

Comparative Activities of Sodium Tetrathiocarbonate and Metalaxyl on *Phytophthora capsici* and Root and Crown Rot on Chile Pepper

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ABSTRACT

Matheron, M. E., and Matejka, J. C. 1995. Comparative activities of sodium tetrathiocarbonate and metalaxyl on *Phytophthora capsici* and root and crown rot on chile pepper. *Plant Dis.* 79:56-59.

The activities of sodium tetrathiocarbonate (STTC) and metalaxyl on the viability of *Phytophthora capsici* within colonized pepper tissue, the recovery of *P. capsici* from soil and the development of root and crown rot on chile pepper were evaluated and compared. *P. capsici* was not recovered from colonized chile pepper stem tissue buried in soil that was drenched with 4,900 $\mu\text{g/ml}$ of STTC. In contrast, recovery of the pathogen from stem tissue buried in soil drenched with 10 $\mu\text{g/ml}$ of metalaxyl did not differ from that obtained for stem pieces from soil drenched with water only. The number of lesions formed on pear fruit bait incubated with field soil containing *P. capsici* and treated with STTC at 12 $\mu\text{g/ml}$ was reduced 36% compared with untreated soil, whereas no lesions developed when soil was treated with metalaxyl at 10 $\mu\text{g/ml}$. Growth of chile pepper seedlings inoculated with zoospores of *P. capsici* in the presence of STTC at 245 $\mu\text{g/ml}$ or metalaxyl at 10 $\mu\text{g/ml}$ was equivalent to that obtained for plants not inoculated with the pathogen. Root and shoot growth of chile pepper seedlings grown in field soil naturally infested with *P. capsici* that was treated 1 wk before planting with either STTC at 4,900 $\mu\text{g/ml}$ or metalaxyl at 10 $\mu\text{g/ml}$ was significantly greater than that of plants grown in untreated soil. These investigations demonstrate the potential benefits of STTC as a management tool for the control of *Phytophthora* root and crown rot of chile pepper.

Phytophthora capsici Leonian causes severe root and crown rot, wilt, and blight of peppers (*Capsicum annuum* L.) (6,26,27). Recently, the incidence of *Phytophthora* root and crown rot on chile peppers has increased in southeastern Arizona and also is a major problem on this crop in New Mexico (4). In Arizona, the crown and root rot phase first appears on isolated plants early in the growing season, following furrow

irrigation. Additional plants become infected during each subsequent irrigation. As the plant canopy develops and covers the row, the aerial blight phase of the disease may occur with the onset of rainfall during July and August. During this time, stems, leaves, and pepper fruit are most frequently attacked.

The efficacy of the systemic fungicide metalaxyl for control of *Phytophthora* root and crown rot of chile pepper has been demonstrated (18,25,29,30). Recently, the inhibitory activity of sodium tetrathiocarbonate (STTC) (Enzone, Unocal Corp., Brea, CA) on sporulation and growth of six species of *Phytophthora* in vitro was reported (20).

When added to water and applied to soil, STTC releases carbon disulfide, a known biocide (1,3,12,23). The use of carbon disulfide as a partial soil sterilant first was recorded in 1894 (14,24). Since then, this material has been reported to have biocidal effects on *Armillaria mellea* (3), *Trichoderma viride* (23), *Clitocybe tabescens*, (7) and species of *Phytophthora* (20,21). Control of *Phytophthora* root and crown rot of citrus by STTC has been demonstrated in greenhouse trials (21). The objectives of this study were to evaluate and compare the effects of STTC and metalaxyl on the viability of *P. capsici* within colonized pepper tissue, the recovery of this pathogen from soil, and the development of root and crown rot on chile pepper. A partial account of this work has been published (22).

MATERIALS AND METHODS

Viability of mycelium of *P. capsici* within pepper tissue buried in soil. Segments of stems 10 mm long and 3–4 mm in diameter from 3-mo-old chile pepper plants (New Mexico 6-4) grown in the greenhouse were collected and allowed to be colonized by an isolate of *P. capsici* (MIS-6C) that originated from a diseased pepper plant collected in southeastern Arizona. The isolate of *P. capsici* was grown on V8 juice agar for 5 days at 24 C. Stem segments were surface-disinfested for 20 min in a 1% solution of NaOCl, then rinsed three times in sterile distilled water. Stem segments were placed adjacent to the margins of actively growing cultures of *P. capsici*

Accepted for publication 30 September 1994.

