Influence of Dinitroanaline Herbicides on Growth, Sporulation, and Infectivity of Four *Phytophthora* spp. Pathogenic to Deciduous Fruit Trees

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ABSTRACT

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Crown rot of potted Mahaleb cherry seedlings, caused by *Phytophthora cryptogea*, *P. cambivora*, and *P. megasperma*, was virtually prevented by applying the herbicide oryzalin at either of two nonphytotoxic rates (1.12 and 2.24 kg/ha) to a porous soil mix (1 volume of sandy loam:2 volumes of vermiculite) prior to infestation with the pathogens. In untreated pots, leaf-disk baits exposed during each of five to nine biweekly flooding periods were colonized at frequencies of 96 to 99, 49 to 95, and 13 to 47% by the respective pathogens, but only 0 to 7% of the baits were colonized in pots containing oryzalin-treated soil mix. At 2.24 kg/ha, pendimethalin similarly reduced both the incidence of disease caused by *P. cambivora* and its frequency of isolation from baits, but pendimethalin had little effect at 1.12 kg/ha. Likewise, pendimethalin had little effect on the incidence of crown rot caused by *P. cryptogea* when applied at either rate and only modestly reduced the frequency of its baiting. When tested in vitro at 0.06 to 4.0 µg/ml, both herbicides significantly affected pathogen activities. At a concentration approximating that found in solutions of treated field soils (0.25 µg/ml), oryzalin inhibited the formation of sporangia by *P. cambivora*, *P. cryptogea*, *P. cactorum*, and *P. megasperma* by 97, 89, 80, and 65%, respectively, whereas pendimethalin inhibited formation by 93, 49, 54, and 74%, respectively, at this concentration. When added to soil extract containing freshly released zoospores of *P. cryptogea*, *P. cactorum*, or *P. megasperma*, both herbicides significantly reduced the motile period, although pendimethalin generally provided the greater reduction at lower concentrations. Oryzalin was much more inhibitory than pendimethalin to mycelial growth of all four pathogens, although reductions were only modest (≤20% relative to the checks) at concentrations ≤0.25 µg/ml.

The influence of herbicides on the development of various soilborne diseases has been studied extensively, and at least three reviews on the subject have been published (1,4,17). In the majority of host-pathogen systems examined, disease incidence and severity have been unaffected or exacerbated by herbicide applications; however, herbicides have limited disease development in some cases, generally by eliciting host defense responses or limiting pathogen activities (1). Perhaps the best-documented example involving the latter mechanism is the suppression of pea root rot caused by *Aphanomyces euteiches* in soils treated with dinitroanaline herbicides (8,9,11,21,22): these compounds restrict both the production and motility of the pathogen's zoospores (8,22). Applications of dinitroanaline herbicides also have reduced the incidence of clubroot of cole crops caused by another zoospore-forming fungus, *Plasmodiophora brassicae* (6).

Root, crown, and collar rots caused by *Phytophthora* spp. are serious and widespread diseases of deciduous fruit trees for which control strategies integrating cultural practices, host resistance, and chemical treatments generally are recommended (5,16). Although the influence of selective herbicide use has not been investigated or discussed within this context, the suppression by dinitroanaline herbicides of diseases caused by other zoospore-forming fungi suggests that the materials might be applicable within such a program. Therefore, the objective of the current study was to examine two dinitroanaline herbicides registered for use on deciduous fruit trees with respect to their influence on four *Phytophthora* spp. that commonly cause root and crown rots on these hosts. A preliminary portion of this work has been published (25).

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MATERIALS AND METHODS

Fungal isolates. Single isolates of the following *Phytophthora* spp. were used: *P. cactorum* (Lebert & Cohn) Schröt. (isolate NY 188), originally recovered from apple; *P. cambivora* (Petri) Buisman (NY 216), from cherry; *P. cryptogea* Pethybr. & Lafferty sensu lato (NY 154, assigned to subgroup J by Mills et al. [18]), from cherry; and *P. megasperma* Drechs. (NY 362), from red raspberry. Working cultures were maintained at 15°C on Difco (Detroit) cornmeal agar (CMA) and transferred every 2 to 3 weeks.

