Effect of Sodium Tetrathiocarbonate, Metalaxyl, and Fosetyl-Al on Development and Control of Phytophthora Root Rot of Citrus

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ABSTRACT

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The activity of sodium tetrathiocarbonate (STTC) on sporulation and growth of several species of *Phytophthora* in vitro has recently been demonstrated. This study was initiated to evaluate and compare the effect of root and soil treatments with STTC, metalaxyl, and fosetyl-Al on the subsequent development of Phytophthora root rot on citrus. Growth of rough lemon (a citrus rootstock) seedlings inoculated with sporangia of *P. citrophthora* and *P. parasitica* in the presence of STTC at 245 μg/ml or metalaxyl at 10μg/ml was enhanced significantly compared with untreated plants. Shoot growth of rough lemon seedlings in soil naturally infested with *P. parasitica* that was treated 1 wk before planting with either STTC at 4,900 μg/ml or metalaxyl at 10 μg/ml was equivalent to that obtained in sterilized orchard soil. STTC, when applied as a soil drench at 2,450 and 4,900 μg/ml, was lethal to *P. citrophthora* and *P. parasitica* on colonized leaf disks of lemon buried in soil, whereas metalaxyl at 10 μg/ml or fosetyl-Al at 3,000 μg/ml did not appreciably affect the viability of the pathogens. Sporangium production on leaf disks of lemon colonized by *P. citrophthora* and *P. parasitica* and buried in soil was reduced at least 90% compared with the untreated control 3 and 6 days after treatment of soil with 2,450 μg/ml of STTC, 10 μg/ml of metalaxyl, and 3,000 μg/ml of fosetyl-Al. Compared with untreated soil, the number of lesions formed on pear fruit bait incubated with citrus orchard soil containing *P. citrophthora* or *P. parasitica* and treated with STTC at 24 μg/ml was reduced 76 and 74%, respectively, whereas no lesions developed when soil was treated with metalaxyl at 10 μg/ml or fosetyl-Al at 3,000 μg/ml. These investigations demonstrate the potential utility of STTC as a fungicide for control of Phytophthora root rot of citrus.

A high incidence of root and crown rot has been observed in commercial citrus plantings in Arizona. *Phytophthora citrophthora* (R. E. Smith & E. H. Smith) Leonian and *P. parasitica* Dastur are consistently isolated from diseased root and bark tissue, as well as from soil adjacent to infected trees (12). Severe losses can occur in orchards established with trees on a susceptible rootstock, such as rough lemon (*Citrus jambhiri* Lush.), or in plantings on resistant rootstocks where the graft union is at or below the soil surface, exposing susceptible scion tissue to the pathogens.

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The efficacy of the systemic fungicides fosetyl-Al (Aliette, Rhone-Poulenc Inc., Monmouth Junction, NJ) and metalaxyl (Ridomil, Ciba-Geigy Corp., Ardsley, NY) for control of Phytophthora root and crown rot of citrus has been demonstrated (3,4,8,15,16). Recently, the activity of sodium tetrathiocarbonate (STTC) (Enzone [Gy-81], Unocal Corp.) on sporulation and growth of six species of *Phytophthora* in vitro has been reported (9). When added to water and applied to soil, STTC releases carbon disulfide, a known biocide (1,2,5,14).

The objective of this study was to compare the effect of root and soil treatment with STTC or metalaxyl on subsequent development of Phytophthora root rot on citrus. The viability of *P. citrophthora* and *P. parasitica* in colonized citrus tissue, recovery of these pathogens from soil, and sporangium production after application of STTC, metalaxyl, or fosetyl-Al to soil was also examined. A preliminary account of this work has been published (10).