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and incubated for 7 days at 24 C. Ten colonized stem segments, which contained only mycelium of the pathogen, were placed between two layers of fiberglass window screen on a 2.5-cm layer of nonsterile field soil (sandy loam: 81% sand, 7% silt, 12% clay) in a 10-cm-diameter × 10-cm-deep plastic pot and covered with an additional 5-cm layer of soil. The soil in each pot was drenched with 200 ml of water containing 10 µg a.i./ml of metalaxyl (Ridomil 2E) or 1,225, 2,450, or 4,900 µg a.i./ml of STTC (Enzone 3.4 E), which thoroughly wet the soil. Control pots were drenched with water only. Pots were allowed to drain freely, then incubated for 4 days at 23–25 C, after which the 10 stem segments within each pot were removed, rinsed in water, placed on a dry paper towel to remove excess moisture, and plated on pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (17) medium. Petri dishes were incubated in darkness at 24 C for 3 days and observed daily for growth of mycelium of *P. capsici* from stem segments. This test was conducted three times, with each trial consisting of five replicate pots per treatment. Standard deviations of treatment means were calculated (15).

Recovery of *P. capsici* from soil. Soil was collected from a field infested with *P. capsici*, and thoroughly mixed in the laboratory, then a 500-cm³ sample of this soil was placed into a series of containers 12 cm wide × 23 cm long × 7 cm deep; 750 ml of water containing 12, 24, 61, 122, or 245 µg/ml of STTC or 10 µg/ml of metalaxyl was added to the containers. Containers used as controls were drenched with water only. Two mature but green, unblemished Bartlett or Anjou pear fruit were placed on the surface of each soil sample, which resulted in a 1- to 2-cm deep aqueous layer at the soil surface. After incubation at 23–25 C for 48 hr, the fruit were removed from the soil, rinsed in tap water, and incubated for an additional 4 days at the same temperature. Firm brown spots developed on pear fruit infected by *P. capsici*. A small piece of tissue from each brown spot was placed on pimaricin-ampicillin-rifampicin-pentachloronitrobenzene medium, incubated at 24 C in darkness, and observed for growth of *P. capsici*. The number of lesions caused by the pathogen was recorded. This series of experiments was performed four times. Standard deviations of treatment means and linear regression analysis were produced using the SigmaStat Statistical Software Package (Jandel Scientific, San Rafael, CA).

Disease development and inoculation with zoospores. Seeds of chile pepper (New Mexico 6-4) were sown and germinated in vermiculite; seedlings were transplanted at the three-leaf stage into sterile potting mix (45% peat/45% vermiculite/10% sand, by volume) in 10-

cm-diameter × 10-cm-deep plastic pots and grown in the greenhouse. Pots containing 2-mo-old pepper seedlings were placed in 11-cm-diameter × 10-cm-deep containers and enough water containing 245 µg/ml of STTC or 10 µg/ml of metalaxyl was applied to the soil so that a 1-cm layer of aqueous suspension covered the potting mix surface in each pot. Control plants were flooded with only water.

A 1.5% nonsterile soil extract was prepared by mixing 15 g of a sandy loam orchard soil in 1 L of distilled water with a magnetic stirrer for 8 hr at 25 C. After an additional 16-hr incubation period without stirring, the suspension was decanted. Further clarification of the soil extract was achieved by filtration through Whatman No. 1 filter paper. Zoospores were produced by growing an isolate of *P. capsici* (MIS-6C) on V8 juice agar at 24 C for 5 days. Four 6-mm-diameter agar disks were removed from the edges of actively growing cultures and placed into each of 10 60-mm-diameter plastic petri dishes containing 7 ml of 1.5% nonsterile soil extract. Numerous sporangia formed after incubation of agar disks for 48 hr at 24 C. Sporangia were induced to release zoospores by chilling at 4 C for 20 min. After rewarming at 25 C for 20 min, the contents of each petri dish were decanted into a 100-ml beaker through cheesecloth to remove agar disks and attached mycelia. The zoospore suspension was quantified using a hemacytometer. Approximately 4,500 zoospores then were added to the edges of each of 10 pots containing only water or aqueous solutions of STTC or metalaxyl. Noninoculated control pots received no zoospore inoculum. After 2 hr, pots containing chile pepper plants were removed from the flooding chambers and allowed to drain freely. Plants were maintained in the greenhouse, watered as needed, and fertilized weekly with water-soluble Miracle-Gro fertilizer (15-30-15; Stern's Miracle-Gro Products, Inc., Port Washington, NY). The mean soil temperature during these tests was 23 C.

