Effect of Soil Structure on Penetration by Metham-Sodium and of Temperature on Concentrations Required to Kill Soilborne Pathogens

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Contribution 932-E, 1983 series, from the ARO.
We are grateful to A. Hadas and Z. Gerstl from the Department of Soil Physics and the Department of Soil Residues Chemistry, respectively, Institute of Soils and Water, ARO, for advice.
Accepted for publication 16 October 1984.

ABSTRACT

Ben-Yephet, Y., and Frank, Z. R. 1985. Effect of soil structure on penetration by metham-sodium and of temperature on concentrations required to kill soilborne pathogens. Phytopathology 75:403-406.

Penetration of metham-sodium and its efficacy against microsclerotia (MS) of *Verticillium dahliae* were evaluated in a loessial soil (20% clay content) with a single-grained structure and a clay soil (54% clay content) with well defined, aggregated structure. The chemical, applied in a dilute solution to 1 m^2 field microplots, killed the MS to a deeper level in the clay soil than in the loessial soil. To corroborate results obtained from field studies, penetration of metham-sodium was also tested in plastic columns filled with sieved or ground samples of soils. In the clay soil, the fungicide killed MS to a deeper level in the sieved samples with large (<6,000 μm) aggregates than in ground samples of the same soil containing smaller aggregates. In the loessial soil, however, the fungicide killed MS to the same depth in both the sieved and ground samples which had similar aggregate sizes. The toxicity of metham-sodium to resting structures of several pathogens was greatest at a high temperature (35 C). However, the temperature coefficient of the fungicide's activity was not constant. The fungicide dose required to kill 50% of the MS of *V. dahliae* at 15 C was 1.5, 3, and 6 times that required at 25, 30, and 35 C, respectively. The ED_{50} for *V. dahliae*, *Fusarium oxysporum* f. sp. *melonis*, and *Sclerotium rolfsii* was half that for *Sclerotinia sclerotiorum*.

Additional key words: methyl-isothiocyanate, percolation, temperature-coefficient (Q_{10}).

Metham-sodium (sodium methyldithiocarbamate) (MES) was selected as a suitable fungicide against soilborne pathogens such as *Verticillium dahliae* Kleb., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Sclerotium rolfsii* Sacc., and *Fusarium oxysporum* Schlecht. because of its low cost and ease of application to the soil via the sprinkler irrigation system (7). The persistent structures of those pathogens are widespread in the upper 40 cm of the soil profile (6). Thus, to achieve efficient control, a soil fungicide must be effective to at least that depth. The fungicidal activity of MES depends upon its conversion to methylisothiocyanate (MIT) in the soil (12).

MIT is partially adsorbed to clay and peat; thus, an increase in clay or peat content of the soil reduced the amount of MIT released (13). In a previous study (2), it was confirmed that 20% more fungicide was required to kill microsclerotia (MS) of *V. dahliae* in ground and sieved (<250-μm particle size) alluvial soil (54% clay) than in loessial soil (20% clay). Recently, Ben-Yephet et al (5) tested MES penetration in loessial soil to a depth of 40 cm and observed that increasing the MES dose applied in dilute solutions did not increase fungicide penetration past the 20-cm depth. They assumed that the limited penetration was due to the retarded rate of percolation and thus the increased rate of adsorption of MIT molecules on clay particles in the unstructured loessial soil.

Soil temperature also is known to affect MES performance. However, MES efficacies at different temperatures were not similar (2,10).

The purpose of the present work was to study the effects of soil structure and aggregate-size fractions on MES penetration and of incubation temperatures on MES concentrations required to kill the persistent structures of several soilborne plant pathogens.

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MATERIALS AND METHODS

Inoculum and fungicide. The inoculum used in all experiments was microsclerotia (MS) (<250 μm in diameter) of *V. dahliae* from potato stems (4). The MS were mixed with air-dried loessial and clay soils at 10^4/g, and 0.5- to 1.0-g aliquots of this mixture were placed in 20-μm-mesh nylon bags. In microplot experiments, these bags were then tied along a string at intervals of 2, 10, 20, 30, and 40 cm from the upper end and buried at those depths from the soil surface following seedbed preparation. In soil column experiments, the MS encased in nylon netting were placed at depths of 4, 8, 16, 20, 24, 28, 32, 36, and 40 cm. After soil treatments, samples were suspended in 50 ml of sterile distilled water, poured onto a filter paper, and rinsed three times with sterile distilled water. To determine viability, fifty MS from each sample were identified under low-power magnification with a dissecting microscope, singly transferred with a botanical needle, and plated on a synthetic medium containing PCNB and sucrose (1).

