

Comparative Fungitoxicity of Captafol and Metalaxyl to *Phytophthora capsici*

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ABSTRACT

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In vitro fungitoxicity of the nonsystemic fungicide *cis-N*-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide (captafol) and the systemic fungicide *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester (CGA-48988, metalaxyl) to five isolates of *Phytophthora capsici* was studied in lima bean agar and broth. Captafol was more effective than metalaxyl in reducing growth in solid and liquid media, inhibiting zoospore release from sporangia, stopping zoospore motility, and inhibiting germination of sporangia and zoospores. Metalaxyl was more effective than captafol in inhibiting production of sporangia and oospores and equally as effective as captafol at low concentrations (less than 2.5 μg active ingredient [a.i.] per milliliter of medium) in inhibiting oospore germination. At fungicide concentrations from 2.5 to 10 $\mu\text{g}/\text{ml}$, metalaxyl

inhibited oospore germination more than captafol. Approximately 40% of oospores of *P. capsici* germinated in distilled water after 16 days of incubation. Water extracts from tops (stems and leaves) and roots of pepper plants that had been drenched with metalaxyl 7, 11, 17, and 24 days before the assay completely inhibited germination of sporangia and reduced growth in liquid medium. Similar extracts from captafol-drenched plants were much less inhibitory. Water extracts from tops of pepper plants that had been drenched with metalaxyl 1, 7, and 21 days before extract preparation reduced or eliminated oospore production. Extracts from captafol-drenched plants did not. A single soil drench containing 3.0 mg a.i. of metalaxyl applied to two-leaf plants was sufficient to effectively protect pepper plants against foliar infection for at least 7 days after drenching.

Additional key words: *Capsicum annuum*, pepper blight, crown rot, root rot.

Phytophthora blight, root rot, and crown rot caused in pepper (*Capsicum annuum* L.) plants by *Phytophthora capsici* Leonian, has become a great threat to pepper production in New Jersey (6), California (8), and many other states. In addition to causing a serious disease on pepper, isolates of *P. capsici* have been reported to attack eggplant, cucumber, honeydew melon, pumpkin, squash,

tomato, and watermelon (Polach and Webster 9). A new papaya fruit rot was also found to be caused by a *Phytophthora* sp. closely resembling *P. capsici* (1). In southern New Jersey, losses to peppers in 1978 because of *Phytophthora* blight averaged about 20% with as high as 100% in some locations (S. A. Johnston, unpublished). In a few very severe cases the growers plowed under the pepper crop before harvest.

Field trials in New Jersey (6) and at Beltsville (J. C. Locke and G. C. Papavizas, unpublished) showed that the systemic fungicide

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N-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester (CGA-48988, metalaxyl, Ridomil® 50W, CIBA-Geigy Corp., Greensboro, NC 27409) and the nonsystemic fungicide *cis-N*-[(1, 1, 2, 2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide (captafol, Difolatan®, Chevron Chemical Co., Richmond, CA 94804) suppressed the crown rot phase and the pepper blight phase, respectively. Metalaxyl also was effective against other species of *Phytophthora* (2,5,7,13).

This investigation presents data on the mechanisms by which captafol and metalaxyl inhibit various phases in the life cycle of *P. capsici*.

MATERIALS AND METHODS

Fungal isolates. Five isolates of *P. capsici* were used in this study. Isolates ATCC 15399 and ATCC 15427, compatibility types A² and A¹, respectively, were obtained from the American Type Culture Collection. Isolate S1 (type A¹) was obtained from infected

