Control of Phytophthora Root Rot of Processing Tomato with Ethazol and Metalaxyl

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ABSTRACT

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Metalaxyl at low concentrations in vitro inhibited mycelial growth and sporangium formation of *Phytophthora parasitica*, the main causal agent of root and crown rot of tomato in California. Germination of chlamydospores and zoospores was relatively unaffected by metalaxyl, but germ tube growth of both was inhibited. Ethazol was more toxic than metalaxyl to mycelial growth and sporangium formation and also inhibited zoospore and chlamydospore germination. Both fungicides at high concentrations reduced duration of zoospore motility, but neither affected zoospore formation and release. In the greenhouse, a single preplant soil treatment with metalaxyl 5G or 50WP at 2–10 μg a.i./ml soil protected tomato from Phytophthora root rot for 7 wk. Higher rates (50–250 μg a.i./ml soil) were phytotoxic. At these rates, metalaxyl was active systemically in leaves but not in stems. Tomato plants grown for 1 mo in soil treated with metalaxyl (100 μg a.i./ml soil), then transplanted in infested, untreated soil, were partially protected against root rot for only 1 wk, whereas lower concentrations were ineffective. Seed treatments with metalaxyl 50WP (7.5–30 g a.i./kg seed) were as effective against root rot as soil treatments, without being phytotoxic. Ethazol was 50–100 times less effective than metalaxyl when applied as soil or seed treatments. Both chemicals, at rates effective for disease control, acted as fungistats rather than fungicides. In the field, preplant soil treatments (30-cm bands) with metalaxyl 5G (0.3–1.5 kg a.i./ha) or ethazol (1.5–15 kg a.i./ha) and seed treatments with metalaxyl 50WP (7.5–30 g a.i./kg seed) provided partial control of Phytophthora root rot during earlier stages of growth, and total yield was increased. Yield increases generally were proportional to the level of apparent disease control. Soil treatments with metalaxyl 5G (0.75–1.5 kg a.i./ha) were the most effective for level and duration of protection and yield increase.

Several species of *Phytophthora* attack tomato (*Lycopersicon esculentum* Mill.), causing preemergence and postemergence damping-off of seedlings, root and crown rot, stem canker, leaf blight, and fruit rot (3,7,8,12,13,19,20). In California, a crown rot of tomato caused by *P. capsici* Leonian was reported in 1955 (3). This disease caused considerable losses in tomato fields in the San Joaquin and Sacramento valleys during 1955–1965 (13). Both *P. capsici* and *P. parasitica* Dast. were isolated from naturally infected plants (13). In recent years, the disease has occurred sporadically in the major processing tomato production areas of the state, causing nearly complete destruction of the crop in some fields. Infection may occur at any time during the growing season. Early infections cause seedling damping-off, whereas later infections cause root and crown rot. Infected plants cease to grow and the foliage gradually wilts and dies. Fruits on plants with severely damaged foliage are smaller, fewer, and more prone to sunburn. Infection of fruit touching the ground (buckeye rot) occurs commonly but is not a major cause of economic loss.

Numerous isolations from infected plants in the field and pathogenicity tests in the greenhouse proved *P. parasitica* responsible for more than 85% of the root rot on processing tomatoes in the state. Furthermore, all isolates studied (more than 100) belonged to a single mating type (A2). *P. capsici*, also reported to be associated with the disease (3,13), was isolated infrequently (N. Ioannou and R. G. Grogan, *unpublished*).

Sonoda et al (14) reported partial disease control in transplanted, fresh-market tomatoes with preplant root-dip treatments of protectant fungicides. Such treatments, however, cannot be applied to processing tomatoes that are direct-seeded in California.

Metalaxyl (CGA-48988, Ridomil) and ethazol (Terrazole) are effective for control of diseases caused by *Phytophthora* and other oomycetes (1,2,4,6,9, 11,16,21). Our work was done to compare activity of these two fungicides against Phytophthora root rot of tomato and to investigate their in vitro effects on the different life stages of *P. parasitica*.

MATERIALS AND METHODS

Laboratory experiments. A tomato isolate of *P. parasitica* (DM30-2) was used in all laboratory tests. Ethazol 35WP and metalaxyl 50WP were used at concentrations from 0.5 to 500 μg a.i./ml to determine effects on mycelial growth, chlamydospore germination, production of sporangia from mycelia and germinating chlamydospores, indirect sporangial germination (zoospore formation and release), zoospore motility, and germination of encysted zoospores. Unless otherwise stated, each experiment was repeated at least once and each treatment was replicated five times.

To determine the effect of the fungicide treatments on radial mycelial growth, 5-mm-diameter inoculum plugs from the margin of a 5-day-old colony grown on cornmeal agar (CMA) were placed in the centers of 9-cm-diameter petri plates containing CMA, and different concentrations of the two fungicides were added to the medium after autoclaving. Inoculated plates were placed in loosely closed plastic boxes to reduce evaporation and incubated at 24 C in the dark. Colony radii were measured at 3, 6, 10, and 15 days; occasionally, incubation was extended to 20 days or longer.

Mycelial growth inhibition by fumigant action of the two fungicides was investigated by placing in the same box 10 inoculated CMA plates containing no fungicide and 10 uninoculated CMA plates containing 10 μg/ml of either ethazol or metalaxyl. The control box contained 10 inoculated and 10 uninoculated CMA plates without fungicide. All plates were incubated at 24 C in the dark, and after 7 days, colony radii were measured.

