

## In Vitro Activity of Sodium Tetrathiocarbonate on Sporulation and Growth of Six *Phytophthora* Species

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### ABSTRACT

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Sodium tetrathiocarbonate releases carbon disulfide when added to water and applied to soils. In vitro tests were initiated to examine growth and sporulation of *Phytophthora parasitica*, *P. citrophthora*, *P. capsici*, *P. cactorum*, *P. cambivora*, and *P. drechsleri* in the presence of sodium tetrathiocarbonate. The duration of zoospore motility for these species was reduced 94% in an aqueous solution containing 12 µg/ml of this chemical. Production of zoospore cysts was reduced 47, 74, and 83% by sodium tetrathiocarbonate at rates of 2.4, 12, and 60 µg/ml, respectively. Zoospore cysts formed in the presence of the compound at 2.4 µg/ml were viable, whereas those formed at 12 µg/ml did not germinate. Sporangium

production in soil by *P. parasitica*, *P. citrophthora*, *P. capsici*, and *P. cactorum* was reduced 23, 65, and 98% in the presence of sodium tetrathiocarbonate at 122, 245, and 490 µg/ml, respectively. However, concentrations of 2,450 µg/ml were needed to completely inhibit mycelial growth by all species. These results suggest that application of sodium tetrathiocarbonate as a soil drench could inhibit zoospore motility and reduce production of sporangia and viable zoospore cysts, thus reducing inoculum production and subsequent new infections by *Phytophthora* species.

Sodium tetrathiocarbonate (GY-81) (Unocal Corporation, Los Angeles, CA) releases carbon disulfide when added to water and applied to soils. The use of carbon disulfide as a partial soil sterilant first was recorded in 1894 (7,15). Since then, carbon disulfide has been reported to have biocidal effects on *Armillaria mellea* (2,5,14), *Trichoderma viride* (14), *Clitocybe tabescens* (3), and plant parasitic nematodes (3).

Currently, sodium tetrathiocarbonate (STTC) is being developed as a nematicide. However, during a recent field trial in two orange groves in Yuma, AZ, application of this material affected the subsequent recovery of *Phytophthora parasitica* Dastur from the treated soil. Samples were collected from the top 7 cm layer of soil within the dripline of 14 orange trees both before and 7 days after treatment with STTC at a concentration of 122 µg/ml in the flood-irrigation water. Using a pear-baiting technique (13), we recovered an average of 12 and four propagules of *P. parasitica* from soil before and after treatment, respectively. Our preliminary findings suggested that STTC may inhibit inoculum production by *P. parasitica*.

*Phytophthora* root and crown rot and citrus nematode (*Tylenchulus semipenitrans*) damage are major soilborne disease problems affecting citrus production, especially in Arizona (1,8). Also, apple (11) and chili pepper plantings (12) in southeastern Arizona are affected by *Phytophthora* spp. and could become affected by nematodes; therefore, the availability of a nematicide that also possesses activity against *Phytophthora* spp. could be particularly useful to growers. Accordingly, this investigation was initiated to determine the in vitro effects of STTC on various stages of the life cycle of *Phytophthora* spp. causing disease losses in Arizona. A partial account of this study was reported earlier (9).

### MATERIALS AND METHODS

**Fungi.** Two isolates each of *P. parasitica*, *P. citrophthora* (Smith & Smith) Leonian, *P. capsici* Leonian, *P. cactorum* (Lebert & Cohn) Schroeter, *P. cambivora* (Petri) Buisman, and *P. drechsleri* Tucker were used. All isolates of *Phytophthora* were recovered from diseased citrus and apple trees or vegetable crops in Arizona.

**Fungicide.** GY-81 contains 408 g of STTC per liter of product, which is equivalent to a carbon disulfide concentration of 168 g per

liter. Solutions of STTC were freshly prepared before each experiment.

**Zoospore motility, encystment, and germination.** Zoospores were produced by growing isolates on V-8 juice agar (V8A; containing commercial V-8 juice, 200 ml; CaCO<sub>3</sub>, 2 g; agar, 17 g; and distilled water, 800 ml) plates at 24 C for 5 days. Four 6-mm-diameter agar disks were removed from the edge of an actively growing culture of each isolate and placed in a 60-mm-diameter plastic petri dish containing 7 ml of 1.5% nonsterile soil extract. This 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 ± 2 C. After an additional 16-hr incubation period, the suspension was decanted. Further clarification of the soil extract was achieved by centrifugation at 5,000 g for 20 min, or by filtration through Whatman No. 1 filter paper. Four such petri dishes were prepared for each isolate of *Phytophthora* tested.