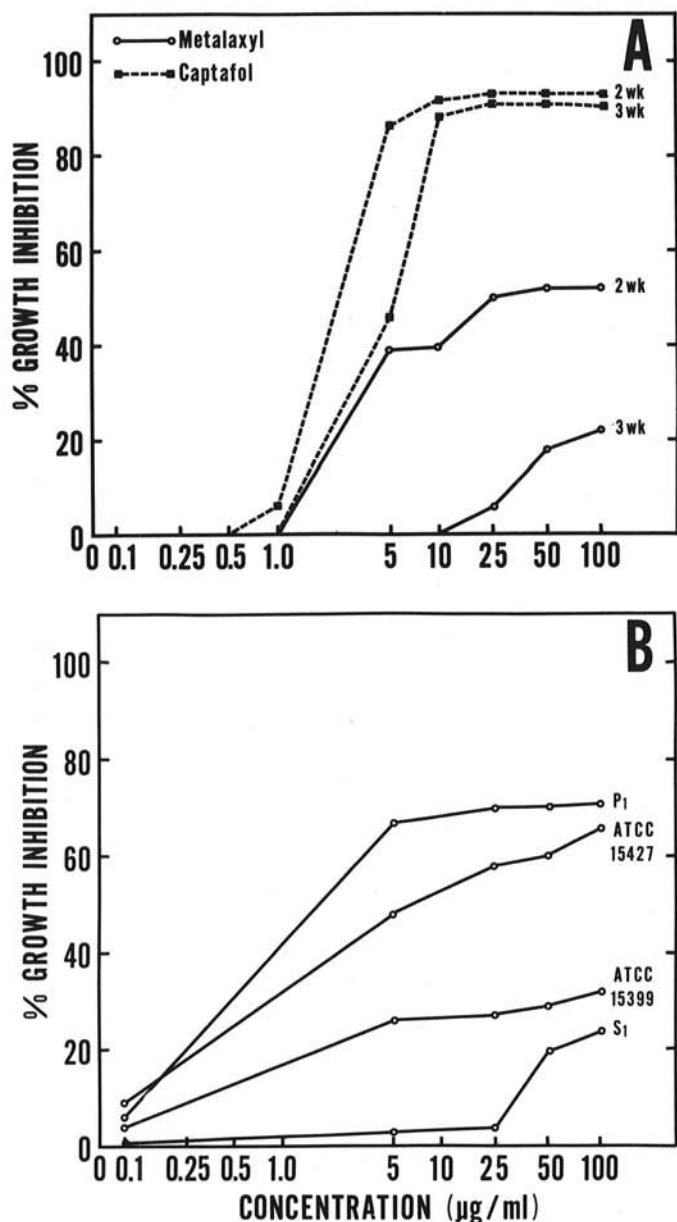


Fig. 1. Rate-response curves of *Phytophthora capsici* in lima bean broth (LBB) amended with captafol or metalaxyl. A, Effect of captafol and metalaxyl on growth of *P. capsici* (isolate ATCC 15399) as measured by dry weight of mycelium in LBB after 2 and 3 wk of incubation. B, Effect of captafol on four isolates of *P. capsici* as measured by dry weight of mycelium in LBB after 3 wk of incubation.

squash in New Jersey in 1978. Isolate P1 (type A²) was obtained from the Rutgers Research and Development Center, Bridgeton, NJ. Isolate S1 × P1 (type A¹) was derived from a cross made between S1 and P1 at the Soilborne Diseases Laboratory, Beltsville, MD. All isolates were maintained on Difco lima bean agar (LBA) and transferred monthly.

Effect of fungicides on growth and sporangial and oospore production. In vitro toxicity of captafol and metalaxyl toward the growth of *P. capsici* was studied in liquid (40 ml/250-ml Erlenmeyer flask) lima bean extract broth (LBB). The liquid medium was prepared by boiling 200 g of lima bean seeds in distilled water for 30 min, straining through a double layer of cheesecloth with a thin cotton pad, and adding glucose (1 g/L) and sitosterol (2 µg/ml) and adding distilled water to make 1 L of LBB. The pH of the medium after autoclaving was 6.2. Captafol (commercial formulation 4F) and metalaxyl (50W) were diluted in sterile distilled water and added in appropriate amounts (w/v) to LBB after it was autoclaved and before it was dispensed to flasks. The two fungicides were tested in LBB at 0.0 (control), 0.1, 0.25, 1.0, 5, 10, 25, 50, and 100 µg active ingredient (a.i.) per milliliter of LBB. Disks (5 mm in diameter) from 6-day-old *P. capsici* colonies (isolate ATCC 15399) grown on fungicide-free LBA were transferred to flasks, and dry weights of mycelial mats of flask cultures were determined at 1, 2, and 3 wk. Each treatment was replicated four times and the experiments were done twice. Toxicity was expressed as percent inhibition of growth relative to the fungicide-free control.

Toxicity of the two fungicides toward sporangial formation was determined by growing isolates S1, ATCC 15399, and S1 × P1 on LBA for 12 days at 25 C. The two fungicides were tested in the solid medium at concentrations up to 25 µg/ml of LBA. Sporangia were counted in single focal planes of 10 microscopic fields (2 mm in diameter) in each of four radial rows per petri dish. Each reading obtained represented the average of three replicate dishes.

Toxicity of captafol and metalaxyl toward oospore formation was determined on clear V-8 juice medium (100 ml V-8 juice, 900 ml distilled water, 3 g CaCO₃, centrifuged). The two fungicides were tested in the solid V-8 juice medium at concentrations up to 25 µg/ml of V-8 juice agar. Oospores were produced on the fungicide-free and fungicide-amended medium by pairing isolate S1 with ATCC 15399 and isolate S1 × P1 with ATCC 15399 on the agar (inoculum plugs placed 2 cm apart) and allowing them to grow in the dark at 25 C for 2 wk. Oospores were counted in single focal planes of eight microscope fields (2 mm in diameter) in each of two plates.