Chlamydospore suspensions in sterile distilled water (SDW) containing about 2.5×10^4 spores per milliliter were prepared as described by Tsao (17,18) and Mircetich et al (10). Viability of chlamydospores was determined by staining with rose bengal (10). Portions (0.2 ml) were transferred to CMA plates (60-mm-diameter) containing the two fungicides, and after 20 hr at 24 C in the dark, chlamydospore germination was estimated by direct microscopic examination of 100 viable spores on each replicate plate. A spore was considered germinated if a microscopically visible germ tube had developed. Duplicate plates were examined after 60 hr of incubation.

Effects of the two fungicides on chlamydospore sporulation (sporangium production) were studied in an autoclaved soil extract (SE) prepared as follows: 100 g (air-dry equivalent) of moist soil collected from a tomato field were added to 1 L of water, stirred for 1 hr with a magnetic stirrer, and centrifuged at 5,000 rpm for 10 min; the supernatant was

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filtered through a Whatman No. 2 filter paper and autoclaved. Chlamydospores were suspended in the autoclaved SE and 0.2-ml portions were pipetted into 0.5-ml microbeakers containing 0.2 ml of SDW (control) or fungicide solution at twice the desired concentrations. The microbeakers comprising each treatment were placed in a petri plate containing a double layer of moist filter paper to decrease evaporation and incubated at 24 C in the dark. Germination was assessed after 24 and 72 hr by placing a drop of the suspension on a microscope slide under a cover glass supported by small pieces of glass to give a sample of constant depth. One hundred spores from each of five replicate microbeakers were observed at each reading.

To determine the effects of the fungicides on sporangium production, mycelial mats were produced in V-8 juice broth (V8JB) (10,18), washed three times for 10 min in SDW, blotted dry, and placed in 60-mm-diameter petri plates. Each plate contained 3 ml of autoclaved SE with the desired fungicide concentration. Plates were incubated at room temperature (20–25 C). After 1, 2, and 3 days, the mats were examined at ×10 for abundance of sporangia and rated on a scale of 0 (no sporangia detected) to 5 (abundant sporulation). Observations also were made of indirect germination of sporangia (zoospore formation and release). After examination on day 3, each mycelial mat was blended in 10 ml water for 10 min (Waring Blendor with a microcontainer), a drop of the resulting suspension was placed on a microscope slide under a supported cover glass, and the numbers of sporangia per 50 microscope fields (×10) were determined.

Effects of the fungicides on zoospore motility were determined by using a microtitration plate with 96 wells, each with 0.1 ml of SDW or fungicide solution at twice the desired concentration. Zoospores released from sporangium-bearing mycelial mats (chilled at 15 C for 1 hr) were adjusted to about 10^4 spores per milliliter and 0.1-ml portions were transferred to each well of the microtitration plates. The time for 100% immobilization was determined by microscopic examination.

To study the effect of the fungicides on germination, zoospores suspended in SDW were induced to encyst by shaking on a vortex mixer, and 0.2-ml portions were pipetted onto CMA plates (60 cm diameter) containing the fungicides. The plates were incubated at 24 C in the dark, and after 12 hr, the percentages of germination were assessed by microscopic examination of 100 spores per replicate plate.

Greenhouse experiments. Activity of the two fungicides against Phytophthora root rot was studied in the greenhouse using artificially infested U.C. soil mix (1:1 mixture of fine sand and peat), naturally infested Yolo fine sandy loam field soil, and field soil amended 1:1 with artificially infested U.C. soil mix. Inoculum for soil infestation was prepared by culturing *P. parasitica* (isolate DM 30-2) on a vermiculite-V8JB substrate in 1-L mason jars. The substrate, consisting of 500 ml of vermiculite plus 450 ml V8JB (10,18) per jar, was autoclaved for 30 min at 121 C for two consecutive days and inoculated with six 5-mm-diameter inoculum plugs from the margin of a 5-day-old colony of *P. parasitica* from V8JB agar plates. The jars were incubated at room temperature (20–25 C), and after about 2 wk, the colonized vermiculite was added to pasteurized U.C. soil mix at 1:25 (v/v) and mixed thoroughly with a small cement mixer. In some experiments, this soil was amended 1:1 with artificially infested U.C. soil mix prepared similarly. Experiments were also conducted in uninfested U.C. soil mix to assess phytotoxicity of the chemicals.

Ethazol (5G + 35WP) and metalaxyl (5G + 35WP) were incorporated into the soil at 2–250 μg a.i./cm^3 or applied as seed treatments (WP formulation only) at 0.3–30 g a.i./kg seed using a seed treater and a predetermined amount of water that was gradually absorbed during a 10-min spray period. Seed was allowed to dry at room temperature before planting.

Four greenhouse experiments were conducted with soil-applied treatments (experiments 1–4) and three with seed treatments (experiments 5–7). The experimental unit in all tests was a 20-cm-diameter plastic pot in which 25 seeds of the tomato cultivar 145-B-7879 were planted. For each treatment, there were five replicate pots. Records of disease incidence and/or phytotoxicity were taken at weekly intervals for about 50 days. Development of disease was separated into three phases: 1) preemergence damping-off, estimated by the percentage of seedling emergence; 2) postemergence seedling damping-off; and 3) root rot, rated on a scale from 0 (all root system healthy) to 5 (all root system rotten). Isolations for *P. parasitica* were made from specimens typical of each phase of disease.