MATERIALS AND METHODS

Disease development—inoculation with sporangia. Rough lemon seedlings were grown in pasteurized potting mix (45% peat:45% vermiculite:10% sand,

v/v/v). Four-month-old seedlings were removed carefully from the potting mix, washed thoroughly in tap water to remove potting mix adhering to the roots, and placed in 8-cm-diameter × 13-cm-deep plastic cups each containing either 400 ml of distilled water alone, distilled water amended with 122 or 245 μg/ml of STTC (Enzone 3.4E), or distilled water amended with 10 μg/ml of metalaxyl (Ridomil 2E). Each cup contained 10 seedlings with roots totally immersed in water. Plants were inoculated with agar disks bearing sporangia of *P. citrophthora* (isolate C-72-S from a citrus tree in Yuma, AZ) or *P. parasitica* (isolate C-19-S from a citrus tree in Mesa, AZ) following procedures described earlier (11). Species of *Phytophthora* were grown on V-8 juice agar (13) and 10 6-mm-diameter agar disks were removed from the edge of an actively growing culture and incubated in nonsterile soil extract in 60-mm-diameter plastic petri dishes (9). Numerous sporangia formed after incubation for 5 days at 24 C and were subsequently induced to release zoospores by chilling for 15 min at 4 C. Immediately after chilling, 10 agar disks containing sporangia were added to each cup containing plants. Uninoculated control plants were incubated in cups without the addition of agar disks. Plants remained in the laboratory for 4 hr at 23–25 C, during which time the agar disks remained on the bottom of the cup.

Inoculum was quantified by microscopically counting the number of sporangia that had formed on each of eight separate agar disks for each species of *Phytophthora*. The final concentration of sporangia during inoculation of plants was approximately nine and 24 per milliliter of water for *P. citrophthora* and *P. parasitica*, respectively.

After the 4-hr inoculation, rough lemon seedlings were removed from the containers, and the contents of each cup were decanted and replaced with distilled water. Roots of inoculated plants were rinsed in tap water, placed into the water in the cups, and incubated for an additional 18 hr at 24 C. Seedlings were then planted individually in potting mix in 10-cm-diameter × 10-cm-deep plastic pots in the greenhouse. Once every 2 wk for the duration of the experiment, one-half of the plants were immersed for 48 hr in water-filled containers so that 1 cm of water covered the surface of the potting mix in each pot, in order to stimulate disease development. Between flooding treatments, seedlings were watered as needed and the potting mix was allowed to drain freely. Rough lemon seedlings were 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 26 C.

Experiments lasted 3 mo, and final disease incidence and severity were evaluated by recording plant mortality and the fresh weight of shoots and roots. Analyses of variance of the resultant data were performed, and Duncan's multiple range test was used to differentiate treatment means when appropriate. Disease was confirmed as resulting from infection by *Phytophthora* by reisolating the pathogen from rough lemon seedlings. Ten rootlets from each of 10 replicate plants per treatment were plated on PARP medium (7). The experiment was performed three times with comparable results; data presented are from only one experiment.

Disease development — preplant drench of naturally infested soil. Soil (sandy loam: 81% sand, 7% silt, 12% clay) infested with *P. parasitica* was obtained from a citrus orchard in Yuma. The soil 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 solution over the surface. An inoculated control soil treatment was drenched with water only. After a 2-hr incubation at 27–29 C, the soil was allowed to drain freely and remained undisturbed for 7 days. Four-month-old rough lemon seedlings were then planted individually in the treated soil in 10-cm-diameter × 10-cm-deep plastic pots. An uninoculated control treatment was established by planting seedlings in orchard soil that was heat-sterilized. Once every 2 wk, 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 30 C.

Final disease incidence and severity were evaluated after 3 mo by recording the fresh weight of shoots and roots. Analyses of variance of the resultant data were performed, and Duncan's multiple range test was used to differentiate treatment means when appropriate. Data presented are derived from the combined results of three experiments. As in earlier tests, disease was confirmed as resulting from infection by *P. parasitica* by reisolating the pathogen from the roots of rough lemon seedlings.

Viability of mycelium of *Phytophthora* in soil. Six-millimeter-diameter leaf disks of lemon (*Citrus limon* (L.) N. L. Burm.) were colonized by *P. citrophthora* (isolate C-72-S) and *P. parasitica* (isolate C-19-S) as described previously (9). Ten colonized leaf disks, which contained mycelium only 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 water containing 1,225, 2,450, or 4,900 μg/ml of STTC, 10 μg/ml of metalaxyl, or 3,000 μg/ml of fosetyl-Al (Aliette 80W) in sufficient quantities to thoroughly wet the soil.

