 100% of the *P. parasitica* propagules. A concentration of 40 μg/g of chloroallyl alcohol in soil was lethal to 91% of the citrus nematodes. Colonies of *P. parasitica*, 2 mm in diameter, were not killed after 48 hr of exposure to 1,000 μg/ml of chloroallyl alcohol, the strongest concentration tested, but new growth at the surface of the colonies was negligible (indicating a fungistatic effect) in 400-1,000 μg/ml. These data indicate that the formation of chloroallyl alcohol by hydrolysis of 1, 3-D in soil does not improve control of the citrus nematode nor of *P. parasitica*, and, that phytotoxic concentrations of chloroallyl alcohol may occur when high concentrations of 1, 3-D are applied.

Additional key words: phytotoxicity of 1, 3-D and of chloroallyl alcohol.

High concentrations of 1, 3-dichloropropene (1, 3-D) are useful for preplant control of the citrus nematode *Tylenchulus semipenetrans* (Cobb), *Phytophthora citrophthora* (Sm. and Sm.) Leonian, and *P. parasitica* Dastur (1). Youngson and Goring (8) reported that the toxicity of chloropropane-chloropropene mixtures to root-lesion, root-knot, and sugar beet nematodes is caused almost solely by the 1, 3-dichloropropene components. Turner (7) showed that control of root-knot nematodes and the yield of cotton and of sugar beets were related directly to the concentration of 1, 3-D in the 1, 2-dichloropropane-1, 3-dichloropropene mixtures that were used. In moist soils, each molecule of 1, 3-D is hydrolyzed (nonbiologically) to a molecule of chloroallyl alcohol, which has fungicidal, nematicidal, and phytotoxic properties. Castro and Belser (4) reported that 50% of Cis-1, 3-dichloropropene in a moist soil at 24C was nonbiologically hydrolyzed to cis-chloroallyl alcohol in 20 days. McKenry and Thomason (5) reported that temperature influenced the rate of hydrolysis more than soil moisture. However, in very dry soil (17 bars moisture tension) the rate of hydrolysis was slower than in wet soil 0.6 bars (bar used refer to -bar). At 15C and 25C, 52% and 83%, respectively, of the 1, 3-D in a sandy loam soil at 0.6 bars was hydrolyzed in 10 days. Chloroallyl alcohols are not hydrolyzed, but are biodegraded by a *Pseudomonas* sp. (3). In addition to killing target soil-pathogens, high concentrations of 1, 3-D may kill microorganisms (*Pseudomonas* sp.) that degrade chloroallyl alcohol, and 1, 2-dichloropropane. Consequently, chloroallyl alcohol may remain in soils for long periods. Moje et al. (6) mentioned that cis-chloroallyl alcohol has lower fungicidal or nematicidal properties than cis-1, 3-D, and was phytotoxic to orange seedlings. The high concentrations of 1, 3-D required to control *Phytophthora* spp. and the citrus nematode (2, 6) occasionally result in phytotoxicity even after a presumably adequate waiting period. Information on the toxicity of 1, 3-D and of chloroallyl alcohol to the citrus nematode, to *P. parasitica*, and to sweet orange seedlings, and the effect of different concentrations of 1, 3-D on biodegradation of chloroallyl alcohol are presented herein.

MATERIALS AND METHODS

Toxicity of 1, 3-D and chloroallyl alcohol to citrus nematode and Phytophthora parasitica.—The survival of citrus nematode second-stage larvae (L2) or small colonies of *P. parasitica* in a nonsterile fine-sandy-loam soil that contained concentrations of 0-37.2 μg/g of 1, 3-dichloropropene was determined. D-D® (Shell Chemical Company, San Ramon, CA 94583) and Telone® (Dow Chemical Company, Midland, MI 48640) that contained 56%, 78%, or 93% cis- and trans-1, 3-D, also cis- and trans-1, 2-dichloropropane and related C_3 hydrocarbons