Herbicides. For all experiments conducted in vitro, initial stock solutions of oryzalin and pendimethalin were prepared by dissolving technical grade herbicide in 95% ethanol, such that 5 ml of stock solution added to 1 liter of medium yielded final herbicide and ethanol concentrations of 4.0 µg/ml and 0.5% (vol/vol), respectively. Additional stock solutions were prepared by serial dilution with ethanol to yield final herbicide concentrations of 2.0, 1.0, 0.50, 0.25, 0.125, and 0.0625 µg/ml when added to media. Fresh stock solutions were prepared immediately prior to each experiment.

Effects on vegetative growth. CMA was amended with 0.0625 to 4.0 µg of oryzalin or pendimethalin per ml by incorporating stock solutions into molten agar cooled to 55°C after autoclaving; two control treatments, amended only with 0.5% ethanol or unamended, also were prepared. Agar plugs (4 mm diameter) of each *Phytophthora* sp. were cut from the margins of colonies growing actively on unamended CMA and transferred to the center of individual 90-mm-diameter petri dishes containing 20 cm^3 of treated

agar. Radial growth of each colony was measured after 7 days of incubation at 22°C in the dark. There were five replicate plates for each *Phytophthora* sp. × herbicide-concentration treatment. For each pathogen, growth measurements from the herbicide-amended treatments were converted and expressed as percentages of the mean growth from the ethanol control treatment. Data were subjected to GLM analysis of variance (ANOVA) (SAS Institute, Cary, NC), using herbicide, concentration, and *Phytophthora* sp. as variables.

Effects on sporangium formation. Nonsterile soil extract was prepared by stirring 15 g of herbicide-free garden soil in 1 liter of distilled water overnight and recovering the supernatant after centrifugation. Subsamples (100 ml) were amended with herbicide stock solutions or ethanol only (control) as above. Sporangia were produced by floating colonized disks of V8-juice agar in 60-mm-diameter petri dishes containing 10 ml of these solutions for 24 h at 19°C under fluorescent light (26). Two dishes were used for each *Phytophthora* sp. × herbicide-concentration treatment. After incubation, single disks were removed from each duplicate petri dish, placed in vials containing 3% formaldehyde solution, and stored in a refrigerator until they could be assayed. Individual disks were transferred to a deep-well slide filled with distilled water, which facilitated counting of sporangia by allowing individual sporangiophores to radiate freely from the floating disk. The total number of sporangia (germinated and ungerminated) in four predetermined fields of view around the disk (i.e., the 3, 6, 9, and 12 o'clock positions) were counted at 100× magnification, and the counts from the two disks per treatment were combined. The experiment was conducted five times for each herbicide to provide five replicate data points per treatment. Data were transformed to reflect the percentage of sporangia formed in each herbicide treatment relative to the ethanol control and were subjected to ANOVA as described for mycelial growth.

Effects on zoospore motility. Sporangia were produced with minor modifications of the procedure outlined above. For *P. cactorum*, each of nine 60-mm-diameter petri dishes containing agar disks in 10.5 ml of unamended soil extract was incubated for 20 h at 22°C, transferred to a refrigerator (4°C) for 1 h, and moved to a lab bench (22°C) to induce zoospore discharge. One hour later, the agar disks were discarded, and the concentration of motile zoospores within each dish was determined. To do so, a 0.5-ml aliquot was transferred to a counting chamber, which consisted of a piece of nylon window screen (1.7-mm mesh) glued to a glass slide. A glass coverslip was overlaid, the number of motile zoospores within each of 10 individual grids was quickly determined at 100× magnification, and the time was recorded ($t = 0$). Immediately thereafter, 50 µl of a stock solution was pipetted into the remaining 10 ml of soil extract to provide the appropriate herbicide concentration, after which another dish was examined and treated with the next concentration; this process was repeated until one dish had been treated for each of the seven concentrations of the herbicide being examined plus the two control treatments (ethanol only and unamended). Additional counts were made at 30-min intervals after the treatment, until 210 min had elapsed.