Table 1. Growth and disease development in rough lemon seedlings inoculated with sporangia of *Phytophthora citrophthora* or *P. parasitica* in the presence of metalaxyl or STTC

	Average growth and disease severity[y]							
	P. citrophthora				*P. parasitica*			
	Fresh weight (g)		Plants	Isolation from	Fresh weight (g)		Plants	Isolation from
Treatment	Shoot	Root	dead	rootlets (%)[z]	Shoot	Root	dead	rootlets (%)[z]
Flooded biweekly								
Inoculated control	1 b	0.2 c	9	17	3 c	1.2 c	1	29
Metalaxyl at 10 μg/ml	13 a	4.2 a	0	0	14 a	3.4 a	0	0
STTC at 245 μg/ml	13 a	4.3 a	0	0	7 b	1.9 b	0	10
Uninoculated control	12 a	3.6 b	0	0	12 a	3.6 a	0	0
Not flooded								
Inoculated control	4 b	1.1 b	4	8	5 b	1.2 b	0	10
Metalaxyl at 10 μg/ml	12 a	2.8 a	0	0	12 a	2.6 a	0	0
STTC at 122 μg/ml	10 a	2.5 a	0	0	11 a	2.4 a	0	2
Uninoculated control	12 a	2.8 a	0	0	12 a	2.8 a	0	0

[y] Each value is the mean of 10 replicate plants per treatment. For flooded or nonflooded plants, numbers withn each column with a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Percentage of rootlets from which *P. citrophthora* or *P. parasitica* was isolated at the termination of the experiment. Values represent the mean of 10 rootlets from each of 10 replicate plants.

Control pots were drenched with water only. Pots were allowed to drain freely, then incubated for 5 days at 23–24 C, after which the 10 leaf disks were removed from each pot, rinsed in water, placed on a dry paper towel to remove excess moisture, and plated on PARP medium. Petri plates were incubated in darkness at 24 C for 5 days and observed daily for growth of mycelium of *Phytophthora* from the leaf disks. This test was conducted twice, with each trial consisting of four replicate pots per treatment each containing 10 leaf disks.

Sporulation of *Phytophthora* in soil. Leaf disks of lemon were colonized by *P. citrophthora* (isolate C-7-S from a citrus tree in Mesa) and *P. parasitica* (isolate C-2-C from a citrus tree in Yuma) and buried in field soil in plastic pots as described earlier. Each pot, which contained five colonized leaf disks, was drenched with water containing 245, 490, 1,225, and 2,450 μg/ml of STTC, 10 μg/ml of metalaxyl, or 3,000 μg/ml of fosetyl-Al in sufficient quantities to thoroughly wet the soil. Control pots were drenched with water only. Pots were allowed to drain freely, then incubated for 3–10 days at 23–25 C in the laboratory. At 3, 6, and 10 days after treatment, leaf disks were removed from the soil, rinsed in 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. This experiment was performed three times.

Recovery of *Phytophthora* from soil. Five hundred cubic centimeters of soil collected from citrus orchards infested with *P. citrophthora* or *P. parasitica* was placed into containers 12 cm wide × 23 cm long × 7 cm deep and drenched with 750 ml of water containing 12, 24, 61, 122, or 245 μg/ml of STTC, 10 μg/ml of metalaxyl, or 3,000 μg/ml of fosetyl-Al. Control containers were drenched with water only. Two ripe but green unblemished Bartlett or Anjou pear fruits were placed on the surface of each soil sample, which established a 1- to 2-cm aqueous layer at the soil surface. After incubation at 23–25 C for 48 hr, the fruits 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 fruits invaded by a *Phytophthora* sp. Small pieces of tissue from each brown spot were placed on PARP medium, incubated at 24 C in darkness, and observed for growth of *P. citrophthora* and *P. parasitica*. The number of lesions caused by each pathogen was recorded. This series of experiments was conducted four times and data were subjected to linear regression analysis when appropriate.