Numerous sporangia formed after incubation of agar disks for 24 hr at 21 C for isolates of *P. drechsleri* and 72 hr for all other isolates. Sporangia were induced to release zoospores by chilling at 4 C for 20 min. After rewarming at 25 C for 20 min, agar disks and attached mycelia were removed from each petri dish, and the volume of the remaining zoospore suspension was determined. Solutions with 4.8, 24, and 120 µg/ml of STTC were added to equal volumes of the zoospore suspensions, giving final concentrations of 2.4, 12, and 60 µg/ml. Control zoospore suspensions received an equal volume of water only. Zoospore suspensions were maintained at 25 C and observed microscopically at 75× to determine the maximum elapsed time for complete cessation of motility. Observations were made at 1-min intervals for the first 15 min, then at 15-min intervals for the next 45 min, and finally at 1-hr intervals until no zoospore movement was detected. This experiment was performed four times.

The majority of zoospore cysts were adhering to the bottom of each petri dish, where the number of cysts with intact cell walls was recorded after 4–6 hr in the presence of STTC. The average number of encysted zoospores in four randomly selected microscope fields (2.2 mm<sup>2</sup>) at 75× was used to derive each replicate count in each dish.

To determine the viability of encysted zoospores adhering to the bottom of each petri dish, the aqueous treatment mixture was decanted and replaced with 5 ml of 10% clarified V-8 juice broth (V-8 juice centrifuged for 10 min at 1,000 g, then the supernatant

was diluted with sterile distilled water). After incubation for 19 hr at 21 C, the average number of zoospore cysts from which germ tubes were emerging in four randomly selected microscope fields at 75X was used to determine percent germination.

**Sporangium formation in soil.** Six-millimeter-diameter leaf disks of lemon (*Citrus limon* (L.) Burm.), apple (*Malus sylvestris* Mill.), and chili pepper (*Capsicum annuum* L.) were separately colonized by two isolates each of *P. parasitica*, *P. citrophthora*, *P. capsici*, or *P. cactorum* as described previously (10). *Phytophthora* species were grown on V8A for 5 days at 24 C. Leaf disks were surface-disinfested for 20 min in a 1% solution of sodium hypochlorite, then rinsed three times in sterile water. Leaf disks of lemon were placed adjacent to the margin of actively growing cultures of *P. parasitica* and *P. citrophthora*, whereas leaf disks of chili pepper and apple were placed adjacent to cultures of *P. capsici* and *P. cactorum*, respectively. Leaf disks were colonized by these pathogens after an incubation period of 48 hr at 24 C. No sporangia were detected on colonized leaf disks at that time. *Phytophthora cambivora* and *P. drechsleri* would not form sporangia with these procedures and, therefore, were not included in the tests with STTC.

Eight colonized leaf disks 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 X 10-cm-deep plastic pot and covered with an additional 5-cm layer of the sandy loam. The soil in each pot then was drenched with water containing 122, 245, or 490 µg/ml of STTC in sufficient quantities to thoroughly wet the soil. Control pots were drenched with water only. Pots were allowed to drain freely, then incubated for 72 hr at 25–28 C. Leaf disks then were removed, rinsed with water, and stained and fixed with acid fuchsin in 85% lactic acid. The number of sporangia along the margins of each leaf disk were counted.

**Mycelial growth.** Each isolate of each *Phytophthora* spp. was grown on V8A for 5 days at 24 C. Five 6-mm-diameter agar disks were removed from the edge of an actively growing culture of each pathogen and placed at the edge of a 9-cm-diameter plastic petri dish containing 20 ml of 5% clarified V-8 juice broth (adjusted to an initial pH 7.0 with KOH) amended with STTC at concentrations of 245, 612, 1,225, 1,837, and 2,450 µg/ml. Control petri dishes contained only broth. Petri dishes containing similar concentrations of STTC were incubated in sealed plastic containers at 27–30 C. After 72 hr, radial growth of mycelia was measured from the edge of each inoculum disk. Two isolates of each *Phytophthora* sp. were tested three times.