To produce zoospores of *P. cryptogea* and *P. megasperma*, petri dishes containing colonized agar disks in 10 ml of unamended soil extract were incubated at 13°C for 15 to 20 h. After ample production of zoospores was confirmed by observation at 50× magnification under a dissecting microscope, the disks were discarded, and the dishes were treated and examined as described above. For each observation time, zoospore motility was expressed as a percentage of the number of motile zoospores counted relative to the number counted in the same dish at $t = 0$. The experiment was run four times for each individual *Phytophthora* sp. × herbicide-concentration series, providing four replicate data points per treatment. Data were analyzed using the GLM procedure for repeated measures ANOVA.

Pathogen activity and disease development in herbicide-treated soil. Mahaleb cherry (*Prunus mahaleb* L.) seedlings were grown in a greenhouse for 13 to 18 weeks in 18 cm (diameter) × 19 cm (deep) clay pots containing pasteurized sandy loam mixed with fine vermiculite (1:2, vol/vol). Aqueous suspensions of commercially formulated oryzalin (Surflan A.S.) or pendimethalin (Prowl 4E) were uniformly misted over the soil surface in designated pots with a hand-pumped atomizer calibrated to deliver 2.1 ml per pot (equivalent to 826 liters/ha), and the pots were thoroughly watered several times. The next day, designated pots were infested by adding 15 sweet cherry (*P. avium* L.) leaf disks (6 mm diameter) that had been colonized within the preceding 24 h by placing them at the edge of an actively growing colony of a *Phytophthora* sp. (27). Leaf disks were inserted at arbitrary locations throughout the soil volume but were inserted at least 4 cm from the bottom of the pot and 2 cm from the stem of the seedling. Three days later and at two-week intervals thereafter, all pots were transferred from the greenhouse to a controlled environment room maintained at 20°C. Pots were flooded for a 48-h period by placing them in plastic containers in which water was maintained 5 to 10 mm above the soil surface, after which they were allowed to drain and were returned to the greenhouse. There were six replicate pots for each *Phytophthora* sp. × herbicide-concentration treatment.

During each of the five to nine flooding episodes (depending on the length of the individual experiments), six 4-mm-diameter Mahaleb cherry leaf disks were floated on the water within each pot to serve as zoospore baits. The disks were recovered immediately after the pots had drained, were rinsed in distilled water, were blotted dry, and were plated on modified P_5ARPH selective medium (26). The frequency of disks from which a *Phytophthora* sp. had grown was recorded after 1 week of incubation at 20°C in the dark.

Three to five months after inoculation, the above-ground portions of all plants were removed, and the roots were washed free of soil. The incidence of crown rot was determined by examining each plant for the presence of a characteristic necrotic lesion in the crown region and was confirmed by isolating a *Phytophthora* sp. on P_5ARPH medium. To determine possible phytotoxic responses to the herbicides, root systems of all uninoculated plants were weighed, and mean weights were compared among treatments. The experiment was conducted six times, using different combinations of *Phytophthora* spp., herbicides, and herbicide rates. Baiting incidence data were analyzed separately for each run of the experiment, using standard ANOVA for randomized block design (SAS Institute, Cary, NC) and the Waller-Duncan k ratio least significant difference variable to separate means among treatments for each pathogen.

RESULTS

Effect of herbicides on vegetative growth. Oryzalin caused negligible to severe reduction of mycelial growth, depending on concentration and *Phytophthora* sp. In contrast, pendimethalin had no effect on the growth of *P. cactorum* or *P. megasperma*, regardless of concentration, and caused only slight suppression of mycelial growth of *P. cryptogea* and *P. cambivora* at the highest concentrations tested (Fig. 1). ANOVA revealed that each main effect (herbicide, concentration, and *Phytophthora* sp.) and all interactions were highly significant ($P = 0.0001$) (Table 1).