Table 2. Effect of chemical preplant treatment of soil naturally infested with *Phytophthora parasitica* on subsequent growth and disease development in rough lemon seedlings

Treatment	Fresh weight (g)[y]		Isolation from rootlets (%)[z]
	Shoot	Root	
Sterilized soil	6.6 a	3.1 a	0
Nonsterilized soil	3.6 b	1.4 c	14
Metalaxyl at 10 μg/ml	6.4 a	2.4 b	32
STTC at 4,900 μg/ml	6.2 a	2.4 b	12

[y] Each value is the mean of 21 replicate plants from three experiments. Numbers in each column with a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Percentage of rootlets from which *P. parasitica* was isolated at the termination of the experiment. Values represent the mean of 10 rootlets from each of 21 replicate plants.

Table 3. Effect of chemical drench on viability of *Phytophthora citrophthora* and *P. parasitica* in colonized leaf disks of lemon buried in field soil

Treatment	Rate (μg/ml)	Isolation from leaf disks (%)[a]	
		P. citrophthora	*P. parasitica*
Water		100	100
Fosetyl-Al	3,000	94	100
Metalaxyl	10	100	84
STTC	1,225	84	96
STTC	2,450	0	6
STTC	4,900	0	0

[a] Percentage of lemon leaf disks from which *P. citrophthora* or *P. parasitica* was isolated at the termination of the experiment. Values represent the mean of 10 leaf disks from each of eight replicate pots containing field soil.

RESULTS

Disease development—inoculation with sporangia. Growth of rough lemon seedlings inoculated with sporangia of *P. citrophthora* or *P. parasitica* in the presence of STTC or metalaxyl was enhanced significantly compared with inoculated control plants (Table 1). In the group of plants inoculated with *P. parasitica*, root and shoot growth was greater in plants treated with metalaxyl than in those treated with STTC when plants were flooded every 2 wk for 48 hr. In contrast, there was no significant difference in growth between the two treatments when plants were not flooded. Likewise, disease suppression, as measured by increased plant growth, was equivalent when rough lemon seedlings were treated with STTC or metalaxyl, inoculated with *P. citrophthora*, and subjected to either irrigation regime. Plant death was prevented by treatment with either chemical, although *P. parasitica* was isolated from some rootlets of rough lemon treated with STTC but not from rootlets treated with metalaxyl. No phytotoxicity was observed with either chemical at the rates used.

Table 4. Formation of sporangia by *Phytophthora citrophthora* and *P. parasitica* on colonized citrus leaf disks incubated in soil for different time intervals after treatment with STTC, metalaxyl, or fosetyl-Al

Treatment	Rate (μg/ml)	Sporangia on leaf disks colonized by					
		P. citrophthora[a]			*P. parasitica*[a]		
		3 days	6 days	10 days	3 days	6 days	10 days
No fungicide	...	267	265	74	182	417	85
STTC	245	80*[b]	383	219	114	298	125
STTC	490	39*	235	174	68*	227	106
STTC	1,225	0**	3**	22*	1**	96*	61
STTC	2,450	0**	0**	0**	0**	0**	12*
Metalaxyl	10	12**	21**	8*	7**	28**	12*
Fosetyl-Al	3,000	0**	19**	18*	1**	18**	8**

[a] Each value is the mean number of sporangia that formed along the margin of 15 replicate leaf disks from three experiments.
[b] Within each column, values followed by * or ** represent at least a 50 or 90% reduction, respectively, in the number of sporangia formed compared with the untreated control.

Microscopic observation of agar disks containing sporangia that were used as inoculum immediately after chilling revealed numerous ungerminated sporangia with no zoospores detected in the surrounding soil extract. After the 4-hr period of incubation with rough lemon seedlings in water, sporangia of *P. citrophthora* and *P. parasitica* had released zoospores, some of which were still motile. Sporangia incubated in metalaxyl or STTC with rough lemon seedlings also released zoospores, but only encysted zoospores were observed, often present in partially empty sporangia.