Effect of fungicides on zoospore release. To determine the effect of captafol and metalaxyl on zoospore release from sporangia of *P. capsici*, we obtained sporangia from 8-day-old LBA cultures of isolates S1 and ATCC 15399 by adding 5 ml of sterile distilled water per petri dish and shaking the cultures vigorously by hand to dislodge the sporangia. The sporangia were freed from mycelia by passing the suspension through sterile Nitex® (Tetko, Inc., Elmsford, NY 10523) nylon monofilament screen fabric with 100-µm mesh opening. We mixed five milliliters of sporangial suspensions in distilled water (3,000 sporangia per milliliter) with 5 ml fungicide suspensions that were calculated to give the following concentrations in the mixtures: 0.0, 0.1, 0.25, 0.5, 1.0, 2.5, 5, 10, and 25 (micrograms per milliliter). The mixtures were incubated at 20 C and examined microscopically after 3, 4, and 7 hr to determine the percentage of sporangia that released zoospores in the presence of the fungicides. One hundred sporangia were observed in each of five replications and toxicity was expressed as percent inhibition of zoospore release. Motility of zoospores was also determined microscopically 2 hr after release.

Effect of fungicides on propagule germination. We studied comparative toxicity of the two fungicides on spore germination of *P. capsici* in liquid drops in 60-mm-diameter petri dishes. For the zoospore germination tests, sporangial suspensions of isolates S1 and ATCC 15399 in sterile distilled water were incubated at 20 C for sufficient time (usually 30–40 min) to allow the majority of the sporangia to release their zoospores. Zoospores were separated from the empty sporangia by passing the liquid through a sterile

nylon monofilament screen fabric with 20- μ m mesh opening. Zoospore suspensions were mixed (1:1, v/v) with sterile fungicide suspensions calibrated to give 0.0, 0.1, 0.25, 0.5, 1.0, 2.5, 5, 10, 25, 50, and 100 μ g a.i./ml of the zoospore-fungicide mixtures. Aliquots (0.2 ml) were pipetted (six per petri dish) and the dishes were incubated at 25 C. After 2, 4, and 6 hr we treated drops containing zoospores with lacto-fuchsin and examined them microscopically.

We tested the toxicity of captafol and metalaxyl toward direct sporangial germination in a manner similar to the method for zoospores except that we used Czapek Dox broth containing 2% yeast extract to prepare the sporangial suspension. In preliminary tests, all the sporangia suspended in this medium germinated directly by germ tubes rather than by release of zoospores. We produced oospores of *P. capsici* by pairing isolates in small batches (30 ml/200-ml Erlenmeyer flask) of clear V-8 juice medium as described before but allowing them to grow in the dark at 25 C for 4 wk. Oospore suspensions were prepared from freshly harvested mycelial mats. The mats were washed with sterile distilled water and then comminuted in a Tekmar Tissumizer for 30 sec. Suspensions were passed through two layers of cheesecloth and one layer of lens paper to remove most mycelial fragments. The resulting oospore suspension was further purified by passing it through the same nylon fabric used to separate the sporangia. Oospores were counted in a hemacytometer, and the suspensions were adjusted to 3,000 oospores per milliliter. The effect of captafol and metalaxyl on oospore germination was studied in a manner similar to that used for zoospores. The petri dishes that contained drops of oospore-fungicide mixtures at desired concentrations were placed in plastic sterilizing pans (13.3 \times 42.5 \times 55.2 cm) and these were covered with clear wrap to prevent drying. The pans were placed under fluorescent light for 7 days and then were transferred to complete darkness for 8 more days. The drops were treated with lacto-fuchsin and examined microscopically on the 16th day.

Our germinability readings of zoospores, sporangia, and oospores as they were affected by captafol and metalaxyl were based on 100 propagules per replication, five replications per treatment. The data were expressed as percent inhibition of germination and plotted on semilogarithmic paper.

Effects of fungicides in extracts of pepper plants on growth, oospore formation, and sporangial germination. Six pepper plants (cultivar California Wonder) were grown in soil in small flats (12.7 \times 17.8 cm). When the plants were 10 cm tall, the flats were drenched either with 250 ml of water (control) or 250 ml of water containing captafol or metalaxyl. The two fungicides were added at 18 mg a.i.

per flat or about 3.0 mg per plant. The pepper plants were removed from the flats at various intervals indicated in each particular experiment, the roots were washed thoroughly, and the plants were divided into roots and tops (leaves and stems, cut 3 cm above the soil surface). Roots and tops were extracted separately with water (1:1, w/v) by blending the mixture for 1 min, filtering it through cheesecloth, and centrifuging it at 13,200 g for 30 min. All extracts were adjusted with distilled water so that 1 ml of extract corresponded to 1 g of tissue. The extracts were sterilized at 121 C for 20 min.