When relative growth was regressed against the log value of the oryzalin concentration, the relationship was strongly linear for *P. cactorum* over the range of 1.0 to 4.0 µg/ml; was strongly linear for *P. cambivora* over the range of 0.06 to 4.0 µg/ml; and was strongly linear for the remaining two species over the range of 0.25 to 4.0 µg/ml (Fig. 1). The ED_{50} values calculated from the linear regression model for each species and the r^2 values for the models were: *P. cambivora*, 0.90 µg/ml, $r^2 = 0.97$; *P. megasperma*, 0.98 µg/ml, $r^2 = 0.99$; *P. cryptogea*, 3.13 µg/ml, $r^2 = 0.99$; and *P. cactorum*, 3.90 µg/ml, $r^2 = 0.89$.

Effect of herbicides on formation of sporangia. The suppression of sporangium formation by oryzalin was dramatic for all four *Phytophthora* spp., although the magnitude of the response varied

according to *Phytophthora* sp. and herbicide concentration. At the lowest concentration tested (0.06 µg/ml), sporangium production was reduced by 93, 89, 76, and 55% relative to the ethanol control treatment for *P. cambivora*, *P. cryptogea*, *P. cactorum*, and *P. megasperma*, respectively. Formation of sporangia by *P. cambivora* was inhibited by 98 to 100% at concentrations ≥0.13 µg/ml, and formation by *P. cryptogea*, *P. cactorum*, and *P. megasperma* was inhibited by >90% at concentrations of 0.50, 1.0, and 4.0 µg/ml, respectively (Fig. 2).

Pendimethalin was generally less inhibitory to sporangium production than was oryzalin, but the relative influence of the two herbicides varied among the four *Phytophthora* spp., as indicated by the highly significant ($P = 0.0001$) herbicide × species interaction (Table 2). For instance, pendimethalin was slightly to moderately more inhibitory than oryzalin with respect to sporangium formation by *P. megasperma* at concentrations ≤1.0 µg/ml and was only slightly less inhibitory than oryzalin to *P. cambivora* at all concentrations. However, pendimethalin was considerably less active than oryzalin against *P. cactorum* and *P. cryptogea*, inhibiting sporangium formation by only 45 to 60% regardless of concentration (Fig. 2).

TABLE 1. Analysis of variance for mycelial growth (colony radius) of four *Phytophthora* spp. on cornmeal agar amended with oryzalin or pendimethalin

Source[z]	df	*F* value	*P* > *F*
Replicate	4	0.5	0.77
Herbicide	1	13,787.5	0.0001
Species	3	2,814.1	0.0001
Herbicide × species	3	1,626.4	0.0001
Concentration	7	876.2	0.0001
Herbicide × concentration	7	540.5	0.0001
Species × concentration	21	59.0	0.0001
Herbicide × species × concentration	21	41.8	0.0001

[z] All *Phytophthora* spp.

Effect of herbicides on zoospore motility. Both herbicides reduced the percentage of zoospores that remained motile over time; however, pendimethalin generally provided the greater reduction at low concentrations, as indicated by the highly significant ($P = 0.0001$) herbicide × concentration interaction for three of the *Phytophthora* spp. (Table 3). For instance, virtually all zoospores of *P. cryptogea* lost motility within 30 min of exposure to pendimethalin concentrations ≥0.50 µg/ml, and 91% lost motility (relative to the ethanol control treatment) after 60 min of exposure to 0.25 µg/ml (Fig. 3); in contrast, after 60 min of exposure to oryzalin at 0.25 µg/ml, motility of *P. cryptogea* zoospores was reduced by only 54% relative to the control (Fig. 4). Zoospores of *P. megasperma* and *P. cactorum* were less sensitive to pendimethalin than were those of *P. cryptogea*, particularly at concentrations ≤0.25 µg/ml. For example, after 210 min at 0.25 µg/ml, the reductions in motility (relative to controls) for *P. cryptogea*, *P. megasperma*, and *P. cactorum* were 97, 32, and 27%, respectively (Fig. 3). However, differences among the three species were relatively minor at higher concentrations, particularly ≥120 min after treatment (Fig. 3).