Sclerotia of *S. sclerotiorum* were obtained from plant residues collected from a field recently affected by lettuce-drop disease. Sclerotia were collected by rinsing residues of diseased plants with tap water and stored at 4 C until required. Ten sclerotia enclosed in a nylon mesh bag were used for each replicate in soil-column experiments. After experimental treatment, sclerotia were washed clean of soil particles in tap water, surface sterilized in sodium hypochlorite (1%) for 8 min, washed twice with sterile distilled water, and plated on potato-dextrose agar (PDA). Chlamydospores of *Fusarium oxysporum* f. sp. *melonis* Leach & Currence and sclerotia of *S. rolfsii* (500–840 μm size range) were produced as described (11,14) and kindly supplied by A. Greenberger, Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel. Aliquots (1 g) of soil mixed with chlamydospores (4,000/g of soil) or sclerotia (25/g of soil) were placed in 20-μm-mesh nylon bags. After experimental treatments, sclerotia of *S. rolfsii* were separated from the soil and plated on filter paper moistened with an aqueous solution of bromocresol green (100 mg/L). Viable germinating sclerotia changed the color of the filter paper from blue to yellow

(11). Viability of chlamydospores of *F. oxysporum* was determined by dilution-plating on a selective medium (14), and five plates from each column were observed for colonies of *Fusarium*.

Commercial-grade metham-sodium (sodium-methyl dithiocarbamate) was used in an aqueous solution containing 32.7% active ingredient (a.i.), pH 9.1. The fungicide dose was calculated as milliliters of product per liter of water per square meter, or as the amount of solution at a particular concentration of metham sodium.

Soils and aggregate-size groups. Two soil types were used in field experiments and in soil columns: a silty loam, loessial sierozem type, pH 8.4, 20% clay (predominantly montmorillonite), 0.5% organic matter, and with a field capacity of 20% (v/v); and a clay, pH 7.9, 54% clay (predominantly montmorillonite), 1.36% organic matter, and with a field capacity of 32% (v/v).

The two soils represented different types of soil structure: the loessial soil was single-grained, poorly structured, and unstable to water; the clay soil had well defined aggregates and was stable to water. To evaluate MES penetration into those soils, experiments were conducted in 1-m^2 field microplots of and in the laboratory in soil columns. Columns were packed with one of three soil aggregate-size groups prepared from the plow layer of each soil type: $<6{,}000$ μm (A), <250 μm (B), and <149 μm (C). In both soils, aggregate size A closely represented aggregate structure in the cultivated field. Aggregate sizes B and C were prepared by grinding loessial soil gently with pestle and mortar. For the clay soil, however, samples were ground by machine (soil grinder toothed shaft and grinding disks (KG D-6072; Karl Kolb, Dreieich, West Germany) as needed to obtain B and C aggregate groups.

Each soil aggregate-size group (A, B, and C) was passed through several sieves to segregate the ranges of aggregate sizes. Times for water percolation in columns of each aggregate group were determined (Table 1). In the loessial soil, the content of the fine aggregates was almost the same in the B and C aggregate groups whereas in the A-group, 20% of the aggregates were 250–6,000 μm. The rates of water percolation was nevertheless similar in all three aggregate groups. In the clay soil, however, the three aggregate groups differed distinctly in the soil aggregate-size distribution and in times needed for water percolation in the columns.

Metham-sodium penetration in soils in field microplots. Microplot experiments were established in the loessial and clay soils following seedbed preparation. MS of *V. dahliae* were inserted to a depth of 40 cm in the middle of each 1-m^2 microplot. Then the soil was leveled and each microplot was bordered with soil ridges to avert possible runoff. Toxicity of MES to MS was tested at 50, 70, 80, and 90 ml of product per square meter. Fungicide at each concentration was suspended in 80 or 100 L/m^2 which are the total water volumes found experimentally to be required to bring the loessial and clay soils, respectively, to field capacity down to 40 cm. Control plots received water without MES. Water with or without MES was applied intermittently with calibrated water cans at eight irrigation periods during 6 hr. Treatments were replicated in three randomized blocks in each of two experiments. Ten to 14 days after soil treatment, propagule samples from fungicide-treated and control plots were processed to determine MS viability.