Root and top extracts from the three treatments (replicated four times) were mixed (1:1, v/v) with suspensions of sporangia of isolate S1 in distilled water (3,000 sporangia per milliliter) and germinability of sporangia was studied in the extracts as before. For growth inhibition studies, root or top extracts were mixed with LBB (15 ml extract: 85 ml LBB) and the mixtures were dispensed in 200-ml Erlenmeyer flasks (30 ml per flask).

For oospore production, top extracts were prepared from treated and nontreated pepper plants 1, 7, and 21 days after soil drenching. The extracts were mixed with the clear V8 juice medium (1:1). The appropriate amount of agar was added to the mixtures and these were autoclaved at 1.1 kg/cm² (16 psi) for 25 min. Oospore production on the sterile extract-V8 juice agar was studied as described before.

RESULTS

Effect of captafol and metalaxyl on growth and sporangial and oospore production. At fungicide concentrations up to 100 μ g a.i./ml of LBB, captafol was more toxic to mycelia of *P. capsici* (isolate ATCC 15399) than was metalaxyl. The ED₅₀ values for captafol were between 1.0 and 5.0 μ g/ml at 2 and 3 wk (Fig. 1A). Results of the 1-wk assay are not shown in Fig. 1A. Although the ED₅₀ values for metalaxyl were similar to those for captafol obtained at the 1-wk assay, after 2 wk of growth the ED₅₀ value was 25 μ g/ml. No ED₅₀ value could be obtained at the 3-wk assay. Even at 100 μ g/ml of LBB metalaxyl caused only ~22% growth inhibition. In addition to *P. capsici*, in one test we also included *P. megasperma* Drechs. var. *sojae* (A. A. Hildeb.) (race 3) in order to compare the sensitivity of the two species to metalaxyl. We found that *P. megasperma* var. *sojae* was much more sensitive to

TABLE 1. Effect of increasing concentrations of captafol and metalaxyl on production of oospores by two crosses of compatible isolates of *Phytophthora capsici* on clear V-8 juice agar

Fungicide concentration (μ g/ml)	No. of oospores per microscopic field ^y	
	S1 \times ATCC 15399	(S1 \times P1) \times ATCC 15399
Captafol		
0	74 a ^z	34 de
0.25	78 a	22 ef
0.5	65 ab	43 cd
2.5	58 b	34 d
5	41 d	63 b
10	55 bc	59 b
25	58 b	53 bc
Metalaxyl		
0	61 b	33 de
0.25	57 b	64 b
0.5	43 cd	77 a
2.5	5 e	13 f
5	13 e	0 g
10	0 e	0 g
25	0 e	0 g

^yOospores counted after 2 wk of incubation.

^zIn each column, values followed by the same letter do not differ significantly according to Duncan's multiple range test, $P = 0.05$.

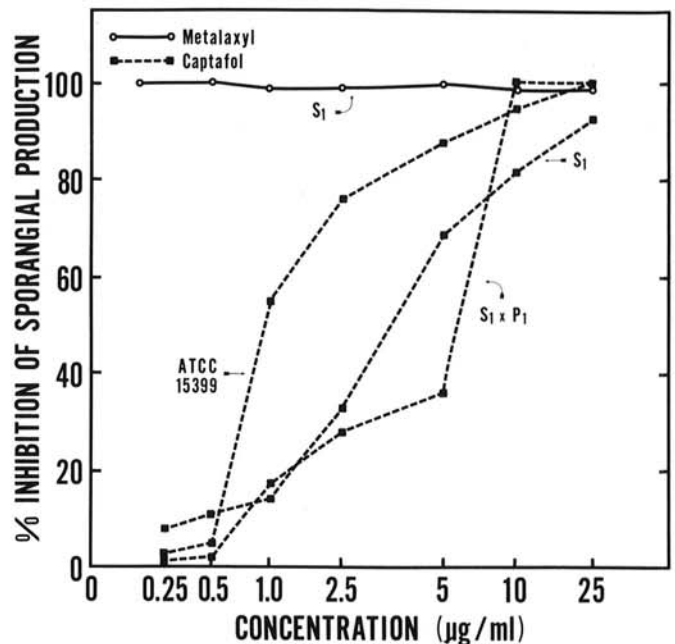


Fig. 2. Effect of increasing concentrations of captafol and metalaxyl on production of sporangia by isolates of *Phytophthora capsici* on lima bean agar (effect of metalaxyl on isolates ATCC 15399 and S1 and P1 was similar to that on isolate S1).