Experiment 1 was conducted in artificially infested U.C. soil mix, using granular and wettable powder formulations of both fungicides. After chemical treatments were applied, pots were filled with infested soil to 5 cm from the top; a layer (1 cm) of uninfested soil with the same chemical treatment was added and seeds were planted on the surface and covered with a 1-cm layer of sand. This procedure obviated preemergence damping-off and provided uniform stands for better assessment of the effect of the fungicides on postemergence damping-off and root rot. Experiment 2 was identical to experiment 1, except the fungicides were applied in uninfested U.C. soil mix for assessment of phytotoxicity and for detection of systemic activity of metalaxyl, by assay of disks and stem sections for fungitoxicity in vitro or by transfer to infested soil of plants that had grown for 1 mo in fungicide-treated soils. Experiment 3 was conducted in naturally infested field soil and experiment 4 in field soil amended 1:1 with artificially infested U.C. soil mix. In both experiments 3 and 4, only the granular fungicide formulations were used and seeds were planted directly onto the infested soil and covered with sand.

Seed treatments were tested in three greenhouse experiments (5–7). In experiment 5, seeds were planted directly onto the artificially infested U.C. soil mix to evaluate the effect of seed treatments on preemergence damping-off. Experiment 6 was identical to 5, except the seeds were planted on a layer of uninfested soil as in experiment 1. Finally, experiment 7 was conducted in uninfested U.C. soil mix to determine phytotoxicity.

Field experiments. Two field experiments were conducted during 1977, the first on artificially infested Yolo fine sandy loam near Davis and the second in a naturally infested field with the same soil type near Woodland, CA. Inoculum was prepared as follows: Field soil collected from the Davis plot was infested with vermiculite inoculum as in the greenhouse experiments, placed in flats, and planted to tomatoes in the greenhouse; after about 50 days, when almost all plants had been killed by *Phytophthora*, the flats were replanted. This procedure was repeated once more, then thoroughly mixed soil from all flats was used as inoculum for field infestation. One week before planting, inoculum was spread by hand at 5 L/m^2 on the surface of 30-cm-wide raised beds. Inoculum was incorporated about 7–8 cm deep into the soil, with a small rototiller. The beds then were reshaped, and subsequent operations were identical to those used for the naturally infested field plot.

Chemical treatments that had shown promise in the greenhouse were retested in field experiments. Granular fungicides mixed with sand were spread by hand on the surface of 30-cm-wide beds at 1 L/8 m of bed length and incorporated about 7–8 cm deep into the soil with a small rototiller immediately before planting. Seed treatments were performed the day before planting as described previously.

Processing tomato cultivar 145-B-7879 was used in both experiments. Seed was sown densely (60–70 seeds/m row) with a small hand-operated planter in rows occupying the center of the beds. Three rows 8 m long and 1.5 m apart comprised an individual plot and each was separated from adjacent plots at both sides by an unplanted row and at the ends by 1 m of unplanted row. A randomized complete block design with three replicates was used in both experiments. Planting was

done on 12 and 17 May for the Davis and Woodland experiments, respectively. Both trials were sprinkle-irrigated until the emerged seedlings reached the second or third true-leaf stage (about 40 days from planting), when they were thinned to about one plant per 20 cm of row. From thinning until the end of the growing season (late September), the plants were furrow-irrigated.

The following records were taken from the central row of each plot: 1) phytotoxicity; 2) preemergence damping-off; 3) postemergence damping-off; 4) incidence of root and crown rot, determined by counting plants showing wilting and/or dying of foliage at regular intervals between thinning and harvest; and 5) total yield from the Woodland experiment only. At each reading of disease incidence, representative diseased plants were taken for in vitro isolation of the pathogen.

RESULTS

Laboratory experiment. Both chemicals at low concentrations greatly decreased mycelial growth of *P. parasitica* with ED_{50} values for growth inhibition between 0.5 and 1 μg/ml. All comparable concentrations of ethazol, however, were more effective than metalaxyl; the ED_{100} dose for metalaxyl was 100 times higher than for ethazol (Table 1). Comparative activity of the two fungicides after 3 and 6 days of incubation was similar to that after 10 days. After 15 and 20 days incubation, however, the activity of metalaxyl diminished, whereas that of ethazol remained about the same as at 10 days. For example, the ED_{50} value of metalaxyl after 20 days was about 10 μg/ml, whereas that of ethazol was 1–2 μg/ml. Similar results were obtained in a repeat experiment.

Both fungicides also inhibited mycelial growth by fumigant action. Growth on CMA plates incubated in the same box with uninoculated CMA plates containing 100 μg/ml of ethazol was reduced 59%. Relative inhibition by fumigant action of metalaxyl was about 37% less than that of ethazol.

