Effects of Soil Water Content and Soil Temperature on Efficacy of Metham-Sodium Against *Verticillium dahliae*

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ABSTRACT

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Soil column studies were conducted to investigate the influence of soil water content and soil temperature on the efficacy of metham-sodium and its degradation product methyl isothiocyanate against *Verticillium dahliae*. The viability of the microsclerotia (MS) of the fungus in the top 30 cm of fumigated and control columns was measured. Temperatures for studies were 2 or 22°C, and the soil water content, expressed as soil matric potential, varied from -23 (wet), -113 (moist), to -2485 J/kg (dry). There was a significant interaction of soil water content and temperature on the efficacy of metham-sodium against *V. dahliae* MS. For the low soil temperature (2°C) the fumigant was more effective against MS of the fungus in wet than in moist or dry soil. Soil water content did not affect fungicidal activity of metham-sodium when the soil columns were maintained at 22°C. These results suggest that the fumigant has a greater efficacy against *V. dahliae* in wet/cold soil conditions compared to the other conditions tested. Consequently, it was recommended that metham-sodium be applied to fields by chemigation in late fall or early spring to obtain efficacious results.

Additional keywords: Busan 1020, chemigation, percent kill, potato early dying, soil fumigant

The soil fumigant metham-sodium (sodium *N*-methylthiocarbamate) is a general biocide used for the control of soil fungi, nematodes, insects, and weeds. The activity of the fumigant depends on its conversion to methyl isothiocyanate (MIT) (9,18), which constitutes 90% of the conversion products in soil (16). Other gaseous degradation products include CS₂, COS, and H₂S (8,23). These gases may play a minor role in the overall effectiveness of the fumigant.

The behavior of MIT and its precursor in soil has been the subject of several investigations over the last three decades. The focus of these studies varied from simple laboratory tests to more comprehensive field experiments involving its efficacy against one or several pathogens (4,5,7,18,19), and its mobility and fate in the soil environment (10,11,24,25).

Although considerable research has been devoted to the activity of the fumigant in soil, few investigations attempted to define the optimum soil conditions for fumigation

with metham-sodium. Conflicting results were reported on the influence of soil temperature on the efficacy of the fumigant against soilborne pathogens. The toxicity of the fumigant was found to be greater at 20°C (13) or 35°C (3) than at other temperatures tested (13). The toxicity of MIT to several resting structures of several pathogens, including *Verticillium* spp., *Sclerotium rolfsii* Sacc., *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Fusarium oxysporum* Schlechtend.:Fr., increased with increasing soil temperature (5). Leistra (14) and Leistra and Smelt (15) suggested that high soil temperatures favor the decomposition of MIT, thus impairing adequate distribution of the gas through the soil, especially wet soils. Others reported that MIT has a good fungicidal activity at low temperatures (20,29).

Metham-sodium was more effective against *V. dahliae* Kleb. in soil at 50 or 100% field capacity than at lower soil water contents (3). Vanachter (28) and Acland (1) showed that metham-sodium quickly dissipated under dry conditions and that MIT diffused less in the gaseous than in the water phase. Ashley et al. (2) reported no significant effect of soil water content on the release of MIT from seven soil types.

The fumigant tends to be erratic in performance when applied through injection. This variability has been a worldwide problem (17,30). Recently, application

through irrigation systems (chemigation) has been a common practice in the central sands region of Wisconsin in efforts to control *V. dahliae*, the prevalent fungal species of the complex causing the potato early dying (PED) disease. Other species involved in PED are *V. albo-atrum* Reinke and Berthier, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmanns Stekhoven, *Erwinia carotovora* (Jones) Bergey et al., *Colletotrichum coccodes* (Wallr.) Huges, and *Rhizoctonia solani* Kühn (12,22).

Most of the research on MIT has focused on its performance when the fumigant was applied to soil through injection (16,27). However, little is known about its behavior when applied by chemigation. The objective of this study was to investigate the effects of variable soil water and temperature conditions on the efficacy of MIT against *V. dahliae* when metham-sodium was applied by chemigation to columns packed with a loamy sand soil.

MATERIALS AND METHODS

Column preparation and experimental setup. A temperature-controlled growth room in the University of Wisconsin-Madison's Biotron was used to run four soil column experiments. The soil column preparation and experimental setup were described earlier (24,25). A set of 12 columns was made from 91.5-cm-long by 20.3 cm I. D. Schedule 40 PVC pipes. The inner walls of columns were lined with 0.79 mm thick Teflon sheets to prevent sorption of lipophilic chemicals to PVC surfaces. The soil used to pack the columns was non-sterile Plainfield sand (mixed, mesic Typic Udipsamment). The field soil profile was divided into three layers and the soil from each layer was collected separately. Columns were packed in the same horization, and bulk densities of soil profiles found in the field. A bulk density of 1.40 g cm⁻³ was used for the upper third of the profile and 1.65 g cm⁻³ for the lower two layers. Columns were packed by adding a 5-cm layer of soil at a time. A short piece of PVC cylinder was fastened to the top of each column and the joint was tightly sealed using a clear silicon-rubber sealant (Dow Corning Inc., Midland, MI) to form a closed system that did not allow MIT vapor loss.