Disease development — preplant drench of naturally infested soil. Shoot growth of rough lemon seedlings in soil infested with *P. parasitica* and treated with 4,900 μg/ml of STTC or with metalaxyl 1 wk before planting was equivalent to that obtained in sterilized orchard soil (Table 2). Root growth in soil treated with either chemical was greater than that recorded in untreated soil but less than the root growth achieved in sterilized orchard soil. At the termination of the experiments, *P. parasitica* was isolated from rootlets of plants grown in untreated soil as well as soil treated with STTC or metalaxyl but not from plants grown in sterilized orchard soil.

Viability of mycelium of *Phytophthora* in soil. *P. citrophthora* could not be recovered from colonized leaf disks of lemon buried in field soil that was drenched with 2,450 or 4,900 μg/ml of STTC (Table 3). *P. parasitica* was recovered from only 6% of the colonized leaf disks treated with 2,450 μg/ml of STTC, and no recovery was obtained at a concentration of 4,900 μg/ml.

Sporulation of *Phytophthora* in soil. Three days after leaf disks of lemon colonized by *P. citrophthora* were buried in soil, a single STTC drench of 245 or 490 μg/ml reduced sporangium formation by at least 50%, compared with leaf disks treated with no fungicide (Table 4). At 6 and 10 days after drenching, these same treatments had no negative effect on sporangium production. Relative to the untreated control, sporangium production by *P. citrophthora* decreased at least 90% at 3 and 6 days after soil was treated with 1,225 and 2,450 μg/ml of STTC, 10 μg/ml of metalaxyl, and 3,000 μg/ml of fosetyl-Al.

Compared with the untreated control, a minimum 50% decrease in sporangium formation was observed 3, 6, and 10 days after leaf disks colonized by *P. parasitica* were buried in soil and treated with 490, 1,225, and 2,450 μg/ml of STTC, respectively (Table 4). Relative to the leaf disks not receiving fungicides, sporangium production was reduced at least 90% with 2,450 μg/ml of STTC, 10 μg/ml of metalaxyl, and 3,000 μg/ml of fosetyl-Al at 3 and 6 days after treatment.

Recovery of *Phytophthora* from soil. There was a significant linear relationship ($r = -0.923$) between the log of the number of lesions developing on pear fruit bait incubated with citrus orchard soil containing *P. parasitica* and the log of the concentration of STTC present in the soil. No such relationship was observed for soil infested with *P. citrophthora* and treated with the rates of STTC used in this study. The number of lesions formed on pear fruit incubated with untreated citrus orchard soil containing *P. citrophthora* was 92% less than in untreated soil infested with *P. parasitica*. The number of lesions induced by *P. citrophthora* declined 15 and 76% by treatment of soil with STTC at 12 and 24 μg/ml, respectively, compared with lesion formation in untreated soil. No lesions developed on pear fruit incubated with soils containing either pathogen and treated with metalaxyl or fosetyl-Al (Table 5).

DISCUSSION

At appropriate concentrations, STTC, metalaxyl, and fosetyl-Al restricted the infection of pear fruits by *P. citrophthora* or *P. parasitica* in soil infested with these pathogens. Likewise, a single treatment with STTC or metalaxyl during the inoculation of roots with zoospores of these pathogens significantly reduced the subsequent development of root rot on rough lemon seedlings. Significant disease control on plants that were not flooded after inoculation was achieved with 122 μg/ml of STTC or 10 μg/ml of metalaxyl. When plants were flooded for 48 hr every 2 wk after inoculation, thus establishing an environment that stimulates repeated infection cycles for Phytophthora root rot, a higher concentration of STTC (245 μg/ml) was needed to achieve significant disease control. Lesion development on pear fruit and root rot development on rough lemon seedlings suggest that STTC concentrations up to 245 μg/ml are necessary to inhibit zoospore activity in soil.