Ethazol was much more inhibitory to chlamydospore germination on CMA than metalaxyl (Table 1). After 20 hr of incubation, ethazol at 2 μg/ml completely inhibited germination of chlamydospores, whereas metalaxyl at 100–250 μg/ml caused less than 50% inhibition (Table 1). On fungicide-free CMA, 70.5% of the chlamydospores germinated and after 20 hr had produced long branching germ tubes. The few chlamydospores that germinated in the 0.5- and 1-μg/ml ethazol treatments had short unbranched germ tubes. Germ tube length and branching were also reduced with increasing concentrations of metalaxyl. At concentrations higher than 10 μg/ml, only simple germ tubes were produced and their length decreased from about 400 μm at 10 μg/ml to about 50 μm at 250 μg/ml. Also, with increasing concentrations of metalaxyl, germ tubes became increasingly distorted and vacuolated. After 60 hr, percentage germination was the same as at 20 hr but germ tube length and branching had increased in all treatments. In a repeat experiment, results were similar except the dose of ethazol required for complete inhibition of germination was 10 μg/ml instead of 2 μg/ml.

In fungicide-free, autoclaved SE, 67% of the chlamydospores germinated within 24 hr and 97% of the germinated chlamydospores produced sporangia. Ethazol caused 78, 92, and 100% inhibition of germination at 1, 5, and 10 μg/ml, respectively; about half of the chlamydospores that germinated in 1 and 5 μg/ml ethazol produced sporangia. Metalaxyl had less effect on chlamydospore germination, about 40% inhibition at 250 μg/ml. It was highly inhibitory to sporangium formation from germinating chlamydospores, and sporangium production was nil at or above 10 μg/ml. Germinated chlamydospores in 10 μg/ml metalaxyl formed short unbranched germ tubes that failed to produce sporangia and had started to lyse after 72 hr. At 1 and 5 μg/ml, chlamydospore germination was the same as in the control but sporangium formation was inhibited 65 and 83%, respectively, and had not changed after 72 hr.

After 72 hr, chlamydospores in all fungicide treatments that had either failed to germinate or germinated via a short germ tube without sporangia were washed five times in SDW by centrifugation and plated on CMA. After 20 hr at 24 C in the dark, 50% of the chlamydospores from all treatments had germinated and germ tubes grew rapidly into branching mycelium, indicating a fungistatic rather than fungicidal action of both chemicals.

At 1 μg/ml, both ethazol and metalaxyl inhibited sporangium production by more than 50%. Relatively high concentrations of metalaxyl had little additional effect, whereas ethazol concentrations as low as 5–10 μg/ml nearly prevented sporangium production (Table 1). Indirect sporangial germination was observed at all ethazol (0.5–10 μg/ml) and metalaxyl concentrations (0.5–100 μg/ml) that allowed sporulation.

Metalaxyl at concentrations up to 100 μg/ml and ethazol at concentrations up to 25 μg/ml had no effect on zoospore motility. In these treatments as in the control, duration of motility extended beyond the maximum observation period of 60 min. Concentrations of metalaxyl higher than 100 μg/ml reduced duration of motility, but even at 250 μg/ml (the highest concentration tested) zoospores

Table 1. Effect of ethazol and metalaxyl on linear mycelial growth, chlamydospore germination, sporangium formation, and zoospore germination of *Phytophthora parasitica* in vitro

Fungicide concentration (μg a.i./ml)	Percent inhibition compared with respective water control[a]							
	Mycelial growth[b]		Chlamydospore germination[c]		Sporangial formation[d]		Zoospore germination[e]	
	Ethazol	Metalaxyl	Ethazol	Metalaxyl	Ethazol	Metalaxyl	Ethazol	Metalaxyl
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
0.5	33.1	29.9	72.4	0.0	31.5	48.2	0.0	0
1.0	62.0	53.4	90.1	0.0	54.9	59.4	0.0	0
2.0	74.3	72.4	100.0	17.4	93.7	65.7	0.0	7
5.0	95.6	79.3	···	26.9	98.8	74.8	4.7	7
10.0	98.9	85.0	···	31.2	99.5	86.3	21.3	8
25.0	100.0	93.5	···	25.2	100.0	86.8	39.7	2
50.0	···	96.2	···	29.8	···	81.7	44.3	5
100.0	···	98.1	···	47.2	···	88.5	61.7	0
250.0	···	100.0	···	45.4	···	97.1	85.0	4

[a] Each figure is the average of five replicates.
[b] Radial growth on cornmeal agar (CMA) after 10 days of incubation at 24 C in the dark. Colony radius of control was 26.2 mm.
[c] Chlamydospore germination on CMA after 20 hr at 24 C in the dark. Germination of the control was 70.5%. Germ tube growth decreased with increasing concentration of either chemical.
[d] Sporangium formation on mycelial mats in autoclaved soil extract after 3 days at 20–25 C. The number of sporangia in the water control was 58.4 per 50 microscope fields (low magnification).
[e] Germination of encysted zoospores on CMA after 12 hr at 24 C in the dark. Germination of control was 100%. Germ tube growth decreased with increasing concentration of either chemical.

remained motile for about 1 min. Ethazol at 50 and 100 μg/ml reduced the motility time to 9 and 1 min, respectively, and at 250 μg/ml, motility ceased immediately.

Metalaxyl up to 250 μg/ml had no effect on zoospore germination. Ethazol caused 50% inhibition at 50–100 μg/ml and 85% inhibition at 250 μg/ml (Table 1). Germ tube length and extent of branching decreased progressively with increasing concentrations of either chemical.