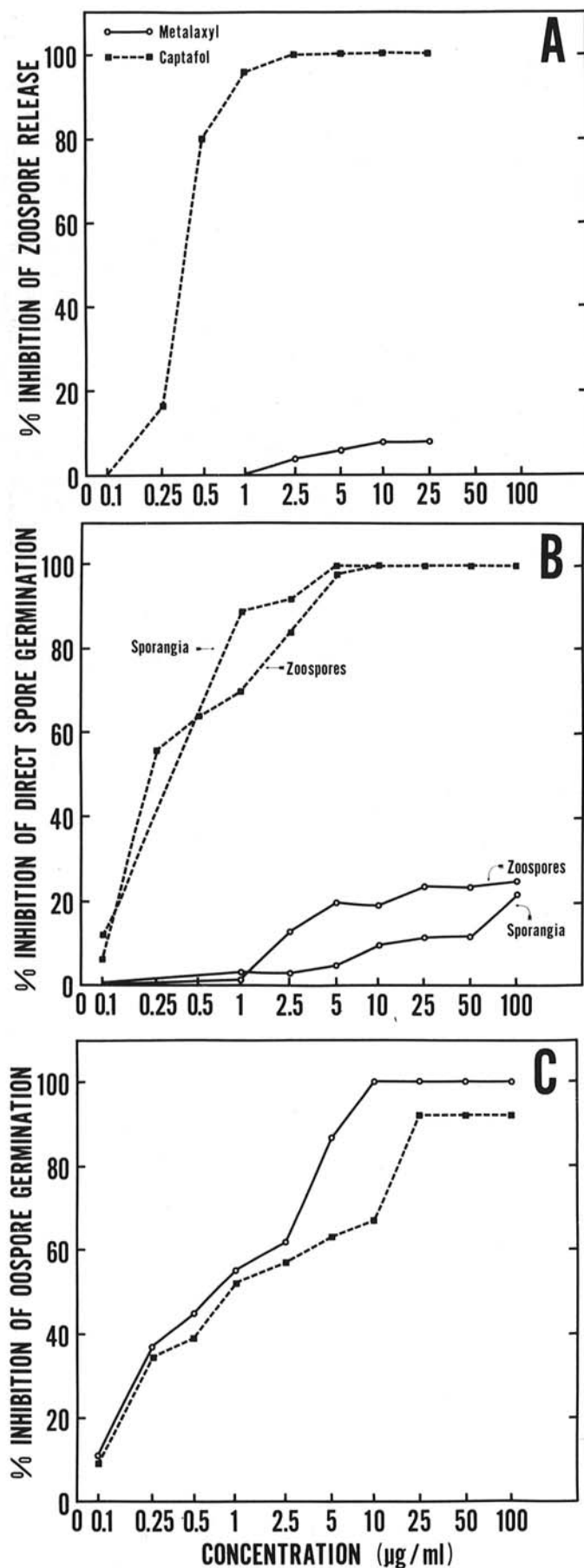


Fig. 3. Effect of increasing concentrations of captafol and metalaxyl on zoospore release and propagule germination in *Phytophthora capsici* (isolate ATCC 15399). A, Zoospore release. B, Germination of zoospores and direct germination of sporangia. C, Oospore germination.

metalaxyl than was *P. capsici*. An ED_{50} value with *P. megasperma* var. *sojae* was obtained at metalaxyl concentrations of less than $0.25 \mu\text{g/ml}$ of LBB at the 3-wk assay.

In a separate experiment we studied the toxicity of metalaxyl in LBB to four isolates of *P. capsici*. Isolate S1 was the least sensitive and isolates P1 and ATCC 15427 were the most sensitive to the fungicide (Fig. 1B). With these two isolates we obtained ED_{50} values at about $1\text{--}5 \mu\text{g/ml}$ after 3 wk of growth. No ED_{50} values were obtained with S1 and ATCC 15399 even at $100 \mu\text{g/ml}$.

The toxicity of captafol and metalaxyl to sporangium production by *P. capsici* on LBA was the reverse of that to growth, and differences in inhibition were more striking (Fig. 2). Metalaxyl completely inhibited sporangial production of isolates S1, ATCC 15399, and S1 \times P1 even at $0.25 \mu\text{g/ml}$ (only data for S1 are shown in Fig. 2). In contrast, ED_{50} values for inhibition of sporangial production by captafol were $0.5\text{--}1.0$, $2.5\text{--}5$, and $5\text{--}10 \mu\text{g/ml}$ for isolates ATCC 15399, S1, and S1 \times P1, respectively. Captafol prevented sporangial formation in each isolate; however, it prevented sporangial formation completely only at concentrations higher than $10 \mu\text{g/ml}$.