were used for the source of 1, 3-D. Since the initial fungicidal, herbicidal, and nematicidal properties of D-D and Telone have been reported to be related primarily to 1, 3-D (6, 7, 8), and cis and trans-1, 3-dichloropropene hydrolyze to chloroallyl alcohols (3, 4), only the concentration of 1, 3-D in the mixtures used is considered in the tests reported herein. The percentage of 1, 3-D in the D-D and Telone that was used is presented in Tables 1 and 2. Also survival of L2 citrus nematode in nonsterilized soil that contained 0-320 μg/g of chloroallyl alcohol was determined. The chemicals were mixed into 600 g of soil (dry wt basis) by tumbling in sealed 946-ml jars. The soil contained approximately 1,251 citrus nematode L2/50 cm^3, and 10.3% water. Since approximately 96% of the 1, 3-D and 100% of the chloroallyl alcohol was in the water phase in the soil, the concentration in the soil-water was calculated to be 9.3 and 9.7 times, respectively, that in oven-dry soil. Colonies of *P. parasitica* were obtained by seeding potato-glucose-$CaCO_3$ broth in 250-ml flasks with zoospores and shaking at 25C. After 2 days, two colonies (2 mm diameter) were enclosed in fine mesh nylon gauze and placed in the soil. Four jars of each treatment were sealed for 4 days with a lid that contained a 'Mylar' plastic liner, and stored at 24C. Then two 50-cm^3 lots of soil from each jar were placed on Baermann funnels for 48 hr and the active citrus nematode larvae that emerged were counted. The *P. parasitica* colonies were removed, placed into firm apples by means of a forceps and the punctures were covered with tape. Then tissue from rots that developed was placed on potato-glucose agar (PGA) and the fungi that grew were identified.

The toxicity of chloroallyl alcohol to *P. parasitica* was tested in water and not in soil, because chloroallyl alcohol has very low vapor pressure and is highly miscible in water. In water, the large size (2 mm diameter) of the *P. parasitica* colonies could be more uniformly treated with the toxicant than was possible in soil. Two small colonies of *P. parasitica*, similar to those described above, were placed in 0, 40, 80, 120, 160, 200, 300, 400, 500, 600, 700, 800, and 1,000 μg/ml of chloroallyl alcohol in 20-ml

TABLE 1. Mortality of citrus nematode larvae and of *Phytophthora parasitica* in nonsterilized soil treated with different concentrations of chloroallyl alcohol or 1, 3-dichloropropene

Concentration		Decrease of citrus nematodes[a] (%)	Kill of *Phytophthora parasitica* colonies[b] (%)
Compound (μg/g)	1, 3-D component (μg/g)		
None	Control	0	0
D-D[c]			
2.5	1.4	66	0
5.0	2.8	98	0
10.0	5.6	100	0
20.0	11.2	100	0
40.0	22.4	100	25
Telone[d]			
2.5	2.3	92	0
5.0	4.6	100	0
10.0	9.3	100	0
20.0	18.6	100	0
40.0	37.2	100	100
Chloroallyl alcohol			
40		91	
80		100	
160		100	
320		100	

[a]The figures are based on four replications and approximately 1,251 active citrus nematode larvae/50 cm^3 of nontreated soil.
[b]The survival is based on four replications.
[c]The D-D mixture contained 56% 1, 3-dichloropropene.
[d]Telone II contained 91-93% 1, 3-dichloropropene.

TABLE 2. Toxicity of nonsterilized and of initially sterilized soil to second-stage citrus nematode larvae and sweet orange seedlings 80 days post-treatment with different concentrations of 1, 3-dichloropropene (1, 3-D) or chloroallyl alcohol

Treatment and rate (μg/g soil)	Seedlings dead or severely injured[a]		Kill of citrus nematodes[b]	
	Nonsterilized soil (%)	Sterilized soil (%)	Nonsterilized soil (%)	Sterilized soil (%)
None (Control)	0	0	0	0
1, 3-D[c]				
31.2	0	75	100	100
62.5	75	100	100	100
125	100	100	100	100
250	100	100	100	100
Chloroallyl alcohol				
40	0	100	86	100
80	0	100	100	100
160	0	100	100	100
320	100	100	100	100

[a]The percent of seedlings dead or nearly dead 21 days after planting in soil 80 days after treatment. The figures represent the condition of four seedlings in replicated pots.
[b]The figures are the average of six jars or replications.
[c]The 1, 3-D in Telone that contained 78% 1, 3-dichloropropene.