In experiments 2 and 4, soil temperature was maintained at 2 ± 0.5°C

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(cold), while in experiments 1 and 3 the temperature was $22 \pm 0.5^\circ\text{C}$ (warm). These temperatures reflect typical cold (early spring and late fall) and warm (early fall) soil conditions in Wisconsin during field application. In all of the experiments, three soil water levels were employed, corresponding to the following soil matric potentials: -23 (wet), -113 (moist), and -2485 J/kg (dry). These soil water contents were established by adding water to each 5-cm layer of soil during packing of columns. Experiments were arranged as randomized complete-block designs with four replications. Each experiment was run for 15 days.

Inoculum production and infestation. High populations of *V. dahliae* (approximately 100 microsclerotia [MS] per gram of soil) were established in each column by mixing in MS produced on rye seed (26).

MS of the fungus were spread and mixed thoroughly in each soil layer before packing of columns. In all four experiments, one replicate of each treatment was used as a non-fumigated control. At the end of each experiment, soil was removed in 5-cm layers from the top 30 cm of the column (corresponding to the plow layer) and two separate sets of samples were taken from each layer. One set of samples was assayed for viability of MS and the other for MIT residues.

Fumigant application. Solutions of metham-sodium at 345 g liter^{-1} (Busan 1020, Buckman Laboratories, Inc., Memphis, TN) were applied to columns at a rate of 480 liters ha^{-1} with 25 mm of water. The theoretical concentration of MIT was equivalent to $520 \text{ mg liter}^{-1}$ assuming 84% conversion of metham-sodium to MIT (25). Thus, the theoretical amount of MIT

applied per column was 428 mg.

Soil assays for MIT residues and *V. dahliae*. Extraction of MIT residues from soil was accomplished by a method described by Leistra et al. (16). Soil samples were collected in 200-ml glass jars that were immediately closed with gas-tight screw-caps. The content of each jar was mixed and kept frozen until the time of extraction. Samples of about 50 g of soil were quickly weighed into 200-ml glass jars each containing 24 ml ethyl acetate and 25 ml water. The jars were sealed with screw-caps and mechanically shaken for 2 h. The ethyl acetate layer was separated by centrifugation at 2000 rpm for 2 min. The solution in ethyl acetate was diluted, if necessary, and MIT residue concentrations were measured using a gas chromatograph (25). MIT extraction and GC sensitivity were 92.5 ± 4.5 and 10 ppb, respectively.

Soil samples collected for assays for *V. dahliae* were dried at room temperature for 2 weeks, then assayed by plating soil dilutions on peptate selective medium (PM) as described by Nicot and Rouse (21). Percent kill of MS of the fungus was calculated from MS counts in the control column and treated columns for each soil water treatment.

Experimental design and analysis of data. Data for percent kill of MS for all four experiments were pooled and evaluated using a two-way analysis of variance (ANOVA). The ANOVA test was performed with SAS procedure GLM (SAS Institute, Cary, NC). Mean separation was accomplished using least square differences.

RESULTS

MIT residue in soil. At 15 days after chemigation, regardless of soil water content, MIT residues were below the detection limit in columns maintained at 22°C . However, MIT persisted for more than 15 days at 2°C , with amounts depending on the soil water content (Fig. 1). In cold/dry soil, MIT residues were low near the soil surface, increased with depth to a peak of $17.4 \mu\text{g g}^{-1}$ soil at 15 cm below the soil surface, then dropped virtually to zero at a depth of 30 cm. Average MIT concentration was $9.6 \mu\text{g g}^{-1}$ of soil in the dry treatment 15 days after chemigation. In cold/moist soil, MIT residue averaged $8.5 \mu\text{g g}^{-1}$ of soil, while in wet soil, MIT leaching through the columns resulted in lower MIT residue concentrations in the top 30-cm layer of the column ($6.2 \mu\text{g g}^{-1}$ of soil).

Influence of MIT on the viability of microsclerotia. Statistical analysis of data revealed significant effects for soil water content and the interaction of soil water content by soil temperature on the efficacy of the fumigant (Table 1). At 2°C , increasing soil water content had a significant positive effect on the efficacy of the fumigant against *V. dahliae* in the upper

Fig. 1. Methyl isothiocyanate residue concentration with depth in the top 30-cm layer of soil columns treated with metham-sodium and maintained at 2°C and various soil water contents after 15 days from chemigation. Open squares for wet (soil matric potential = -23 J/kg), solid squares for moist (soil matric potential = -113 J/kg), and open circles for dry (soil matric potential = $-2,485$ J/kg) treatments. Error bars are standard errors of means.