The date when initial disease symptoms were first observed on each plant was recorded during the course of the study. Experiments were terminated after 3 mo, when final disease severity was determined by recording the number of dead (permanently wilted) plants and the fresh weights of shoots and roots. Infection by *P. capsici* was confirmed by reisolating the pathogen from infected stems and roots. Analysis of variance (ANOVA) of the data was performed using the MSTAT-C Statistical Software Package (Michigan State University) and Duncan's multiple range test was used to differentiate treatment means when appropriate. This experiment was conducted twice with comparable results; data presented are from only one

experiment.

Disease development and preplant drenching of naturally infested soil. Field soil naturally infested with *P. capsici* was placed in plastic trays 20 cm wide × 25 cm long × 13 cm deep and drenched with enough water containing 10 µg/ml of metalaxyl or 4,900 µg/ml of STTC to saturate the soil and maintain a 1-cm layer of suspension over the surface. An infested control treatment was drenched with water only. After a 2-hr incubation period at 30–33 C, the soil was allowed to drain freely and left undisturbed for 7 days. Six 2-mo-old chile pepper seedlings then were planted individually in the treated soil from each treatment in 10-cm-diameter × 10-cm-deep plastic pots. A noninfested control treatment was established by planting seedlings in field soil that had been heat-sterilized (121 C, 102 kPa, 60 min). Once every 2 wk for the duration of the experiment, plants were flooded for 48 hr to stimulate disease development. Seedlings were fertilized weekly as described earlier. The average soil temperature during these experiments was 22 C. The date when initial disease symptoms were first observed on each plant was recorded during the experiment. Final disease severity was determined after 3 mo by recording the fresh weights of shoots and roots. Variances among the three runs of this experiment were homogeneous; therefore, a combined ANOVA was calculated on pooled data using MSTAT-C. Duncan's multiple range test was used to compare treatment means. As in earlier tests, disease incidence was confirmed by reisolating *P. capsici* from infected stem and root tissue of chile pepper plants.

RESULTS

Viability of mycelium of *P. capsici* within pepper tissue buried in soil. *P. capsici* was recovered from 100 and 23% of the colonized stem segments of chile pepper buried in soil drenched with 1,225 and 2,450 µg/ml of STTC, respectively,

Table 1. Effect of chemical drench on viability of *Phytophthora capsici* within colonized pepper stem segments buried in field soil

Treatment	Rate (µg/ml)	Isolation of <i>P. capsici</i> from stem segments (%) ²
Water	...	100
Metalaxyl	10	93 ± 13
STTC	1,225	100
STTC	2,450	23 ± 23
STTC	4,900	0

²Percentage of pepper stem segments (± standard deviation) from which *P. capsici* was isolated at termination of experiment. Values represent mean of 10 stem segments from each of five replicate pots for each of three runs.

while the pathogen was not recovered from soil drenched at 4,900 µg/ml (Table 1). Recovery of *P. capsici* from colonized stem tissue buried in soil drenched with 10 µg/ml of metalaxyl was equivalent to that recorded for stem pieces from soil drenched with STTC at 1,225 µg/ml or water.

Recovery of *P. capsici* from soil. There was a significant linear relationship ($R^2 = 0.956$) between the number of lesions that developed on pear fruit bait incubated with field soil containing *P. capsici* and the log of the concentration of STTC present in soil. No lesions developed on pear fruit incubated with soil containing the pathogen and treated with metalaxyl at 10 µg/ml, while treatment of soil with STTC at 12 µg/ml led to a reduction in the number of lesions induced by *P. capsici* of only 36%, compared with the number of lesions formed for untreated soil (Table 2).