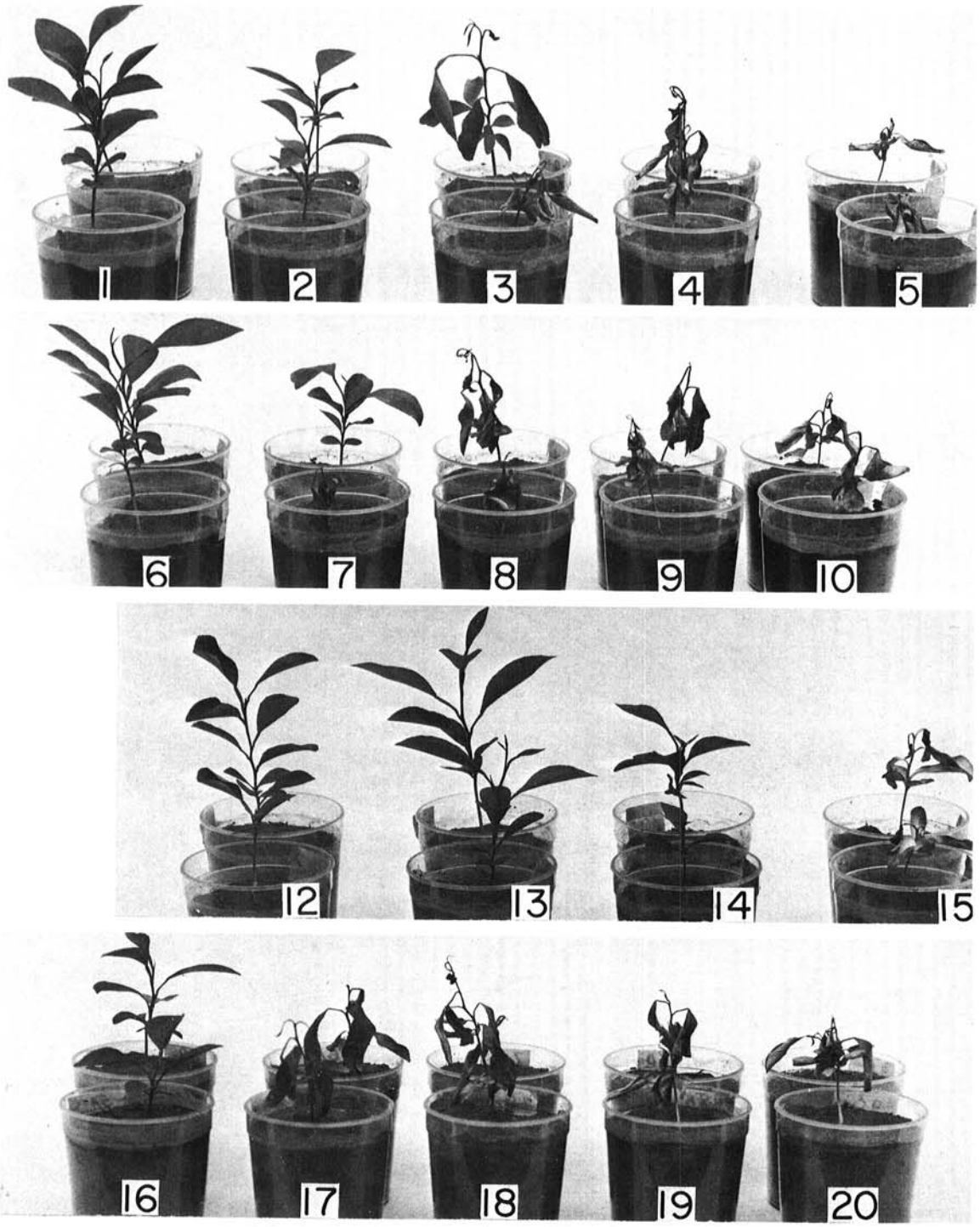

Fig. 1. Sweet orange seedlings planted 80 days after treatment of soils with different concentrations of chloroallyl alcohol or Telone. The two seedlings selected per treatment comprise the range of toxicity symptoms that developed on four seedlings 7 days after planting. Pots 1-5: nonsterilized soil that was treated with 0, 31.2, 62.4, 125, or 250 μg/g of 1, 3-D, respectively. Pots 6-10: steam-sterilized soil that was treated with 0, 31.2, 62.4, 125, or 250 μg/g of 1, 3-D, respectively. Pots 12-15: nonsterilized soil that was treated with 40, 80, 160, or 320 μg/g chloroallyl alcohol, respectively. Pots 16-20: steam-sterilized soil that was treated with 0, 40, 80, 160, or 320 μg/g chloroallyl alcohol, respectively.

bottles. The highest concentrations of chloroallyl alcohol used is 3.3 times that which might accrue from 37.2 μg/g of 1, 3-D in soil with 10.3% water. The bottles were sealed with Teflon-lined plastic caps and shaken slowly for 24 or 48 hr. There were four bottles of each treatment. The viability of *P. parasitica* was determined by placing rinsed colonies into firm apples as described above.