Greenhouse experiments. Because soil treatments with the wettable powder and granular formulations of the fungicides in experiment 1 gave similar results, only results with the granular formulations are reported. Uniformly high levels of seedling emergence (80–90%) were recorded after 10 days in all treatments; however, significant differences in incidence of postemergence damping-off occurred among the treatments 2 wk after planting (Table 2). Metalaxyl at all concentrations provided excellent control of the disease during the 7-wk experimental period. Comparable levels of control were obtained only with the two high concentrations of ethazol, 100 and 250 μg/ml. At 50 μg/ml, ethazol gave partial control of the disease, whereas at 2–10 μg/ml, it was completely ineffective or only reduced the rate of infection, and disease incidence was 100% after 7 wk (Table 2). At the end of the experiment, the root systems of plants in all metalaxyl treatments as well as the uninfested control were completely free of root rot. No root rot was evident at 250 μg/ml ethazol, whereas at 50 and 100 μg/ml, the root rot index was 3.2 and 1.7, respectively (Table 2).

Soil treatments with the granular or wettable powder formulations of ethazol up to 250 μg/ml had no phytotoxic effects on germination or subsequent growth (experiment 2). Soil treatments with granular or wettable powder formulations of metalaxyl at 50 μg/ml or higher were phytotoxic; however, the percentage of, and time needed for, seedling emergence were not affected. A few days after emergence and before any true leaves had developed, cotyledons were wilted, yellowed, and burned. At 50 μg/ml, about 25% of the seedlings lost their cotyledons. Later, however, all plants recovered, and after 7 wk, only slight reduction in growth and a slight yellowish appearance of the foliage were evident. At 100 μg/ml, all seedlings lost their cotyledons and subsequently developed marginal necrosis and general yellowing on the true leaves. Plant growth was reduced to less than 50% of that in the control. About 30% of the plants were killed within 3–7 wk of planting in the 250 μg/ml metalaxyl treatment.

Plants grown for 1 mo in uninfested and 0–250 μg/ml WP metalaxyl-treated U.C. soil mix were sampled for systemic activity with laboratory bioassays (1), using 5-mm leaf disks and about 1-mm-thick sections of stems. No systemic activity in stems was detected in any treatment, but systemic activity was readily detected in leaf disks from metalaxyl treatments higher than 50 μg/ml.

Systemic activity of metalaxyl was assessed further using plants grown for 1 mo in uninfested U.C. soil mix treated with metalaxyl (WP) at 0, 10, 50, and 100 μg/ml and transplanted individually into pots with infested, untreated U.C. soil mix. Although disease development was slower in plants that had been exposed to 100 μg/ml metalaxyl, all plants from all treatments were killed within 3 wk of

Table 2. Effects of soil treatments with granular formulations of ethazol and metalaxyl on postemergence seedling damping-off and root rot of processing tomato cultivar 145-B-7879 grown in U.C. soil mix artificially infested with *Phytophthora parasitica* (greenhouse experiment 1)

Fungicide[u]	Rate[v] (μg a.i./ml soil)	Percentage of dead plants[w,x] 2 Wk	4 Wk	7 Wk	Average root rot index on surviving plants[y]
Metalaxyl 5G	2	0.0 e	1.6 c	1.6 c	0.0
	5	0.0 e	3.3 c	3.3 c	0.0
	10	0.0 e	0.0 c	0.0 c	0.0
	50	0.0 e	0.0 c	0.0 c	0.0
	100	0.0 e	0.0 c	0.0 c	0.0
	250	0.0 e	0.0 c	0.0 c	0.0
Ethazol 5G	2	100.0 a	100.0 a	100.0 a	...[z]
	5	87.8 b	100.0 a	100.0 a	...
	10	54.1 c	100.0 a	100.0 a	...
	50	11.1 d	68.9 b	72.2 b	3.2
	100	0.0 e	3.5 c	7.0 c	1.7
	250	0.0 e	0.0 c	0.0 c	0.0
Infested check	...	100.0 a	100.0 a	100.0 a	...
Uninfested check	...	0.0 e	0.0 c	0.0 c	0.0

[u] Both granular and the wettable powder (WP) formulations were used. Results from the WP were similar to those reported for the granulars.
[v] Fungicides were incorporated into the soil mix just before potting and planting.
[w] Preemergence damping-off was eliminated by planting seeds on a layer of uninfested soil (see text).
[x] In each column, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Severity of root rot was rated on a scale from 0 (no evident root rot) to 5 (entire root system rotted).
[z] None survived.