**Effect of pH on sporulation and growth.** Concentrations of STTC used in these studies raised the pH of resultant treatment solutions. To determine the effect of pH alone on sporulation and growth of *Phytophthora* spp., the pH in a series of experiments without STTC was adjusted to values found in the tests involving this material. Sporangium formation and zoospore motility, encystment, and germination were evaluated as previously described at pH values of 7.5, 7.7, 7.8, 8.1, 8.3, 8.5, and 8.8, which

TABLE 1. Effect of sodium tetrathiocarbonate (STTC) on the duration of zoospore motility of six species of *Phytophthora*<sup>2</sup>

<i>Phytophthora</i> species	Zoospore motility without STTC (min)	Percent inhibition of the duration of zoospore motility with STCC (µg/ml) at		
		2.4	12	60
<i>P. parasitica</i>	196	96 a	97 a	97 a
<i>P. citrophthora</i>	82	92 a	93 a	94 a
<i>P. capsici</i>	257	38 bc	96 a	96 a
<i>P. cactorum</i>	155	92 a	96 a	95 a
<i>P. cambivora</i>	67	46 bc	86 a	87 a
<i>P. drechsleri</i>	314	55 b	93 a	92 a
Combined average values	179	70	94	94

<sup>2</sup> Each value is an average of eight replicate determinations from four experiments; numbers within each column with the same letter do not differ significantly at  $P = 0.05$  according to Duncan's multiple range test.

correspond to the pH of STTC concentrations of 0, 2.4, 12, 60, 122, 245, and 490 µg/ml, respectively. Mycelial growth was examined in 5% clarified V-8 juice broth adjusted to pH values of 7.0, 8.0, 8.5, 8.8, 9.0, and 9.1, which were the pH values of STTC concentrations of 0, 245, 612, 1,225, 1,837, and 2,450 µg/ml, respectively. The pH of water or 5% clarified V-8 juice broth was adjusted by addition of 1 N KOH.

All experiments were established in a completely randomized design. The experiments were conducted two to four times and analyses of variance performed on the data from each trial. When error variances were homogeneous, a combined analysis of variance was performed on pooled data from all trials. Percent data were transformed to arcsins for statistical analysis, but actual percentages are presented in the tables. Duncan's multiple range test was used to determine differences between mean values at selected concentrations of STTC.

## RESULTS AND DISCUSSION

**Zoospore motility, encystment, and germination.** Sodium tetrathiocarbonate currently is being developed as a nematocide. Results of this study reveal that this compound also affects various stages in the life cycle of six species of *Phytophthora*. The zoospore appears to be highly sensitive to the chemical. Sodium tetrathiocarbonate at concentrations of 2.4, 12, and 60 µg/ml had a marked effect on the duration of motility of zoospores of all *Phytophthora* spp. tested (Table 1). Complete cessation of zoospore motility occurred after 16–18 min for *P. drechsleri* and after 4–6 min for the other species in the presence of STTC at 12 or 60 µg/ml. At 2.4 µg/ml, inhibition of zoospore motility for *P. parasitica*, *P. citrophthora*, and *P. cactorum* was significantly greater than *P. capsici*, *P. cambivora*, and *P. drechsleri*. At 12 and 60 µg/ml, zoospore motility was comparably reduced for all *Phytophthora* spp. tested (Table 1).

The decreasing duration of zoospore motility in the presence of

TABLE 2. Effect of sodium tetrathiocarbonate (STTC) on production of zoospore cysts of six species of *Phytophthora*<sup>2</sup>

<i>Phytophthora</i> species	Zoospore cyst production without STTC (No./2.2 mm <sup>2</sup> )	Percent reduction in number of zoospore cysts with STTC (µg/ml) at		
		2.4	12	60
<i>P. parasitica</i>	288	20 bc	61 cd	100 a
<i>P. citrophthora</i>	281	21 bc	84 ab	69 bc
<i>P. capsici</i>	322	22 bc	75 abc	100 a
<i>P. cactorum</i>	77	97 a	98 a	98 a
<i>P. cambivora</i>	16	37 b	32 d	51 c
<i>P. drechsleri</i>	66	83 a	96 a	78 ab
Combined average values	175	47	74	83

<sup>2</sup> Each value is an average of 16 replicate counts from two experiments; numbers within each column with the same letter do not differ significantly at  $P = 0.05$  according to Duncan's multiple range test.