The pattern of toxicity of the two fungicides toward oospore production resembled that toward sporangial formation. Metalaxyl completely inhibited oospore formation by two crosses of compatible isolates at 10 and $25 \mu\text{g/ml}$ and reduced oospore numbers at $2.5 \mu\text{g/ml}$ (Table 1). Captafol had no effect on oospore formation even at $25 \mu\text{g/ml}$.

Effect of the fungicides on zoospore release and motility. Almost 20 and 80% of sporangia of *P. capsici* never released zoospores at captafol concentrations of 0.25 and $0.5 \mu\text{g/ml}$, respectively (Fig. 3A). Captafol completely prevented zoospore release at concentrations of $1 \mu\text{g/ml}$ or more. In contrast, metalaxyl prevented less than 10% of sporangia from releasing zoospores even at $25 \mu\text{g/ml}$.

The two fungicides also differed in ability to immobilize zoospores. Metalaxyl did not stop motility of zoospores of isolates S1 and ATCC 15399 even when these were exposed to $100 \mu\text{g/ml}$ of the fungicide for 2 hr. Captafol prevented motility at $0.5 \mu\text{g/ml}$. At this concentration, zoospores immediately were immobilized and lysed within 2–3 hr.

Effect of captafol and metalaxyl on propagule germination. Captafol prevented 50% of zoospores and sporangia of isolates S1 and ATCC 15399 from germinating at about $0.25 \mu\text{g/ml}$, and completely prevented germination at $5 \mu\text{g/ml}$ (Fig. 3B). In contrast, metalaxyl prevented only about 20% of zoospores and sporangia from germinating even at $100 \mu\text{g/ml}$. The pattern of germination inhibition by either fungicide was similar on both kinds of propagules.

At concentrations up to $2.5 \mu\text{g a.i./ml}$ the two fungicides were equally toxic to oospore germination (Fig. 3C). The ED_{50} values for both fungicides were $1.0 \mu\text{g a.i./ml}$ or less. At concentrations from 2.5 to $10 \mu\text{g/ml}$, metalaxyl was more toxic to oospore germination than was captafol, a reversal of the effect on zoospore and sporangium germination. Maximum inhibition of oospore germination occurred with $10 \mu\text{g}$ metalaxyl per milliliter and with $25 \mu\text{g}$ captafol per milliliter, but captafol did not prevent germination completely even at $100 \mu\text{g/ml}$.

Although all sporangia germinated by releasing zoospores in distilled water-fungicide suspensions, 100% of sporangia of both isolates germinated directly by producing germ tubes in the Czapek Dox medium containing 2% yeast extract (Fig. 4A). The germ tubes grew rapidly and developed into submerged colonies without producing new sporangia.

With the technique we used, about 40% of the oospores from the cross S1 \times ATCC 15399 germinated in fungicide-free distilled water by producing sporangia borne on pedicels coming out of the oospores (Fig. 4B). The pedicels varied in length; some were no longer than three times the length of the sporangia, whereas others were as long as $100 \mu\text{m}$, and bore single elongated sporangia.

Effect of captafol and metalaxyl in extracts from pepper plants on growth, oospore formation, and sporangial germination. Pepper plants drenched with 3.0 mg metalaxyl or captafol per plant were harvested 7, 11, 17, and 24 days after treatment and water

extracts of the plant tops and roots were used to study the germinability of sporangia of *P. capsici*. Earlier we found that in pepper plant tissue extracts sporangia of isolates S1 and ATCC 15399 germinated directly (Fig. 4A). Top extracts from metalaxyl-treated plants harvested 7, 11, 17, and 24 days after drenching almost completely inhibited sporangial germination of the two isolates (Fig. 5). Very little inhibition occurred with top extracts from captafol-drenched plants. We obtained identical results with root extracts. The inhibitory effect of extracts from metalaxyl-drenched plants, however, was temporary. After 4 hr of incubation in these extracts there was only an average 10–20% inhibition of sporangial germination instead of the near 100% inhibition at 2 hr.

We also studied growth inhibition by top and root extracts collected 7 days after drenching in the previous experiment. Top extracts from metalaxyl-drenched plants reduced growth in LBB by 57% and root extracts by 23%. Extracts from captafol-drenched plants caused no reduction in growth. In another experiment, metalaxyl-drenched and nondrenched pepper plants were harvested 3 and 7 days after treatment, and top extracts were tested against growth in LBB and compared with known amounts of metalaxyl added directly to LBB. Top extracts from the 3- and 7-day harvest inhibited growth 75 and 89%, respectively. Metalaxyl added to LBB at 1.0 and 5.0 $\mu\text{g/ml}$ of medium inhibited growth 43 and 63%, respectively.