Table 3. Effects of soil treatments with granular formulations of ethazol and metalaxyl on preemergence and postemergence damping-off and root rot of processing tomato cultivar 145-B-7879 grown in naturally infested field soil (greenhouse experiment 3) or in field soil amended 1:1 with U.C. soil mix, artificially infested with *Phytophthora parasitica* (greenhouse experiment 4)

		Naturally infested field soil			Field soil amended 1:1 with artificially infested U.C. soil mix		
Fungicide[x]	Rate of application[x] (μg a.i./ml soil)	Seedling emergence[y,z] (%)	Percentage of dead plants[z] 3 Wk	7 Wk	Seedling emergence[y,z] (%)	Percentage of dead plants[z] 3 Wk	7 Wk
Metalaxyl 5G	2	97 a	0.0 b	0.0 c	96 a	0.0 c	1.4 c
	5	84 a	0.0 b	0.0 c	90 a	0.0 c	0.0 c
	10	94 a	0.0 b	0.0 c	88 ab	0.0 c	0.0 c
Ethazol 5G	10	81 a	3.1 b	61.0 a	57 c	63.0 b	95.0 a
	50	91 a	1.5 b	32.0 b	66 bc	2.0 c	36.0 b
	100	90 a	0.0 b	4.4 c	81 ab	5.0 c	8.0 c
Untreated check	...	93 a	18.6 a	68.0 a	54 c	100.0 a	100.0 a

[x] Fungicides were incorporated into the soil just before potting and planting.
[y] Seeds were planted directly onto the infested soil and covered with a 1-cm layer of sand.
[z] In each column, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

transplantation into infested soil.

In naturally infested field soil (experiment 3), the relative activity of the two chemicals was similar to artificially infested U.C. soil mix (experiment 1, Table 2). No appreciable preemergence damping-off occurred, and plant stands were uniformly high in all treatments and similar to those of the control, with more than 90% emergence (Table 3). Seedling emergence in the control was reduced to 54% by preemergence damping-off in field soil amended 1:1 with artificially infested U.C. mix (experiment 4). Soil treatments with metalaxyl at 2, 5, and 10 μg/ml effectively controlled preemergence damping-off and increased plant stands to about 90% (Table 3). Ethazol at 100 μg/ml improved seedling emergence, but at 10 and 50 μg/ml, there was very little effect (Table 3). At 10 μg/ml ethazol, disease was delayed somewhat but final incidence was similar to that in the control. At 50 μg/ml ethazol, final disease incidence was reduced significantly, and at 100 μg/ml, disease control was very good (Table 3). Metalaxyl at all concentrations provided better disease control than the higher rates of ethazol. In fact, the lowest concentration of metalaxyl (2 μg/ml) provided better protection than the highest concentration of ethazol (100 μg/ml).

Ethazol seed treatments at concentrations of 0.3–15 g/kg seed were ineffective in reducing preemergence damping-off (Table 4) and at 30 g/kg seed, emergence was 38% (Table 4). Metalaxyl at 1.5 g/kg seed or higher resulted in plant stands comparable to the uninfested check. At 0.3 g/kg seed, metalaxyl was only partially effective.

In experiment 6, seedling stands were uniformly high in all treatments and in the infested check. Within 3 wk of planting, however, 80% of the seedlings in the infested check and the ethazol treatments at 0.3–15 g/kg seed were killed by *P. parasitica* (Table 4). Ethazol at 30 g/kg seed and metalaxyl at 0.3 g/kg seed provided very low levels of protection and only during the first 3 wk of growth. Metalaxyl at 1.5 and 3.0 g/kg seed provided partial protection for the first 7 wk, and 7.5 g/kg seed or higher provided effective protection during the whole experimental period (Table 4). None of the seed treatments were phytotoxic.

Field experiments. In both trials, seedling emergence was complete by 2 wk after planting. Plant stands were uniform, with about 50 seedlings per meter of row in all treatments. None of the treatments affected germination or subsequent growth of plants.

Postemergence damping-off started within 1 wk of seedling emergence in both trials. Percentages of seedling damping-off in the various treatments during the first 6 wk are summarized in Tables 5 (Davis) and 6 (Woodland). In both trials, soil treatments with metalaxyl gave the best results. Seed treatments with metalaxyl had little effect on postemergence damping-off, but soil treatments with ethazol provided satisfactory control.

Because of the dense planting, it was possible to remove infected plants at thinning and obtain apparently healthy plants spaced at 20 cm in all treatments. Incidence of root and crown rot was higher in the naturally infested field (Woodland) than in the artificially infested soil (Davis). Comparative efficacy of the treatments was similar in both trials (Tables 5 and 6).

Soil treatments with all three metalaxyl rates provided season-long protection (Table 5). Ethazol soil treatments and seed treatment with metalaxyl at 15 g/kg seed provided protection for the first month after thinning. Thereafter, disease incidence in these treatments was the same as in the control (Table 5).

In the naturally infested field (Woodland) soil treatments with metalaxyl at 0.75 and 1.5 kg/ha provided the best control. Although these treatments provided some season-long protection, activity was most evident during the first month after thinning (Table 6). Final disease control was two or three times less than that obtained with the same treatments in artificially infested soil (Table 5). Soil treatments with ethazol and seed treatments with metalaxyl at 15 kg/ha and 30 g/kg seed delayed disease development compared with the control but had no effect on final disease incidence (Table 6).

All chemical treatments significantly increased total yield of red and green fruit compared with the control (Table 6). Yield increases were generally proportional with the overall level of disease control effected by the treatments. Thus, soil treatments with metalaxyl, which provided the most effective control of the disease, also resulted in highest yields (Table 6). About 100% yield increase was obtained with ethazol at 15 kg/ha, a treatment that also gave satisfactory control of the disease, particularly during the first 2 mo after thinning. Smaller yield increases, 35–70% higher than the control, resulted from other treatments.