Effect of the concentration of 1, 3-D on degradation of chloroallyl alcohol in soils.—Different concentrations of 1, 3-D or chloroallyl alcohol, added to nonsterilized and sterilized fine-sandy-loam soil (65% sand, 26.2% silt, and 8.8% clay), were assayed for toxicity to citrus nematode larvae and to sweet orange [*Citrus sinensis* (L.) Osbeck] seedlings after 80 days. Concentrations of 31.2 - 249.8 μg/g 1, 3-D (oven-dry basis) or 40-320 μg/g chloroallyl alcohol (oven-dry basis) were added to nonsterilized and to steam-sterilized soil. The sterilized soil was prepared by autoclaving [1.05 kg/cm^2 (15 psi) for 40 min] 600g (oven-dry basis) of the soil in 946-ml wide-mouth jars that were closed by a lid with a Mylar plastic liner. Samples of soil from four jars of sterilized soil plated on soil-extract agar were sterile. The 1, 3-D was pipetted onto 600g (oven-dry basis) of nonsterilized and sterilized soil, and the chloroallyl alcohol onto sterilized soil in jars. Immediately after the chemicals were added, the jars were closed and shaken and tumbled by hand to mix the chemicals into the soil. Different concentrations of chloroallyl alcohol, which is nonvolatile, were mixed into nonsterilized soil in a twin-shell dry blender, and then 600g (oven-dry basis) lots of the soil were placed into jars. The moisture content of the soil in all of the jars was increased from 4% to 9% and uniformity was obtained by shaking and tumbling. Six jars of each treatment including controls were sealed for 80 days at 21-24 C. Then approximately 10,560 citrus nematode L2 and 1440 adult males were pipetted onto the soil in each jar and uniformly distributed in the soil by tumbling and shaking the jars by hand. After 4 days, two 50-cm^3 subsamples of soil from each jar were placed on Baermann funnels for 2 days and the number of citrus nematode L2 and males that had emerged were counted. Homosassa sweet orange seedlings 7-14 cm high were planted in 473-ml plastic pots, one per pot, with soil from four jars of each treatment. The trees were placed in a controlled temperature tank at 27 C in a greenhouse (21-27C), and the condition of the trees was observed frequently during 21 days.

RESULTS

Toxicity of 1, 3-D and of chloroallyl alcohol to citrus nematode or Phytophthora parasitica.—Concentrations of 2.3 and 2.8 μg/g of 1, 3-D in a nonsterilized fine-sandy-loam soil killed or inactivated 92 and 98%, respectively, of the L2 citrus nematode (Table 1). Concentrations of 40 or 80 μg/g of chloroallyl alcohol killed or inactivated 91 and 100% of the L2 citrus nematode, respectively. In this test, 1, 3-D was approximately 17 times more toxic to the citrus nematode than was chloroallyl alcohol. Mycelial colonies of *P. parasitica* were killed in 25% and 100% of the jars of soils that contained 22.4 μg/g or 37.2 μg/g of 1, 3-D, respectively. All of the colonies of *P. parasitica* exposed to 0-1,000 μg/ml of chloroallyl alcohol for 1 or 2 days were viable. However, little or no new mycelium extended from the surface of the colonies in 400-1,000 μg/ml of chloroallyl alcohol; these concentrations of chloroallyl appeared to be fungistatic. Between 8- and 10-fold higher concentrations of 1, 3-D were required to kill small colonies of *P. parasitica* than citrus nematode larvae. The toxicity of D-D and Telone to citrus nematode larvae and to *P. parasitica* was directly related to the concentration of 1, 3-D. A concentration of 22.4 μg/g of 1, 3-D (40 μg/g of D-D) or 37.2 μg/g of 1, 3-D (40 μg/g Telone) in soil killed approximately 25% and 100% of *P. parasitica*, respectively. These data indicate that the 100% kill of *P. parasitica* mycelial colonies buried 4 days in soil that contained 37.2 μg/g (oven-dry basis) or 361 μg/ml of 1, 3-D in the soil water was caused by 1, 3-D and not by chloroallyl alcohol.