Top extracts prepared from metalaxyl-drenched plants prepared 1, 7, and 21 days after drenching and mixed with a clear V-8 juice agar almost completely prevented oospore formation of S1 \times ATCC 15399 and (S1 \times P1) \times ATCC 15399 (Table 2). Extracts from captafol-drenched plants caused no reduction in numbers of oospores produced. Microscopic observations of mycelia of these isolates on the extract-V-8 juice agar showed many malformations even with the 21-day extracts (Fig. 6).

DISCUSSION

In the present study, we observed several differences and a few similarities between the nonsystemic fungicide captafol and the new systemic fungicide metalaxyl. Both fungicides had similar patterns of toxicity toward growth of *P. capsici* in its early stages (1-wk assay). At 2 and 3 wk, however, the toxicity values for captafol were two to four times higher than those for metalaxyl. The magnitude of differences between the two fungicides appeared to be due not only to the concentration used and the time of incubation, but also to the isolates of *P. capsici* tested. Inhibition of oospore germination also was similar, especially at low fungicide

TABLE 2. Effect of water extracts from pepper plants (leaf and stem tissue) grown in soil drenched with captafol or metalaxyl and assayed 1, 7, and 21 days after treatment on production of oospores by two crosses of compatible isolates of *Phytophthora capsici* on clear V-8 juice agar fortified with the extracts

Time from soil treatment to assays (days)	Soil treatment ^x	Oospores per microscope field ^y	
		S1 \times ATCC 15399	(S1 \times P1) \times ATCC 15399
1 Day	None (control)	61 a ^z	56 a
	Captafol	63 a	59 a
	Metalaxyl	4 b	0 b
7 Days	None (control)	58 a	54 a
	Captafol	60 a	62 a
	Metalaxyl	0 b	2 b
21 Days	None (control)	60 a	52 a
	Captafol	66 a	62 a
	Metalaxyl	5 b	6 b

^xThree milligrams of active ingredient per plant.

^yOospores counted after 2 wk of incubation.

^zIn each column, values followed by the same letter do not differ significantly according to Duncan's multiple range test, $P = 0.05$.

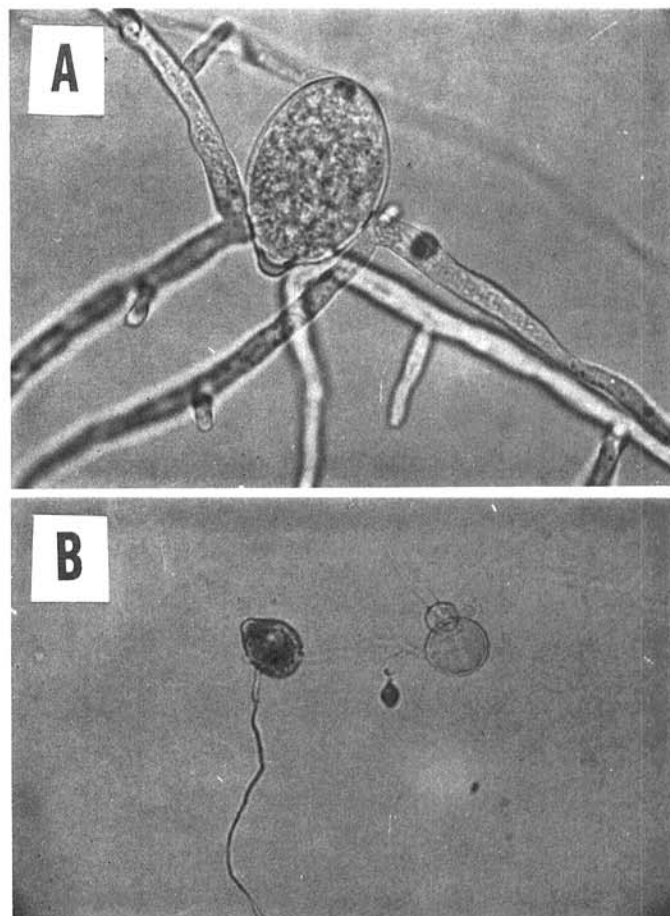


Fig. 4. A, Direct germination of a sporangium of *Phytophthora capsici* (isolate S1) in Czapek Dox broth containing 2% yeast extract. B, Indirect germination of an oospore of *P. capsici* after being incubated in distilled water 7 days under fluorescent light and 8 days in the dark.