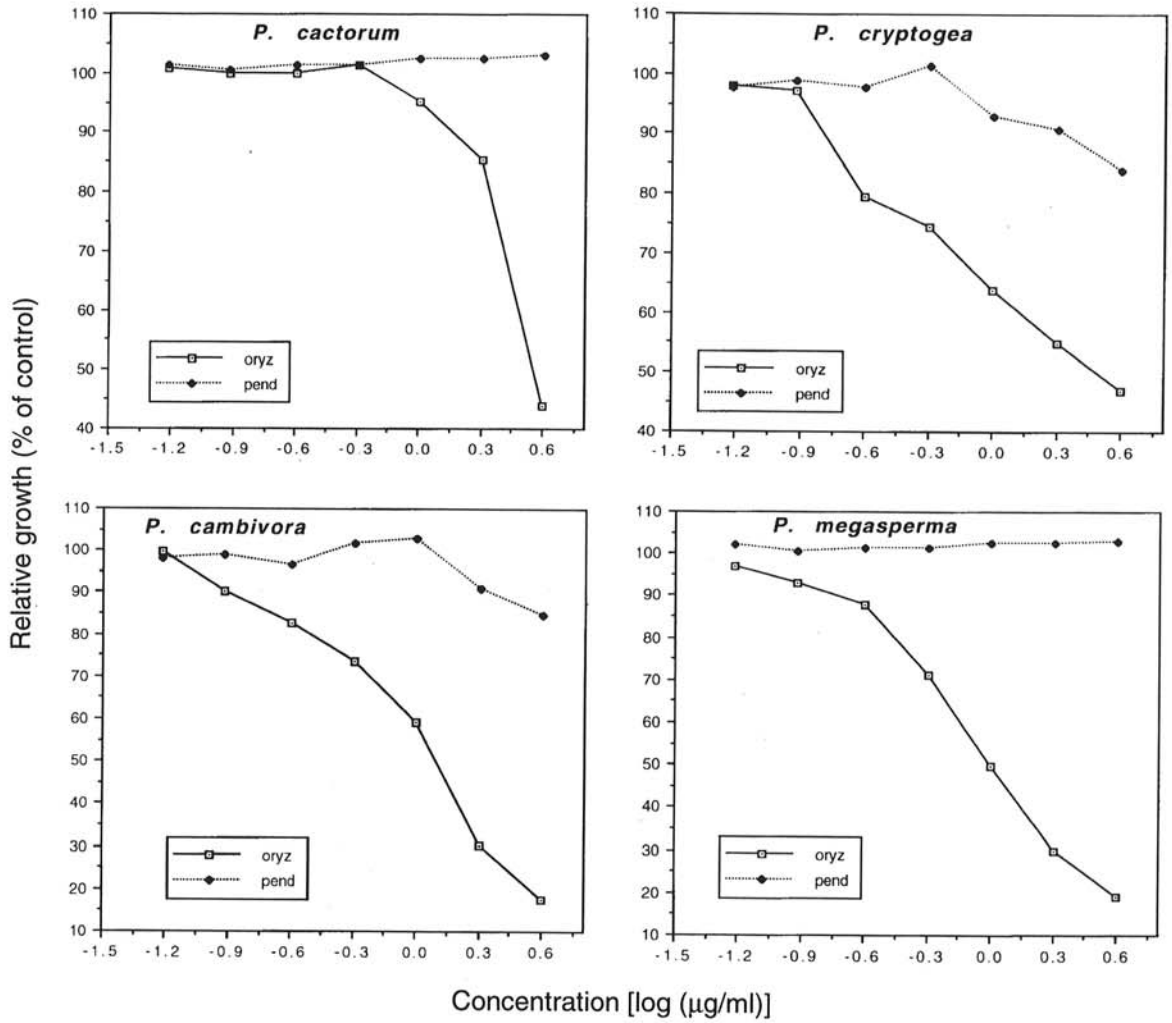

Fig. 1. Mycelial growth of four *Phytophthora* spp. after 7 days at 22°C on cornmeal agar (CMA) amended with oryzalin or pendimethalin at concentrations of 0.0625 to 4.0 µg/ml, expressed as a percentage of the colony radius produced by each species on control medium (CMA amended with 0.5% ethanol).

Like pendimethalin, oryzalin was more inhibitory to motility of *P. cryptogea* zoospores than to those of *P. cactorum* or *P. megasperma* (Fig. 4). Although zoospores of the three *Phytophthora* spp. reacted similarly to oryzalin at 4.0 µg/ml, those of *P. megasperma* and *P. cactorum* were less affected by lower concentrations, and zoospores of *P. megasperma* were the least sensitive to concentrations ≤0.50 µg/ml. For example, 120 min after treatment with oryzalin at 0.06 µg/ml, the reduction in zoospore motility (relative to controls) for *P. cryptogea*, *P. cactorum*, and *P. megasperma* was 39, 19, and 2%, respectively (Fig. 4).

Even in the absence of a dinitroanaline herbicide, motility of zoospores of *P. cambivora* was severely inhibited by ethanol only (or 0.1% acetone in preliminary attempts to identify an alternative solvent); thus, no data were obtained for this species. In general, the ethanol control treatment reduced zoospore motility of the other *Phytophthora* spp. only modestly (0 to 20%) relative to the unamended control treatment (data not shown).

Effects of herbicides on development of crown rot. Crown rot of potted Mahaleb cherry seedlings was virtually prevented by applications of oryzalin at rates of 1.12 to 3.36 kg/ha. Over the three experiments that examined its effects in soil infested with *P. cryptogea*, no crown rot developed on the 24 plants in oryzalin-treated soil, whereas 15 of 18 plants in untreated soil developed the disease (Table 4). Similarly, *P. cambivora* caused crown rot on 