Effect of the concentration of 1, 3-D on the degradation of chloroallyl alcohol in soils.—Steamed and nonsteamed soils, 80 days after being treated with 31.2 μg/g or 62.5 μg/g of 1, 3-D, respectively, were moderately toxic to sweet orange seedlings (Table 2). Concentrations of 62.5-250 μg/g in initially sterilized soil and 125 μg/g and 250 μg/g of 1, 3-D in nonsterilized soil were highly toxic to sweet orange seedlings (Fig. 1, pots 1-10). Concentrations of 40-160 μg/g of chloroallyl alcohol in nonsterilized soil were not toxic, but 40-320 μg/g of chloroallyl alcohol in steam-sterilized soil were highly toxic to sweet orange seedlings that were planted 80 days after treatment (Fig. 1, pots 12-20). The nonsterilized soil contained organisms that metabolized chloroallyl alcohol. Heating the soil or treating it with 62.5-250 μg/g of 1, 3-D or with 320 μg/g of chloroallyl alcohol apparently inactivated or killed these organisms, and led to the accumulation of phytotoxic concentrations of chloroallyl alcohol (Fig. 1, pots 3-5). All of the citrus nematode L2 that were placed in nonsterilized or in sterilized soils 80 days after treating with 31.2-250 μg/g of 1, 3-D or 40-320 μg/g of chloroallyl alcohol (except 14% of those in nonsterilized soil that was treated with 40 μg/g of chloroallyl alcohol) were killed or inactivated. Probably some of the chloroallyl alcohol in the nonsterilized soil that was treated with 40 μg/g of chloroallyl alcohol was biodegraded.

DISCUSSION

Compounds toxic to sweet orange seedlings in steam-sterilized soil 80 days after treatment with Telone were mainly cis- and trans-chloroallyl alcohol and 1, 2-dichloropropane, presumably because microorganisms that biodegrade chloroallyl alcohol were killed during sterilization. Sterilized soil treated with 31.2 μg/g of 1, 3-D for 80 days should contain 26.0 μg/g of chloroallyl alcohol. This soil was less phytotoxic than that to which 40 μg/g of chloroallyl alcohol had been added (Fig. 1, pots 7, 17). Injury of sweet orange seedlings, especially at the low concentrations of 1, 3-D used in these tests, is not attributable to 1, 2-dichloropropane, since Moje et al. (6) reported that 1, 2-dichloropropane at low concentrations was not phytotoxic to sweet orange seedlings.

In nonsterilized soil, a concentration of 31.2 μg/g of 1, 3-D was not phytotoxic 80 days after treatment (Fig. 1, Pot 2). Apparently hydrolysis of 1, 3-D and biodegradation of chloroallyl alcohol (3, 4) was completed. Treatment of nonsterilized soil with 62.5-250 μg/g 1, 3-D, or with 320 μg/g chloroallyl alcohol

apparently killed microorganisms that degrade chloroallyl alcohol and this led to concentrations of chloroallyl alcohol that were toxic to sweet orange seedlings 80 days after treating (Fig. 1, Pots 3-5 and 15). The fungicidal and nematicidal properties of 1, 3-D were not enhanced by hydrolysis to chloroallyl alcohol. Differences in toxicity of the treated soils to sweet orange seedlings suggest that biodegrading microorganisms tolerated higher concentrations of chloroallyl alcohol (160 μg/g) than of 1, 3-D (31.2 μg/g) (Fig. 1, Pots 2 and 14). When 560-1,680 liters/ha (500-1,500 lbs/acre) or more of 1, 3-D are used, 62-186 μg/g or more of 1, 3-D could occur in the top 5-60 cm of soil. Phytotoxic concentrations of chloroallyl alcohol may be produced near the point of injection by hydrolysis of 560 liters/ha 1, 3-D, since 62 μg/g or more of 1, 3-D in a closed system interfered with biodegradation of chloroallyl alcohol. McKenry and Thomason (5) reported that 1, 3-D should be readily hydrolyzed in warm moist soils, but can be expected to persist in the soil atmosphere at concentrations greater than 0.01 μliter/liter, or 0.27 μg/ml in the soil water, for 2-4 mo. However, these concentrations would not be biocidal or phytotoxic.

Telone II (92% 1, 3-D) was more toxic to citrus nematode L2 and *P. parasitica* than D-D (56% 1, 3-D), or chloroallyl alcohol. This appears to be directly related to the concentrations of 1, 3-D (2, 6, 8). The 1, 3-D was approximately eight fold more toxic to L2 citrus nematode L2 than to *P. parasitica*.

Since 40 μg/g or more of chloroallyl alcohol in a fine-sandy-loam soil was highly toxic to sweet orange seedlings and is not easily detectable by odor or by chemical analysis, bioassay methods for detection are recommended before planting soils that have been treated with high concentrations of 1, 3-D.

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