Preparation of soil columns and fungicide. Columns 7.5 cm in diameter and 48 cm long were prepared by connecting four plastic segments. For each soil type, three columns were prepared with samples of each aggregate-size group. All columns were prepared by successively packing aliquots of 100 ml of air-dried soil to a bulk density of 1.28 g/cm^3. Nylon bags containing MS of *V. dahliae* were placed at various depths along the column, usually at 4, 8, 16, 20, 24, 28, 32, 36, and 40 cm. The volume of water or fungicide solution added was adjusted to wet 2.1 kg of soil to field capacity down to 41 cm in the columns. The fungicide concentration used, 103 mg MES a.i. per 2.1 kg of soil, was equivalent to 770 L of product per ha diluted in either 800 m^3 of water per hectare for the loessial soil or in 1,000 m^3/ha for the clay soil. Columns were continuously irrigated with calibrated separatory funnels for 6 hr, except in the columns packed with aggregate group C of the clay soil, into which the solutions were infiltrated for 10–12 hr. For control treatments, columns were filled with sieved samples of each soil (aggregate size $<6{,}000$ μm) and irrigated with water only. The experiment was conducted three times under similar conditions. Four days after irrigation, MS of *V. dahliae* were separated and plated on the synthetic medium as described above.

Effect of incubation temperature on metham-sodium concentrations required to kill soilborne plant pathogens. Soil and columns were equilibrated at the experimental temperatures. Then columns 4.4 cm in diameter and 10 cm long were packed with air-dried loessial soil, 180 g per column. Structures of the pathogens (*V. dahliae*, *S. rolfsii*, *F. oxysporum* f. sp. *melonis*, and *S. sclerotiorum*) were placed at mid-depth in the columns and the soil was irrigated with 25 ml of either water or fungicide solutions at 25, 50, 100, 200, 300, 400, 600, 800, and 1,000 mg of MES a.i. per liter of water. Water temperatures were adjusted before the columns were irrigated. Subsequently, the incubation periods were adjusted to the half-life time of MIT at each temperature to obtain toxicity data of the different concentrations on a comparable time-temperature basis. Dependence of MIT decomposition, in three soils, on temperature was calculated as $Q_{11} = 2$ (15). The decomposition energies in the range of 4–35 C are usually constant (9). Based on the half-life time of MIT as determined at 20 C (8), the incubation periods were extrapolated to be 8, 4, 3, and 2 days at 15, 25, 30, and 35 C, respectively. To evaluate the effect of incubation time on the toxicity of MIT to MS of *V. dahliae*, soil in the columns was irrigated with 25 ml of either water or fungicide solution (100 and 200 mg of MES a.i. per liter of water) and incubated for 4, 8, and 12 days at 25 C and 8, 16, and 24 days at 15 C. All treatments were replicated three times in each of three experiments.

RESULTS

Penetration of metham-sodium into soils in field plots. In the clay soil, the fungicide killed MS to a depth of 40 cm at 70, 80, and

TABLE 1. Time required for water percolation in columns of three aggregate size groups obtained from loessial and clay soils

Soil type	Aggregate size group[x] (μm)	Distribution (%) of soil aggregates by size[y] (μm)				Time (hr)[z] required for water to percolate down to:		
		250–6,000	149–250	74–149	<74	30 cm	35 cm	40 cm
Loessial	A <6,000	20	1.6	29	49.4	9	18	60
	B < 250	0	2	36	62.0	10	20	64
	C < 149	0	0	37	63.0	10	20	64
Clay	A <6,000	86	9	2.8	2.2	7	9	12
	B < 250	0	32	22	43.0	8	12	30
	C < 149	0	0	32	68.0	24	144	268

[x] In both clay and loessial soil, aggregate size group A was obtained by sieving soil collected from the field through a 6,000-μm screen, and it closely represents aggregation that occurs in the field. Groups B and C were obtained by grinding samples of group A until soil passed through the appropriate screen, 250 or 149 μm. The loessial soil was ground with pestle and mortar and the clay soil in a machine.

[y] Distribution of soil aggregates by size was determined by sieving each group through the appropriate screens.

[z] Time of water percolation includes the 6-hr period during which water was gradually applied.