TABLE 3. Effect of sodium tetrathiocarbonate (STTC) on sporangia formation in soil by four species of *Phytophthora*<sup>2</sup>

<i>Phytophthora</i> species	Sporangia without STTC (No./buried leaf disk)	Percent inhibition of sporangia formation with STTC (µg/ml) at		
		122	245	490
<i>P. parasitica</i>	350	28 ab	46 c	100 a
<i>P. citrophthora</i>	378	22 abc	69 ab	96 a
<i>P. capsici</i>	270	31 a	65 abc	100 a
<i>P. cactorum</i>	23	11 bc	80 a	96 a
Combined average values	255	23	65	98

<sup>2</sup> Each value is an average of 32 replicate counts from two experiments; numbers within each column with the same letter do not differ  $P = 0.05$  according to Duncan's multiple range test.

TABLE 4. Effect of sodium tetrathiocarbonate (STTC) on mycelial growth on six species of *Phytophthora*<sup>1</sup>

<i>Phytophthora</i> species	Mycelial growth without STTC (mm)	Percent inhibition of mycelial growth with STCC ( $\mu\text{g/ml}$ ) at				
		245	612	1,225	1,837	2,450
<i>P. parasitica</i>	12	26 a	29 bcd	65 abc	100 a	100 a
<i>P. citrophthora</i>	10	8 b	38 abc	68 ab	92 a	100 a
<i>P. capsici</i>	14	12 b	14 e	47 cd	73 b	99 a
<i>P. cactorum</i>	8	26 a	46 a	76 a	98 a	100 a
<i>P. cambivora</i>	9	10 b	22 de	74 a	100 a	100 a
<i>P. drechsleri</i>	10	1 b	42 ab	46 d	84 ab	100 a
Combined average values	10	14	32	63	91	100

<sup>1</sup> Each value is an average of 20 replicate measurements from two experiments; numbers within each column with the same letter do not differ significantly at  $P = 0.05$  according to Duncan's multiple range test.

increasing STTC concentrations usually was associated with a corresponding decrease in the quantity of zoospore cysts having intact cell walls (Table 2). Zoospores of *P. cactorum* and *P. drechsleri* were highly sensitive to STTC. In the presence of STTC at 2.4  $\mu\text{g/ml}$ , the percent reduction in the number of zoospore cysts of *P. cactorum* and *P. drechsleri* was significantly greater than that for all other species. At 60  $\mu\text{g/ml}$ , the percent reduction in the number of zoospore cysts of *P. parasitica*, *P. capsici*, *P. cactorum*, and *P. drechsleri* were comparable (Table 2). The percent reduction in the number of zoospore cysts of *P. cambivora* was among the lowest of all species at all concentrations of STTC.

In the presence of 10% clarified V-8 juice broth, essentially all zoospore cysts of *P. parasitica*, *P. citrophthora*, and *P. capsici* germinated when formed in soil extract or in the presence of STTC at 2.4  $\mu\text{g/ml}$ , but no cysts germinated when formed in the presence of the compound at 12 and 60  $\mu\text{g/ml}$ . For *P. cactorum* and *P. drechsleri*, germination was reduced approximately 50 and 75%, respectively, when zoospore cysts were formed at 2.4  $\mu\text{g/ml}$ , whereas no germination occurred when the concentration was increased to 12  $\mu\text{g/ml}$ .

**Sporangia formation in soil.** Inhibition of sporangia production required higher concentrations of STTC. A soil drench with three concentrations of STTC resulted in decreasing production of sporangia on buried leaf disks colonized by *P. parasitica*, *P. citrophthora*, *P. capsici*, and *P. cactorum* (Table 3). At 122  $\mu\text{g/ml}$ , *P. cactorum* was significantly less sensitive to the chemical than *P. capsici* but did not significantly differ from *P. parasitica* and *P. citrophthora*; however, at 245  $\mu\text{g/ml}$ , *P. cactorum* was among the most sensitive of the fungi. Inhibition was almost complete for the four *Phytophthora* species tested at a STTC concentration of 490  $\mu\text{g/ml}$ .