Disease development and inoculation with zoospores. Root and shoot growth of chile pepper seedlings inoculated with zoospores of *P. capsici* in the presence of STTC at a concentration of 245 µg/ml or metalaxyl at 10 µg/ml was equivalent to that of noninoculated plants and significantly greater than growth on inoculated control plants (Table 3). Also, the average time of survival of inoculated plants treated with STTC or metalaxyl was equivalent to that of noninoculated plants and significantly greater than the time of survival of inoculated plants not receiving chemical treatment. No phytotoxicity was observed with either chemical at the rates and with the methods of application used.

Disease development and preplant drenching of naturally infested soil. Growth of roots and shoots of chile pepper seedlings in soil infested with *P. capsici* and treated with 4,900 µg/ml of STTC or 10 µg/ml of metalaxyl 1 wk before planting was significantly greater

than that obtained in nontreated field soil infested with the pathogen (Table 4). On the other hand, plant growth in soil treated with either chemical was less than that observed in sterilized field soil. The period of survival of chile pepper plants grown in soil infested with *P. capsici* and treated with 4,900 µg/ml of STTC was equivalent to that of plants grown in sterilized field soil and significantly greater than that of plants grown in nontreated soil (Table 4). No phytotoxicity was observed on plants grown in soil treated with STTC or metalaxyl at the rates and with the methods used.

DISCUSSION

At appropriate concentrations, STTC and metalaxyl limited the infection of pear fruit bait by *P. capsici* in soil infested with the pathogen. Also, a single treatment with STTC or metalaxyl during the inoculation of roots with zoospores of this pathogen significantly reduced the development of crown and root rot on chile pepper plants. Development of crown and root rot on chile pepper plants as well as development of lesions on pear fruit suggest that a STTC concentration in the range of 245 µg/ml is necessary to inhibit zoospore activity in soil.

STTC, applied as a soil drench at a rate of 4,900 µg/ml, was lethal to mycelium of *P. capsici* in stem tissue buried in soil and suppressed development of root rot on chile pepper seedlings planted in soil naturally infested with the pathogen. In contrast, the viability of mycelium of *P. capsici* was not appreciably affected by 10 µg/ml of metalaxyl, while a preplant drench with the same concentration of this material suppressed development of root rot on chile pepper seedlings planted in soil naturally infested with the pathogen. The apparent differences in activities of STTC and metalaxyl on mycelium of *P. capsici*, compared with disease development on chile pepper plants in naturally infested soil, may be partially explained by the activity and fate of each compound when applied to soil. The formulation of STTC tested rapidly releases carbon disulfide, the active biocide, when diluted in water and applied to soil. Apparently, STTC at the concentration tested killed propagules of *P. capsici* within the naturally infested soil; thus, subsequent chile pepper growth and plant health were significantly increased compared with that of plants grown in untreated soil. On the other hand, although it initially did not kill propagules of *P. capsici* in soil, metalaxyl has been shown to inhibit mycelial growth and formation of sporangia of *P. citrophthora*, *P. parasitica*, (9) and *P. palmivora* (13). Metalaxyl is taken up by plant tissues and in citrus can provide protection from colonization of bark by *P. citrophthora* and *P. parasitica* for at least 3 mo (19). When chile pepper plants were grown in soil naturally infested with *P. capsici*, significant disease control presumably was achieved with a single soil drench of metalaxyl because sufficient chemical remained in the soil to repress growth and spore germination of the pathogen

Table 3. Growth and disease development in chile pepper seedlings inoculated with zoospores of *Phytophthora capsici* in the presence of STTC or metalaxyl

Treatment	Plant growth and disease severity		
	Fresh weight (g) ¹		Duration of plant survival ²
	Shoot	Root	
Inoculated control	2 b	3 b	15 b
Metalaxyl at 10 µg/ml	39 a	20 a	61 a
STTC at 245 µg/ml	29 a	16 a	62 a
Uninoculated control	37 a	18 a	63 a

¹Each value is mean of 10 replicate plants per treatment. Numbers within each column with a different letter are significantly different ($P = 0.01$) according to Duncan's multiple range test.