DISCUSSION

Our laboratory results showed that ethazol was more detrimental than metalaxyl to most stages of *P. parasitica*. Yet, in greenhouse experiments, metalaxyl was 50–100 times more effective than ethazol for control of Phytophthora root rot (Tables 2–4). Metalaxyl has been more effective than ethazol against several other diseases caused by soilborne species of *Phytophthora* and *Pythium* (1,6,9,21).

Benson (1) observed a 1,000-fold reduction in the activity of ethazol against mycelial growth of *P. cinnamomi* in soil tests compared with agar and attributed the difference to immobility of ethazol in soil. Reduced soil mobility may also have contributed to the difference in activity of ethazol in our laboratory and greenhouse tests. Because activity of ethazol in soil was not improved by uniform soil incorporation, however, soil mobility alone cannot explain differences in activity of ethazol in soil and agar. Furthermore, Benson (1) reported that ethazol activity in soil was not enhanced by supplemental water. Other factors such as degradation of ethazol in soil may be involved in reduction of activity.

Metalaxyl may control *P. parasitica*

Table 4. Effects of seed treatments with ethazol and metalaxyl on preemergence and postemergence damping-off of processing tomato cultivar 145-B-7879 grown in U.C. soil mix infested artificially with *Phytophthora parasitica* (greenhouse experiments 5 and 6)

Fungicide	Rate of application (g a.i./kg seed)	Seedling emergence[x,y] (%)	Percentage of dead plants[y,z] 3 Wk	7 Wk
Metalaxyl 50WP	0.3	49.0 b	63.0 b	97.0 a
	1.5	81.0 a	8.3 c	46.0 b
	3.0	84.0 a	11.5 c	23.0 bc
	7.5	87.0 a	0.0 c	5.3 c
	15.0	79.0 a	0.0 c	4.8 c
	30.0	81.0 a	0.0 c	5.4 c
Ethazol 35WP	0.3	7.0 c	85.0 a	100.0 a
	1.5	8.4 c	78.0 ab	100.0 a
	3.0	5.6 c	79.0 ab	100.0 a
	7.5	6.2 c	83.0 a	100.0 a
	15.0	15.0 c	76.0 ab	100.0 a
	30.0	38.0 b	61.0 b	96.0 a
Infested check	...	11.0 c	86.0 a	97.0 a
Uninfested check	...	84.0 a	0.0 c	0.0 c

[x] Seedling emergence was determined in greenhouse experiment 5, in which seeds were sown directly onto the infested soil (see text).

[y] In each column, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Numbers of dead plants were determined in greenhouse experiment 6 in which seeds were sown on a layer of uninfested soil to prevent preemergence damping-off.

root rot of tomato by preventing the formation of sporangia from mycelia and germinating chlamydospores and thus prevent the production of zoospores, the main infective propagules. Inhibition of mycelial growth, including growth of chlamydospores and particularly zoospore germ tubes, is of sufficient magnitude to reduce infection and disease development. The effects of metalaxyl on chlamydospore germination, indirect sporangial germination, and zoospore motility and germination were either insignificant or very slight in vitro and thus may not be involved in its mode of action in soil. Our results are in agreement with those of Staub and Young (15) for the biological mode of action of metalaxyl against tobacco black shank caused by *P. parasitica* var. *nicotianae* but do not support the suggestion by Farih et al (5) that metalaxyl controls *Phytophthora* diseases by interfering with all stages of the life cycle. In the latter study (5), indirect sporangial germination and zoospore germination of *P. parasitica* and *P. citrophthora* from citrus were not appreciably inhibited by metalaxyl concentrations higher than required for effective disease control. Similarly, metalaxyl had little effect on direct sporangial germination and on zoospore release, motility, and germination of *P. capsici* (11). However, chlamydospore germination of *P. parasitica* from citrus (5) was more sensitive to metalaxyl than was our tomato isolate. This difference may be attributable to variation in the sensitivity of different isolates (11) as well as to different criteria for germination used in the two studies. We did not examine chlamydospore or oospore formation, which have also been reported highly sensitive to low concentrations of metalaxyl (1,5).

Both ethazol and metalaxyl inhibited mycelial growth of *P. parasitica* through their fumigant action, indicating that in soil these chemicals may affect the pathogen without direct contact. In addition to its possible practical significance, volatility of the chemicals also may be a source of error in laboratory experiments if adequate care is not taken to avoid vapor exchange between treatments.

In agreement with our results, low rates of metalaxyl incorporated into the soil before planting or applied as soil drenches, seed treatments, foliar sprays, or stem paints also have controlled other soilborne and airborne diseases caused by different oomycetes on a wide variety of crops (1,2,4,6,9,11,16,21).