Table 1. Summary of the analysis of variance for percent kill of microsclerotia of *Verticillium dahliae* in soil columns chemigated with metham-sodium at three water regimes and two soil temperatures

Source of variation	df ^a	MS ^b	P value ^c
Soil layer	5	5,203.9	0.0001
Temperature (T)	1	136.8	0.6200
Water content (W)	2	4,827.6	0.0005
T $\times$ W	2	10,775.7	0.0001
Error	26		

^a Degrees of freedom based on two-way ANOVA.

^b Type III mean square.

^c P value from F test based on degrees of freedom.

third of the soil column, with higher MS mortality in wet soil than in any other soil water treatment. In cold, moist soil only 50% of the MS of *V. dahliae* were killed. Dry soil and low temperature conditions reduced the efficacy of the fumigant against the pathogen, and resulted in the lowest MS mortality compared to the other treatments. At high soil temperature, soil water saturation had no effect on the efficacy of the fumigant. However, there was a trend for lower MS mortality in dry than in wet or moist soil conditions at 22°C (Fig. 2).

DISCUSSION

Most of the research on metham-sodium has focused on MIT gas diffusion, since the fumigant is often injected directly into the soil. However, application to sandy soils in Wisconsin has recently switched to soil-drenching chemigation. This report is the first to deal with the effect of both soil water content and soil temperature on the efficacy of metham-sodium against *V. dahliae* when the fumigant was applied by chemigation to soil columns.

Higher mortality of MS of *V. dahliae* was observed in the top 30 cm of columns packed with wet soil and maintained at 2°C. These results could be explained by the fact that MIT has higher partitioning in water than in air (27). Thus, in wet conditions, MIT gas diffusion may be slow (16), but most of the MIT was in the water phase and therefore was able to move by mass flow throughout the plow layer, allowing more fumigant contact with the MS. However, fumigant activity was high in columns packed with dry soil and kept

at 22°C. Although the movement of MIT with mass flow is slow in a dry soil (25), high soil temperature and low soil water-saturation may have enhanced MIT gas diffusion in soil, resulting in more even MIT distribution and enhanced activity. A slightly lower kill of MS of the fungus was observed at 22°C and high soil water saturation. This could be attributed to the fast MIT decomposition in warm/wet soil (17), where the proportion of MIT available to move to and act on the pathogen was drastically decreased.

At moist soil water content, soil temperature has a minor effect on efficacy of the fumigant: intermediate levels of MS mortality were observed in both cold/moist and warm/moist soils. Both lower activity of the fumigant and partially retarded distribution due to adsorption of the fumigant was expected in a cold/moist soil. At high soil temperature and intermediate soil water saturation, efficacy of MIT may be partly offset by the high decomposition of MIT.

When the fumigant was applied to a dry/cold soil, MIT distribution throughout the plow layer was retarded, apparently due to the slow movement of the fungicide solution through the soil (25). This retarded movement prolonged the time for MIT to be sorbed by the soil. As a result, the lowest percent kill of the MS was observed in the dry/cold soil. Soil temperature is known to increase the efficacy of MIT in the soil environment (3,5,6,13). This effect was verified in the present study, since the efficacy of MIT was enhanced by increasing soil temperature from

2 to 22°C in dry soil conditions. The increase in MIT efficacy at high temperature may be due to a combination of factors: a thermodynamic increase in MIT activity, enhanced activity of any antagonists that may have tolerated the combination of the temperature and the fungicide, and decreased viability of resting structures or weakening of the pathogen by high temperature (5,6).

Eradication (100% kill) of MS was obtained by Ben-Yephet et al. (7) in a field study using a dose of 800 liter ha⁻¹ of metham-sodium applied by soil drenching (chemigation). In the present study, eradication was nearly achieved with a dose of only 480 liter ha⁻¹ metham-sodium in a wet/cold soil. The results obtained suggest that pathogen levels may be drastically reduced with relatively moderate doses of the fumigant through chemigation. This difference in the required doses could be attributed to the fact that the columns used in this study, unlike the field study by Ben-Yephet et al. (7), were essentially closed systems that did allow MIT loss by volatilization. Prevention of MIT volatilization from soil columns has improved the activity of the fumigant near the soil surface, correcting the problem of low fumigant efficacy near the soil surface observed by other researchers (5,30). However, the results of eradication of MS of *V. dahliae* obtained in this study with such an application dose cannot be applied to chemigation under field conditions, where tarping is not practiced or practical. Under "open" field conditions, MIT loss through volatilization might lead to reduced efficacy.

Based on the enhanced activity of MIT under wet/cold conditions, it is recommended that metham-sodium be applied in late fall or early spring by chemigation to control the soil pathogen *V. dahliae*.

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Fig. 2. Percent mortality of microsclerotia of *Verticillium dahliae* at 2°C (open columns) and 22°C (solid columns) soil temperatures at various soil water contents in soil columns chemigated with metham-sodium. Treatments with the same letters are not significantly different at $P = 0.05$ by least square difference.

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