²Each value represents average elapsed time in days from inoculation to death of plants. Plants were considered dead when leaves were wilted permanently.

Table 4. Effect of chemical preplant treatment of soil naturally infested with *Phytophthora capsici* on subsequent growth and disease development in chile pepper seedlings

Treatment	Plant growth and disease severity		
	Fresh weight (g) ¹		Duration of plant survival ²
	Shoot	Root	
Sterilized soil	33 a	18 a	75 a
Nonsterilized soil	1 c	2 c	7 c
Metalaxyl at 10 µg/ml	17 b	7 b	57 b
STTC at 4,900 µg/ml	20 b	9 b	66 ab

¹Each value is mean of six replicate plants from each of three experiments. Numbers in each column with a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

²Each value represents average elapsed time in days from planting in test soil to death of plants. Plants were considered dead when leaves were wilted permanently.

Table 2. Development of lesions on pear fruit incubated with field soil containing *Phytophthora capsici* and drenched with STTC or metalaxyl

Treatment	Rate (µg/ml)	Number of lesions on pear fruit ²
Untreated	...	63 ± 31
STTC	12	40 ± 23
STTC	24	34 ± 19
STTC	61	22 ± 12
STTC	122	10 ± 10
STTC	245	10 ± 10
Metalaxyl	10	0

²Each value is mean number of lesions (± standard deviation) that formed on two replicate pear fruit from each of four experiments. Lesion development confirmed as resulting from colonization by *P. capsici* by reisolating pathogen from test fruit. Linear regression equation of number of lesions per pear fruit (y) against the log of concentration of STTC (x) is $y = -25.4x + 67.5$, $R^2 = 0.956$ ($P = 0.004$).

for the duration of the experiments.

The ultimate value of any chemical compound for control of *Phytophthora* root and crown rot of chile pepper is defined by the effect of the material on the life stages of *P. capsici* as well as the persistence of the material in the soil or plant. Of all the stages in the life cycle of soilborne species of *Phytophthora*, sporangium formation and zoospore release provide the greatest opportunity for an explosive increase in the number of infective propagules and increased disease development (8,16,31). Saturated soil conditions resulting from irrigation or rainfall can increase the severity of root and crown rot by stimulating release of zoospores from sporangia (2,6,28). STTC at a rate of 245 µg/ml killed zoospores and reduced the severity of root and crown rot; however, maintenance of optimum disease control throughout the life of a chile pepper planting would require application of this nonpersistent material at each irrigation or significant rainfall event. A slow-release formulation of STTC, which would release carbon disulfide only when soil was saturated with water, could provide sustained inhibition of zoospore activity and more effective disease control with less applications of the compound. Another alternative would be a single preplant treatment of an infested field with STTC at a concentration of 4,900 µg/ml, which in greenhouse trials decreased the subsequent decline of shoot and root growth and increased plant survival compared with nontreated soil.

Much higher concentrations of STTC than of metalaxyl were required to kill zoospores of *P. capsici* and control root and crown rot of chile pepper seedlings. However, STTC may have some beneficial qualities as part of an overall pest management program. STTC releases carbon disulfide, which has been reported to have biocidal effects on plant-parasitic nematodes (7). STTC is being developed as a nematicide and fungicide, offering the possibility of a single material for management of nematodes as well as *Phytophthora* root and crown rot on pepper. Recent evidence has been presented on the apparent insensitivity of some isolates of soilborne species of *Phytophthora* to metalaxyl (5,10,11). As a general nonselective biocide, the carbon disulfide released by STTC should be equally lethal to all isolates of a species of *Phytophthora*, whether they are susceptible or resistant to

metalaxyl. STTC could play a role in management strategies to prevent or delay development of resistance by species of *Phytophthora* to metalaxyl in the field. Further studies are needed to determine the efficacy, optimum rate, and timing of application of STTC under diverse soil and environmental conditions in the field.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Unocal Corporation as well as state and Hatch funds allocated to the Arizona Agricultural Experiment Station.

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