EVALUATION OF ACROLEIN, ETHYLENE SULFIDE, FORMALDEHYDE, AND PROPYLENE OXIDE FOR NEMATICIDAL PROPERTIES

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ABSTRACT

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Tests were conducted to determine efficacy of acrolein, ethylene sulfide, formaldehyde, and propylene oxide to control *Meloidogyne incognita* on tomato in three greenhouse trials and *Rotylenchulus reniformis* on cotton in a field trial. The chemicals were applied at rates of 100, 200, and 400 mg a.i./kg soil and compared with the 1,3-dichloropropene (1,3-D) standard at 120 mg a.i./kg. They were injected into soil contained in polyethylene bags, and the bags were sealed for four days. In the field trial, holes were made 20-cm-deep, the chemicals applied, and the holes were sealed with soil. At test termination, nematodes were extracted from 100 cm^3 subsamples of soil and counted. Root-gall ratings were estimated on a 0-10 scale in the *M. incognita* greenhouse trials. Foliar weight of cotton in the field trial was recorded. In greenhouse tests, acrolein and formaldehype resulted in stunted and chlorotic tomato plants and were subsequently dropped from further testing. Ethylene sulfide and propylene oxide significantly reduced *M. incognita* juvenile soil populations and root galling in the three greenhouse tests. In the cotton field test, *R. reniformis* soil population densities were significantly reduced by the propylene oxide, ethylene sulfide and 1,3-D treatments compared to the nontreated controls. Foliar weight of cotton was increased significantly when using these chemicals. In these tests, ethylene sulfide and propylene oxide compared favorably in nematicidal activity to the 1,3-D nematicide standard.

Key words: acrolein, ethylene sulfide, formaldehyde, *Meloidogyne incognita*, nematicide, propylene oxide, reniform nematode, root-knot nematode, *Rotylenchulus reniformis*, ozone depletion, methyl bromide.

RESUMEN

Rahi, G. S., y J. R. Rich. 2003. Evaluación de acroleína, sulfida de etileno, formaldehído y oxido de propileno para propiedades nematicidales. Nematropica 33:165-170.

Ensayos fueron llevados a cabo para determinar la eficacia de acroleína, sulfida de etileno, formaldehído y oxido de propileno para controlar *Meloidogyne incognita* en tomate en tres ensayos de invernadero y para controlar *Rotylenchulus reniformis* en algodón en un ensayo de campo. Los químicos fueron aplicados a 100, 200, y 400 mg de ingrediente activo por kilogramo de suelo, y fueron comparados con el estándar de 1,3-dicloropropano (1,3-D) de 120 mg de ingrediente activo por kilogramo. Estos fueron inyectados en el suelo contenido en bolsas de polietileno, y las bolsas fueron selladas por cuatro días. En el ensayo de campo, se hicieron agujeros de 20 cm de profundidad, los químicos fueron aplicados, y los agujeros fueron cubiertos con tierra. Al final de cada ensayo, se extrajeron y se contaron nemátodos de sub-muestras de 100 cm^3. Índices de agalladuras de las raíces fueros estimados usando una escala de 1-10 en los ensayos de invernadero de *M. Incognita.* El peso foliar de algodón fue registrado. En los ensayos de invernadero, acroleína y formaldehído resultaron en plantas de tomate esmirriadas y cloróticas, y fueron consecuentemente omitidos de los ensayos siguientes. Sulfido de etileno y oxido de propileno redujeron significativamente las populaciones en el suelo y las agalladuras en las raíces en los ensayos de invernadero. En el ensayo de campo de algodón, densidades de poblaciones de *R. reniformis* en el suelo fueron reducidas significativamente por los tratamientos de oxido de propileno, sulfida de etileno y 1,3-D, comparados con los controles sin tratamiento. El peso foliar del algodón fue aumentado significativamente usando estos químicos. En estos ensayos, sulfida de etileno y oxido de propileno tenían actividad nematicidal comparable al del tratamiento de control de 1,3-D.

Palabras clave: acroleína, sulfida de etileno, formaldehído, *Meloidogyne incognita*, nematicida, oxido de propileno, nemátodo reniforme, nemátodo agallador, *Rotylenchulus reniformis*, reducción de ozono, bromuro de metilo.