90 ml of product per square meter; the 50-ml concentration killed MS down to 30 cm. In the loessial soil, however, MS were killed to only 20 cm with all of these MES concentrations. Results suggested that a deeper penetration of MES in the clay soil may have resulted from the well-defined structure of this soil which enabled more rapid percolation compared to that in the loessial soil. The column experiments were conducted to test this hypothesis.

Penetration of metham-sodium into soil aggregate-size groups in columns. The viability of MS following fungicide applications to soil columns containing soil of each of the three aggregate-size groups is summarized in Table 2. In the loessial soil, MS were killed to the same depth (28 cm) in all three aggregate-size groups. With the coarse aggregates (A) (<6,000 μm), however, the fungicide penetrated slightly deeper than with the two other aggregates (B and C), and this was reflected by delayed germination of MS recovered from the 32-cm depth. In the clay soil, MS were killed at deeper depths as the soil aggregate sizes were increased. MS were killed to depths of 36, 28, and 20 cm in the aggregation groups of <6,000 μm, <250 μm, and <149 μm, respectively. Increasing incubation times from 4 to 8 or 12 days after fungicide or water applications did not cause death of MS in deeper layers than with 4 days of incubation in either the loessial or the clay soil.

TABLE 2. Metham-sodium (MES) penetration as measured by percentage of microsclerotia (MS) of *Verticillium dahliae* killed in columns filled with different aggregate size groups derived from loessial and clay soils[y]

Depth in columns (cm)	MS of *V. dahliae* killed (%)[z]					
	Clay soil aggregate size (μm)			Loessial soil aggregate size (μm)		
	<6,000	<250	<149	<6,000	<250	<149
0–20	100	100	100	100	100	100
24	100	100	70	100	100	100
28	100	100	0	100	100	100
32	100	0	0	0	0	0
36	95	0	0	0	0	0
40	0	0	0	0	0	0

[y] Columns each filled with 2.5 kg of soil of designated aggregate sizes were irrigated with water or fungicide solution to wet 2.1 kg to field capacity. Each column received 103 mg of MES a.i., diluted in 520 ml of water for clay soil or in 370 ml of water for loessial soil.

[z] For control treatments, columns were filled with sieved loessial and clay soil, (<6,000 μm aggregate size) and irrigated with water only. Germination was not reduced in the controls at any depth following 4 days of incubation. Fifty MS per treatment replicate were plated on a selective medium and percentage germination was determined. Results are means of three successive replications (one column per treatment per replication).

Effect of incubation temperatures on metham-sodium concentrations required to kill persistent structures of soilborne plant pathogens. Increasing the MES concentrations increased mortality of the structures of the four fungi at all temperatures tested. As incubation temperature decreased, higher concentrations were required to kill the pathogens. The ED_{50} concentration at 15 C was 1.5 times that needed at 25 C, and that in turn was 4 times the concentration needed at 35 C. The MES concentration required to kill 100% of the resting structures was nearly twice that of the ED_{50}. The ED_{50} concentration for *V. dahliae*, *F. oxysporum* f. sp. *melonis*, and *S. rolfsii* was the same and about half that required for *S. sclerotiorum*. ED_{50} and ED_{100} concentrations were calculated from the actual data presented in Table 3. Increasing the incubation time, with water or the fungicide, beyond the half-life time of MIT at 15 and 25 C did not raise the percent kill of MS of *V. dahliae*.

DISCUSSION

Soil structure and soil temperature are evidently important factors that affect MES penetration into soil and its efficacy against soilborne plant pathogens. It has been reported (13) that MIT is partially adsorbed to clay particles and that the adsorption rate increases with higher clay contents. When the fungicide is adsorbed to clay particles it is not effective against soilborne plant pathogens, and it was expected that higher concentrations would be required to kill pathogens in the clay soil than in the loessial soil. However, microplot and laboratory experiments indicated that a lower dose of MES was required to kill MS of *V. dahliae* in the natural or coarsely aggregated clay soil with 54% clay content than in the loessial soil with 20% clay content. These results are in good agreement with those obtained from field studies when MES was applied to loessial and clay soils by sprinkler irrigation system (5; and *unpublished*).