**Mycelial growth.** Growth of mycelia was not appreciably affected by concentrations of STTC that reduced zoospore motility and the number and viability of zoospore cysts. Mycelial growth of *P. parasitica* and *P. cambivora* was completely inhibited by 1,837  $\mu\text{g/ml}$  STTC, and that of *P. citrophthora*, *P. cactorum*, and *P. drechsleri* by 2,450  $\mu\text{g/ml}$ . Slight growth by *P. capsici* was observed at the highest rate of the compound (Table 4). In the presence of 245  $\mu\text{g/ml}$  of STTC, mycelial growth of *P. parasitica* and *P. cactorum* was significantly less than that of *P. citrophthora*, *P. capsici*, *P. cambivora*, and *P. drechsleri*.

**Effect of pH on sporulation and growth.** In the absence of STTC, the duration of zoospore motility, production of encysted zoospores, and germination of zoospore cysts did not change as pH was increased from 7.5 to 8.1. Likewise, mycelial growth was not inhibited as the pH of 5% clarified V-8 juice broth was increased from 7.0 to 9.1. In contrast, sporangium formation in soil by *P. parasitica*, *P. citrophthora*, *P. capsici*, and *P. cactorum* was reduced 1, 62, 41, and 70%, respectively, as the pH of the water drench was increased from 7.5 to 8.3 (pH of water and STTC at a concentration of 122  $\mu\text{g/ml}$ , respectively). No further reduction in sporangia formation occurred from pH 8.3 to 8.8 (pH of STTC at concentrations of 122 and 490  $\mu\text{g/ml}$ , respectively).

Inhibition of sporangia formation by *P. citrophthora*, *P. capsici*, and *P. cactorum* in soil drenched with a solution of potassium hydroxide could result from a direct pH effect or from a change in

the chemical status of the soil. Sporangia of *Phytophthora* spp. form within a pH range of 4–9, although the optimum pH for sporangium formation varies among species and among isolates of the same species. According to Ribeiro (16), it often is not possible to separate the effects of pH from the effects of salt components; therefore, interpretation of data in terms of a pH effect is difficult. Inhibition of sporangia formation in soil by STTC may involve the combined effects of the chemical, the change in pH, and the resultant changes in the chemical status of the soil.

In contrast to the activity of sodium tetrathiocarbonate, *in vitro* tests with the systemic fungicides metalaxyl and oxadixyl revealed that mycelial growth and sporangia formation were more sensitive to these compounds than the germination of zoospore cysts (6). Fuller and Gisi (6) reported that metalaxyl at 10  $\mu\text{g/ml}$  caused no significant decrease in germination of zoospore cysts of *P. palmivora*, whereas concentrations of 0.7 and 1.4  $\mu\text{g/ml}$  caused a 90% reduction in mycelial growth and sporangia formation, respectively. Farih et al (4) showed that metalaxyl at 100  $\mu\text{g/ml}$  caused a 7 and 51% reduction in the germination of zoospores of *P. parasitica* and *P. citrophthora*, respectively, whereas the same concentration of fungicide reduced linear mycelial growth of *P. parasitica* and *P. citrophthora* by 92 and 96%, and reduced sporangia formation by 90 and 99%, respectively.

As a postplant nematocide, STTC is being evaluated at concentrations of 122–245  $\mu\text{g/ml}$  (50–100  $\mu\text{g/ml}$  equivalent concentration of carbon disulfide). Findings from this investigation suggest that these concentrations of the chemical could be highly inhibitory under field conditions to zoospore motility and lethal to zoospore cysts of *P. parasitica*, *P. citrophthora*, *P. capsici*, *P. cactorum*, and *P. drechsleri*. Sporangia production by *P. parasitica*, *P. citrophthora*, *P. capsici*, and *P. cactorum* also could be inhibited at these chemical concentrations, but mycelial growth would not be affected appreciably.

Sodium tetrathiocarbonate decomposes when diluted in water, releasing carbon disulfide. After STTC was applied to a sandy loam soil in a citrus grove in Yuma, AZ, the carbon disulfide concentration decreased daily and could not be detected after 7 days (M. E. Matheron, unpublished). This suggests that multiple applications would be required to suppress zoospore motility and viability as well as sporangia production.

The possibility of a single pesticide with activity against *Phytophthora* spp. and nematodes is an intriguing prospect, especially for citrus production where *Phytophthora* root and crown rot and citrus nematode damage are major soilborne problems. Further evaluation of STTC as a potential fungicide for control of diseases caused by *Phytophthora* spp. is now in progress.

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