Soil treatments with metalaxyl at 50

Table 5. Effects of soil and seed treatments with fungicides on Phytophthora root rot of processing tomato cultivar 145-B-7879 in an artificially infested field at Davis, CA[v]

Fungicide	Method and rate of application		Disease incidence (%)[x]			
	Soil[w] (kg a.i./ha)	Seed (g a.i./kg)	Seedling damping-off[y]	Root and crown rot[z] (no. months after thinning) 1	2	3
Ethazol 5G	7.50	...	3.0 c	8.6 bc	21.3 b	26.7 ab
	15.00	...	4.5 c	8.1 bc	12.0 cd	17.3 c
Metalaxyl 5G	0.30	...	2.4 c	1.4 cd	1.4 e	6.7 d
	0.75	...	0.4 c	2.6 cd	6.0 de	7.3 d
	1.50	...	0.6 c	0.0 d	0.0 e	4.7 d
Metalaxyl 50WP	...	7.5	19.6 b	14.0 b	15.3 bc	20.7 bc
	...	15.0	20.8 b	8.0 bc	18.0 bc	24.0 abc
Untreated check	...	...	28.3 a	26.6 a	28.7 a	31.3 a

[v] The field was infested with *P. parasitica* 1 wk before planting (see text). Planted (direct field-seeding) 12 May, seedling emergence 20–30 May, thinning 21 June, and termination of experiment 23 September.
[w] Granular fungicides were applied on 30-cm-wide bands and incorporated 7–8 cm deep into the soil.
[x] Each number is the average of three replicates. Numbers in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Percentage of seedlings killed by the fungus during the period from emergence to thinning.
[z] Percentage of plants with foliar symptoms (wilting and/or dying) during the period from thinning to the end of the growing season.

Table 6. Effects of soil and seed treatments with fungicides on Phytophthora root rot and total fruit yield of processing tomato cultivar 145-B-7879 in a naturally infested field at Woodland, CA[v]

Fungicide	Method and rate of application		Disease incidence (%)[x]				Total fruit yield (T/ha)[x]
	Soil[w] (kg a.i./ha)	Seed (g a.i./kg)	Seedling damping-off[y]	Root and crown rot[z] (no. months from thinning) 1	2	3	
Ethazol 5G	1.50	...	3.3 c	15.8 bc	23.9 bc	50.3 ab	38.1 bcde
	7.50	...	3.5 c	23.2 b	31.4 b	55.8 a	33.3 bcdef
	15.00	...	2.0 c	7.4 cd	18.5 cde	36.9 bc	43.7 abcd
Metalaxyl 5G	0.30	...	1.4 c	7.1 cd	9.9 def	36.7 bc	47.9 ab
	0.75	...	0.6 c	0.7 d	8.7 ef	19.2 d	47.7 abc
	1.50	...	1.1 c	0.2 d	6.5 f	25.9 cd	56.8 a
Metalaxyl 50WP	...	7.5	14.4 ab	15.0 bc	23.7 bc	42.0 ab	29.9 def
	...	15.0	10.3 b	15.1 bc	17.4 cdef	39.0 bc	36.7 bcdef
	...	30.0	14.7 ab	9.1 cd	20.3 bcd	40.0 bc	31.9 def
Untreated check	...	...	17.6 a	35.4 a	43.5 a	48.8 ab	22.3 f

[v] Planting (direct field-seeding) 17 May, seedling emergence 25 May–5 June, thinning 28 June, and harvest 26 September.
[w] Granular fungicides were applied on 30-cm-wide bands and incorporated 7–8 cm deep into the soil.
[x] Each number is the average of three replicates. Numbers in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Percentage of seedlings killed by the fungus during the period from emergence to thinning.
[z] Percentage of plants with foliar symptoms (wilting and/or dying) during the period from thinning to harvest.

μg/ml or higher were phytotoxic to tomato cultivar 145-B-7879. Such rates, however, were five to 125 times higher than required for effective disease control. Benson (1) also reported phytotoxicity of metalaxyl to azalea.

Systemic activity of metalaxyl was demonstrated using a leaf-disk bioassay but only when the chemical was applied at rates in the phytotoxicity range (50–250 μg/ml). Similar results have been reported for systemic translocation of metalaxyl in azalea (1). Zaki et al (22) found low concentrations of metalaxyl in stems of tomato and avocado plants after leaf or root applications. In our study, no systemic activity could be detected in stems, even at the highest concentrations tested (250 μg/ml), indicating that the chemical mainly accumulates in the leaves. Such acropetal translocation of the chemical has been reported to occur rapidly in tomato (within 1 hr) and to provide effective protection of the leaves against infection by *P. infestans* (2).

Tomato plants grown for 1 mo in soil treated with metalaxyl at 10 and 50 μg/ml were not protected against *P. parasitica* when transplanted into infested untreated soil. Only the highest metalaxyl rate (100 μg/ml) provided partial and short-term (1 wk) protection. In similar experiments with avocado seedlings (22), most of the absorbed fungicide was translocated to foliage and the amount remaining in roots provided only partial protection against *P. cinnamomi* when seedlings were transplanted into infested, untreated soil. Thus, long-term action of metalaxyl against Phytophthora root rots results primarily from its long residual life in soil, which enables activity of the chemical against the pathogens in the soil (1) and also systemic activity, after uptake by the roots (15). The fungicide acts systemically against root infections only as long as there is an adequate supply in the rhizosphere to ensure continuous uptake and maintenance of effective concentrations in the roots and stems.

For unknown reasons in both field trials, seed treatments with metalaxyl had little effect on damping-off, whereas the same treatments were highly effective under greenhouse conditions. After thinning, however, seed treatments, especially at the higher rates, delayed development of root rot even though they did not appreciably reduce the final disease incidence.

All chemical treatments increased total fruit yield even though none provided season-long protection. Apparently, the protection provided during early and midseason was responsible for increased production. Indeed, yield increases were generally proportional to the apparent level of disease control provided by the different treatments during this period of plant development.

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