INTRODUCTION

Methyl bromide, a widely used broad spectrum soil fumigant, is reportedly 50 times more destructive to the stratospheric ozone than chlorine (Raloff, 1995). As such, it is scheduled for phaseout from use in the U.S.A. on 1 January 2005 (Anonymous, 2000). U.S agriculture could lose millions of dollars to soil borne pests and diseases without viable methyl bromide alternatives (Anonymous, 2001). Intensive research has been under way in Florida, U.S.A. and elsewhere to evaluate methyl bromide substitutes. These research efforts have included use of other registered fumigants, solarization, sod-based rotations, plant resistance and combinations of these practices (Locascio *et al.*, 1997; Chellemi *et al.* 1996a & b; Rich and Olson, 1999). In Florida, the most promising single practice has been use of alternative fumigant materials such as 1,3-D + chloropicrin (Gilreath *et al.*, 1998).

In his review, Altman (1970) noted that soil fumigants like formaldehyde, carbon disulfide, chloropicrin or methyl bromide are effective against soil-borne pathogens since they can partially sterilize the soil. Formaldehyde, chloropicrin and hydrogen cyanide were also found to be good biocides by Dalton and Hurwitz (1948) and were used extensively in nursery seedbed preparation and soil treatment. Dalton and Hurwitz (1948) and Clark (1950) showed that ethylene oxide is an effective soil fumigant. However, research on use of ethylene oxide in soil was limited because of toxicity and health risks.

Propylene oxide, which has better handling properties than ethylene oxide, was also shown to be effective against pathogenic organisms (Bartlett and Zelazny 1967; Skipper and Westerman 1972). However, propylene oxide reduced germination of wheat (Ark, 1947). In another study, the chemical required rigorous detoxification steps for proper plant growth (Skipper and Westerman, 1972). Ralph and Khair (1977) observed that propylene oxide had nematicidal activity against newly hatched juveniles of *Meloidgyne hapla* from tomatoes. The present studies were conducted with acrolein, ethylene sulfide, formaldehyde and propylene oxide to evaluate their nematicidal properties. These chemicals were selected for their known biocidal, fumigation, and potentially less environmentally abrasive characteristics.

MATERIALS AND METHODS

Greenhouse Tests:

Sandy loam soil (80% sand, 8% silt, 12% clay, 2% O.M.) from a field site was removed from the top 15 cm, covered with polyethylene and fumigated with methyl bromide at 200 mg a.i./kg soil. The soil was subsequently air dried and water was added to bring it to field capacity. Soil was left undisturbed for 4 days under a polyethylene cover for water equilibrium at room temperature. Afterwards, 9000 eggs and second-stage juveniles (J2) of *Meloidogyne incognita* were added and manually mixed into 6 kg soil of each intended treatment. Nematode inoculum was obtained from tomato roots and extracted with sodium hypochlorite (Hussey and Barker, 1973). The soil was then placed into 10-cm-diam plastic pots lined with double 1 L polyethylene bags (6-mil thickness). Acrolein, ethylene sulfide, formaldehyde, and propylene oxide were injected with a syringe into the soil to 8-cm-deep, and applied at 400 mg a.i./kg soil. Bags were immediately sealed, placed in the greenhouse (high air temperature, 30 C), and covered with newspaper to prevent direct contact with sunlight. Soil was left undisturbed for four days to allow chemical diffusion. Polyethylene bags were opened and left in the greenhouse for 8 days to release any residual chemical fumes. Bags containing the soil were covered loosely with newspaper to avoid excessive moisture loss and direct contact with sunlight. Soil was removed from bags and placed into 10-cm-diam plastic pots, and one 'Rutgers' tomato seedling (12.5 cm high) was transplanted into each pot. Pots were placed on a greenhouse bench in a completely randomized design containing five replicates for each treatment. Plants were watered daily and fertilized with a N-P-K (20-20-20) weekly. Observations for phytotoxicity were made throughout the test period. After 45 days, soil was gently shaken from roots in each pot, and a 100 cm^3 sample removed for *M. incognita* extraction (Jenkins, 1964). Roots were rinsed and rated for galling on a 0-10 scale, where 0 equaled no root galling and 10 equaled 100% of the root system galled.