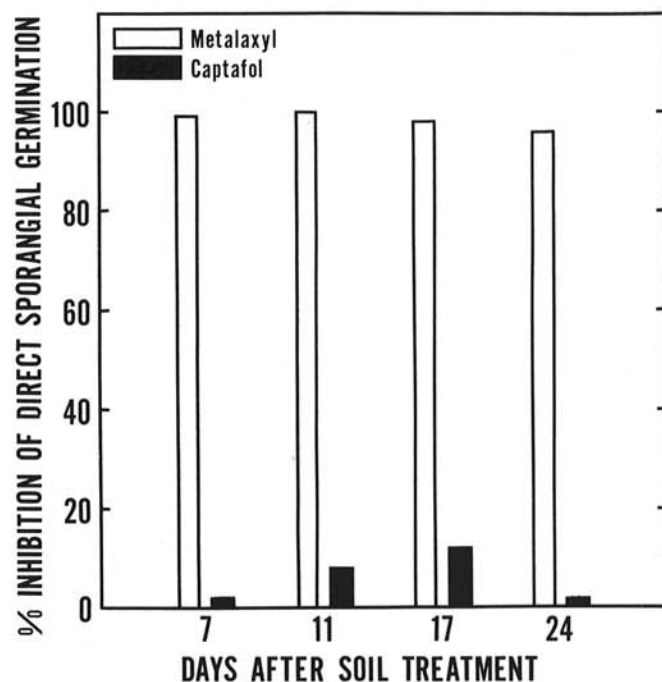


Fig. 5. Effect of water extracts from pepper plants (leaf and stem tissue) grown in soil drenched with captafol or metalaxyl (3 mg active ingredient per plant) and assayed 7, 11, 17, and 24 days after treatment on direct germination of sporangia of *Phytophthora capsici* (isolate S1) after incubation for 2 hr.



Fig. 6. Malformed mycelium of *Phytophthora capsici* (ATCC 15399) grown on clear V-8 juice agar containing extract from tops (leaves and stems) of peppers grown in soil that had been drenched with metalaxyl 7 days before extract preparation.

concentrations (less than $2.5 \mu\text{g a.i./ml}$).

The two fungicides differed in every other aspect described in this paper. Captafol was more toxic than metalaxyl to those phases in the life cycle of *P. capsici* that are normally completed in a relatively short period of time (zoospore release from sporangia, zoospore motility, and direct germination of sporangia and zoospores). These stages in the life cycle are essential for successful foliar infections. Metalaxyl was more inhibitory than captafol to oospore and sporangial production in vitro, and plant extracts containing metalaxyl were more inhibitory to growth, sporangial germination, and oospore production than those containing captafol. These findings may explain why captafol is effective against the foliar blight of pepper, which is presumably initiated by germinating zoospores and sporangia disseminated onto the plants by rain, irrigation water, and wind. On the other hand, if the crown rot and root rot phases are initiated by surviving oospores (whose germinability is strongly inhibited by metalaxyl), a likely but as yet unproven hypothesis, it is easy to understand why metalaxyl is so effective against these two phases of the pathogen.

Our research with extracts of tops (stems and leaves cut 3 cm above the soil surface) from pepper plants prepared several days after drenching the soil suggested that metalaxyl, but not captafol, is translocated upward and remains effective against *P. capsici* within the plant for at least 24 days under greenhouse conditions. Metalaxyl is also known to be translocated acropetally in tomato plants (3,11) and basipetally in avocado plants (13). It also is of interest that greater inhibition of growth of *P. capsici* was observed in lima bean broth fortified with top extracts of pepper plants that had previously received 3 mg of metalaxyl by drench than in LBB containing known amounts (1.0 and $5.0 \mu\text{g/ml}$) of the fungicide. We believe that the differences are real, but we have no explanation at this time. Neither do we know whether the toxicity of the extracts was due to the entire molecule of metalaxyl in pepper plants or to

breakdown products of the fungicide. Further studies are needed of the in vivo mechanism of action of metalaxyl.

In preliminary field tests (6), soil application of metalaxyl at transplanting (May or June) protected pepper plants from crown and root rot, presumably initiated by surviving oospores of *P. capsici* in soil, but not from foliar blight that is initiated by sporangia and zoospores and appears in August or September. This may be because metalaxyl is more fungitoxic to oospores than to sporangia which are responsible for the foliar blight. A more logical explanation is that the blight prevention effectiveness of the fungicide decreases with time. Perhaps it is unrealistic to expect a fungicide to suppress a disease pathogen on the foliage almost 3 mo after its application to soil.

In studies with distilled water we obtained 40% germination of oospores that were produced as a result of mating squash isolate S1 (compatibility type A¹) with pepper isolate ATCC 15399 (type A²). All germinating oospores produced one or more sporangia after germination. The highest percentages of oospore germination reported previously in *P. capsici* was about 20% (10,12). Divinagracia (4) was unable to obtain germination of *P. capsici* oospores; Polach and Webster (9) obtained 8% germination. The high percentage of oospore germination in our tests may be due to the particular mating combination used, to the method of germination employed, or to both.

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