In these studies, metalaxyl and STTC induced zoospores of *P. citrophthora* and *P. parasitica* to encyst. Earlier experiments (9) demonstrated the ability of STTC at 12 μg/ml to reduce zoospore motility to 3–4 min and to kill resulting zoospore cysts, while this same concentration of STTC did not significantly inhibit sporangium formation or mycelial growth. On the other hand, Fuller and Gisi (6) reported that metalaxyl at 10 μg/ml caused no significant decrease in germination of zoospore cysts of *P. palmivora*, whereas concentrations of 0.7 and 1.4 μg/ml caused a 90% reduction in mycelial growth and sporangium formation, respectively. Farih et al (4) showed that metalaxyl at 100 μg/ml reduced linear growth of *P. parasitica* and *P. citrophthora* by 92 and 96%, respectively, whereas the same concentration of fungicide caused only a 7 and 51% reduction in the germination of zoospores. These earlier studies suggest that the viability of zoospore cysts is more sensitive than sporangium formation or mycelial growth to STTC (9). On the other hand, sporangium formation and mycelial growth apparently are more sensitive to metalaxyl than the germination of zoospores (4,6). Although the relative effect of each chemical on various stages of the life cycle of *P. citrophthora* and *P. parasitica* apparently is different, the disease was controlled in both situations.

STTC, applied as a soil drench at a rate of 2,450–4,900 μg/ml, was lethal to mycelium of *P. citrophthora* and *P. parasitica* buried in soil, whereas the viability of both fungi was not appreciably affected by 1,225 μg/ml of STTC, 10 μg/ml of metalaxyl, or 3,000 μg/ml of fosetyl-Al. In comparison, a preplant soil drench with 1,225 μg/ml of STTC or 10 μg/ml of metalaxyl was able to suppress development of root rot on rough lemon seedlings planted in soil naturally in-

Table 5. Development of lesions on pear fruit incubated with citrus orchard soil containing *Phytophthora parasitica* or *P. citrophthora* and drenched with STTC, metalaxyl, or fosetyl-Al

Treatment	Rate (μg/ml)	Lesions on pear fruit incubated with soil containing	
		P. parasitica[a]	*P. citrophthora*[b]
Untreated	...	70	5.5
STTC	12	31	4.5
STTC	24	18	1.3
STTC	61	14	...
STTC	122	4	...
STTC	245	1	...
Metalaxyl	10	0	0
Fosetyl-Al	3,000	0	0

[a] Each value is the mean number of lesions that formed on eight replicate pear fruits from four experiments. Lesion development was confirmed as resulting from colonization by *P. parasitica* by reisolating the pathogen from test pear fruits. Linear regression equation of the log of lesions per pear fruit (y) against the log of concentration of STTC (x) is: $y = 0.623x + 2.036$, $r = -0.923$. r is significant at $P = 0.01$.

[b] Each value is the mean number of lesions that formed on six replicate pear fruits from three experiments. Lesion development was confirmed as resulting from colonization by *P. citrophthora* by reisolating the pathogen from test pear fruits.

fested with *P. parasitica*. It is possible that the naturally infested soil, which included feeder roots from orchard trees, contained propagules of the fungus other than active mycelium, which were more sensitive to STTC and metalaxyl.

STTC and metalaxyl provided comparable levels of disease control under our experimental conditions. The tested formulation of STTC rapidly releases carbon disulfide, the active biocide, when diluted in water. The quick release of carbon disulfide apparently provides short-term inhibition of *Phytophthora* spp. For example, sporangium formation by *P. citrophthora* and *P. parasitica* was reduced at least 50% 3 days after these fungi were treated with STTC at 245–490 μg/ml, a concentration range that is not phytotoxic to rough lemon seedlings. On the other hand, by 6 and 10 days after drenching, these same treatments had no negative influence on sporangium production. STTC at concentrations of 1,225–2,450 μg/ml were needed to inhibit sporangium formation for at least 10 days (levels shown to be toxic to the host plant as well) (M. E. Matheron, *unpublished*). A slow-release formulation of STTC could provide sustained inhibition of sporangium formation and zoospore activity in the orchard, which would lead to more effective control of Phytophthora root rot of citrus.

Currently, STTC is being developed as a nematicide. This chemical also has potential as a control agent for Phytophthora root rot of citrus. The possibility of a single pesticide with activity against *Phytophthora* and nematodes is especially appealing for citrus production, where Phytophthora root and foot rot and citrus nematode damage are major soilborne problems that often occur together in the same orchard.

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