In a second greenhouse test, soil was prepared, inoculated and treatments were applied as in the first test. Treatments consisted of three different rates of acrolein, ethylene sulfide, and propylene oxide at 100, 200, and 400 mg a.i./kg soil. A 1,3-D treatment of 120 mg a.i./kg served as the nematicide standard. Tomatoes were allowed to grow for 55 days before removing a 100 cm^3 soil sample from each pot for J2 extraction. The experimental design was completely randomized and contained five replications of each treatment. The second test was repeated using the same methods and materials but without acrolein and formaldehyde and with one rate (200 mg a.i./kg soil) of propylene oxide and ethylene sulfide. Tomatoes were allowed to grow for 45 days at which time root gall ratings and soil samples were taken.

Field Test:

Based on results from greenhouse studies, ethylene sulfide and propylene oxide were selected for use in a cotton field trial. The site was located at the University of Florida NFREC near Quincy, Florida that had been continuously planted to cotton for over five years. Soil was a sandy loam (82% sand, 10% silt, 8% clay) naturally infested with reniform nematodes, *Rotylenchulus reniformis* Lindford & Oliveira. Field plots were randomly selected in a pre-established 3-week-old cotton crop. Plants were removed and 1.82-m-wide x 0.91-m-long plots were placed over two rows. On 7 July 2000, two days after a 2.0 cm rainfall event, five holes (0.5-cm-diam) to 20-cm-deep were made in each plot row. Three holes, 23-cm-apart in the row middles, and two holes spaced 23-cm-apart were placed on either side of the center hole [making a plus (+) sign pattern]. Treatments in the test included propylene oxide and ethylene sulfide applied at the surface broadcast rate of 450 kg a.i./ha, 1,3-D at 270 kg a.i./ha, and an untreated control. The chemicals were poured into each hole and holes were covered with soil immediately afterwards. Treatments were replicated five times in the test and treatments placed in a completely randomized design. The test site did not receive rainfall or irrigation for one week after chemical application. Two weeks after test initiation, 'DP454BRR' cotton seed were planted manually, and 10 days later, plants were thinned to 9 plants per m of row. Five soil cores (2.5-cm-diam) were taken to 25-cm-deep from each cotton plot 43 days after planting and soil extracted. Fresh foliar weight of the cotton also was measured on the same day.

Data analyses were conducted using ANOVA and means separated with the Duncan's multiple range test.

Table 1. Influence of four chemicals on *Meloidogyne incognita* J2 soil population density and root galling on tomato in a greenhouse study, Test 1.[w]

Treatment[x]	Juveniles/100 cm^3 soil	Root galling index[y]
Ethylene sulfide	5 a[z]	1 a
Acrolein	5 a	0 a
Propylene oxide	10 a	0 a
Formaldehyde	150 b	8 b
Control	433 c	9 b

[w]Data are means of 5 replications per treatment.
[x]Chemicals were applied at the rate of 400 mg a.i./kg soil.
[y]Root galling was rated on 0-10 scale with 0 equaling no root galling and 10 equaling 100% of the root system galled.
[z]Column means followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

RESULTS AND DISCUSSION

In the initial greenhouse trial, acrolein, ethylene sulfide, and propylene oxide significantly ($P \leq 0.05$) suppressed *M. incognita* J2 populations in soil and reduced tomato root galling compared to the control (Table 1). However, formaldehyde was not as effective, and additionally, tomato plants showed symptoms of phytotoxicity including stunting and chlorosis. Formaldehyde was subsequently dropped from further testing. In the second greenhouse test, ethylene sulfide and propylene oxide reduced *M. incognita* J2 soil population densities as well as the nematicide standard (Table 2). The acrolein treatment resulted in J2 soil population densities higher than the other chemical treatments, and tomato plants showed symptoms of phytotoxicity including poor plant growth and chlorosis. Thus, acrolein was also excluded from further tests. Nematode population densities were not different between the ethylene sulfide and propylene oxide treatments and the 1,3-D standard. In the third greenhouse test, ethylene sulfide and propylene oxide reduced J2 soil population densities and root galling compared to the untreated control (Table 3).