The more rapid percolation of water in clay than in loessial soil can be attributed to the aggregated, water-stable structure of the clay soil as contrasted to the single-grained lack of aggregated structure of the loessial soil. The wider range of pore radii of the clay soil in the field and in columns (aggregate size A) enabled percolation of the MES solution to deeper depths in a fingerlike pattern. This presumably allowed subsequent penetration of aggregates. In contrast, the narrow range of pore radii in the loessial soil and the instability of its weak structure to water prevented the fingerlike, deeper penetration of the MES solution. Reduced macropore frequency in ground clay soil (B and C aggregate groups), and stoppering of pores by imbibed microaggregates in aggregate group C, may have retarded infiltration and reduced MES penetration.

TABLE 3. Mortality of resting structures of *Verticillium dahliae* and *Sclerotinia sclerotiorum* induced by different concentrations of metham-sodium (MES) in columns of loessial soil at four temperatures[w]

Temperature (C)[x]	Pathogen[y]	Percentage of resting structures killed by MES at (mg a.i. per liter of water)[z]								
		25	50	100	200	300	400	600	800	1,000
15	*V. dahliae*	0	0	24	78	97.5	100	...	...	...
	S. sclerotiorum	0	0	5	10	49	77.5	98.5	98.5	100
25	*V. dahliae*	0	0	42	99	100	...	...	...	...
	S. sclerotiorum	0	0	7	53	75	87	100	100	100
30	*V. dahliae*	0	4	89	100	100	...	...	...	...
	S. sclerotiorum	0	2	50	96	100	...	...	...	...
35	*V. dahliae*	50	94	100	100	100	...	...	...	...
	S. sclerotiorum	5	42	85	100	100	...	...	...	...

[w] Each column was filled with 180 g of loessial soil and was irrigated with 25 ml of water or fungicide solution (sufficient to moisten 150 g soil to field capacity).

[x] The incubation periods were adjusted to obtain the half-life time of methyl-isothiocyanate (MIT) at each temperature and were 8, 4, 3, and 2 days at 15, 25, 30, and 35 C, respectively.

[y] Resting structures were microsclerotia of *V. dahliae* and sclerotia of *S. sclerotiorum*. Results similar to those for *V. dahliae* also were obtained with sclerotia of *Sclerotium rolfsii* and chlamydospores of *Fusarium oxysporum* f. sp. *melonis*.

[z] At all temperatures tested during the relevant incubation periods, germinability of the resting structures was not reduced in the control treatments and was about 100%.

It might appear likely that in soil with a high clay content and good structure, the rapid percolation of the solution will leave only a small quantity of MIT to penetrate into the clods. However, because soil pathogens exist mainly along root systems that grow through spaces between and within clods, sufficient MIT is available to kill pathogens in those spaces. Gerstl et al (8) reported a half-life of MIT of 4–5 days at 20 C in loessial and clay soils. As the time required for the penetration of the fungicide increases (Table 1), more MIT molecules are adsorbed to clay particles and decompose; thus, fewer MIT molecules arrive at the deeper layers of the loessial soil or of the experimental fine aggregate groups (B and C) of the clay soil.

Toxicity of MES was reported to be greater at 20 C than at other temperatures that were tested (10). In a previous study, however, toxicity of MES to *V. dahliae* in soil columns was higher at 35 C than at lower temperatures for the same incubation period (2). This trend was verified in the present study: different fungi and several MES concentrations were employed for incubation periods that were deliberately adjusted to the half-life of MIT at each temperature. These comparable incubation periods were used to enable similar active concentrations to affect the pathogens tested. Smaller MES concentrations were required to kill the pathogens at the higher temperatures than at the lower ones, but the relation was not linear. The dramatic increase in MIT efficacy at 35 C was possibly due to a combination of several factors: a thermodynamic increase of MIT activity, enhanced activities of any antagonists that may have tolerated the particular combination of temperature and fungicide, and decreased vitality of the resting structures. Although percentage germination of pathogen structures was not reduced following 2 days of incubation at 35 C, the latter possibility is still indicated from results of another study in which 0, 50, and 100% kill of *S. sclerotiorum* sclerotia was observed following incubation at 35 C for 2, 4 and 8 days, respectively (Y. Ben-Yephet, J. Katan, and Y. Sharoni, *unpublished*).

Based on the enhanced activity of MIT at higher temperatures, it has already been recommended (3) that MES be applied in the hot summer or as early as possible in autumn, instead of in cool weather, to control *S. sclerotiorum* in soil prior to planting.

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