In the cotton field test, both ethylene sulfide and propylene oxide (450 kg a.i./ha) reduced *R. reniformis* soil population densities compared to the untreated control (Table 4). Propylene oxide reduced *R reniformis* population densities more than the 1,3-D standard and population densities in the ethylene sulfide treatment populations were not different from those two treatments. The three chemical treatments improved cotton foliar weight compared to the control and did not differ from one another.

Propylene oxide has been found to be an efficient soil fumigant due to ease of handling (Bartlett and Zelazny 1967; Skipper and Westerman 1972), good soil penetration, desirable vaporization temperature (Ralph and Khair 1977), and effectiveness against pathogenic bacteria, fungi, and act-

Table 2. Influence of four chemicals on *Meloidogyne incognita* J2 soil population densities on tomato in a greenhouse study, Test 2.[y]

Treatment	Rate mg a.i./kg	Juveniles/ 100 cm^3 soil
1,3-D	120	4 a[z]
Propylene oxide	400	175 a
Propylene oxide	200	153 a
Propylene oxide	100	78 a
Ethylene sulfide	400	11 a
Ethylene sulfide	200	51 a
Ethylene sulfide	100	123 a
Acrolein	400	946 b
Acrolein	200	1114 b
Acrolein	100	1452 b

[y]Data are means of 5 replications per treatment.
[z]Column means followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3. Influence of two chemicals on *Meloidogyne incognita* J2 soil population densities on tomato in a greenhouse study, Test 3.[w]

Treatment[x]	Juveniles/ 100 cm^3 soil	Root galling index[y]
Ethylene sulfide	8 a[z]	1.3 a
Propylene oxide	11 a	0.0 a
Control	367 b	9.0 b

[w]Data are means of five replications per treatment.
[x]Chemicals were applied at the rate of 200 mg a.i./kg soil.
[y]Root galling was rated on 0-10 scale with 0 equaling no root galling and 10 equaling 100% of the root system galled.
[z]Column means followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

inomycetes (Lopes and Wollum 1976). Epoxides such as propylene oxide sterilize soil by alkylation of functional groups in microbial proteins (Skipper and Westerman 1972). Results of present tests suggest that propylene oxide also possesses nematicidal activity and compares well with 1,3-D at similar rates. However, further studies are required to determine interaction with the soil and concomitant effects on a short and long-term basis. For example, Bartlett and Zelazny (1967) found that propylene oxide increased soil pH slightly. Little information is available on the activity of ethylene sulfide as a biocide. The chemical, however, exhibited good nematicidal properties. More studies are needed to evaluate the effects of both ethylene sulfide and propylene oxide on plant-parasitic nematodes and other soilborne pathogens.

Table 4. Influence of three chemicals on *Rotylenchulus reniformis* soil population densities and cotton foliar weights in a field trial.[y]

Treatment	Rate kg a.i./ha	Nematodes/100 cm^3 soil	Foliar wt. (g)
Propylene oxide	450	100 a[z]	1400 a
Ethylene sulfide	450	142 ab	1439 a
1,3-D	270	221 b	1544 a
Control	—	472 c	805 b

[y]Data are means of five replications per treatment.
[z]Column means followed by the same letter are not significantly different according to Duncans's multiple range test ($P \leq 0.05$).

Propylene oxide has less environmentally abrasive by-products than methyl bromide (Blanchard and Hanlin, 1973) and poses no threat to stratosphere ozone. It has good soil penetration ability and should not be more difficult to handle for application to soil as compared to methyl bromide. For longer contact with soil and greater effectiveness, the area to be treated could be covered with polyethylene similar to that of methyl bromide. The present studies, however, indicate that propylene oxide exhibits good nematicidal activity and confirms work of Ralph and Khair (1977). As such, the chemical has potential as a methyl bromide alternative. However, additional studies on environmental fate and toxicology of both propylene oxide and ethylene sulfide are needed.

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