24 of 30 untreated plants in the five experiments that examined the effects of oryzalin on this pathogen but caused crown rot on only 1 of 42 plants grown in treated soil. In these same five experiments, *P. megasperma* caused crown rot on 13 of 30 untreated plants, but no crown rot developed on plants grown in infested soil treated with oryzalin (Table 4).

In contrast, pendimethalin had little or no apparent effect on the incidence of crown rot caused by *P. cryptogea* when applied at either 1.12 or 2.24 kg/ha nor on disease caused by *P. cambivora* in the one experiment that tested the 1.12 kg/ha rate. However, *P. cambivora* caused no crown rot on the 18 plants grown in soil treated with pendimethalin at 2.24 kg/ha, whereas 14 of the 18 untreated plants developed the disease in these same three experiments (Table 4). The effects of pendimethalin on crown rot caused by *P. megasperma* could not be evaluated because the pathogen caused little disease even on untreated plants in the experiments that examined this herbicide.

Differences in crown rot incidence among treatments were closely related to differences in pathogen baiting frequencies among the same treatments. For example, *P. cryptogea* was recovered from 96 to 99% of the leaf-disk baits in untreated pots; from 86% and 56 to 63% of the baits in pots treated with pendimethalin at 1.12 and 2.24 kg/ha, respectively; but from only 0 to 7% of the baits in pots treated with oryzalin, depending on the experiment (Table 4). Similarly, *P. cambivora* was recovered from 49 to 95% of baits from untreated pots; from 77% and 11 to 50% of baits from pots treated with pendimethalin at 1.12 and 2.24 kg/ha, respectively; but from only 0 to 2% of baits from pots treated with any of the three oryzalin rates. For *P. megasperma*, the respective frequencies were 20 to 47% for the untreated pots; 23% and 0 to 35% for

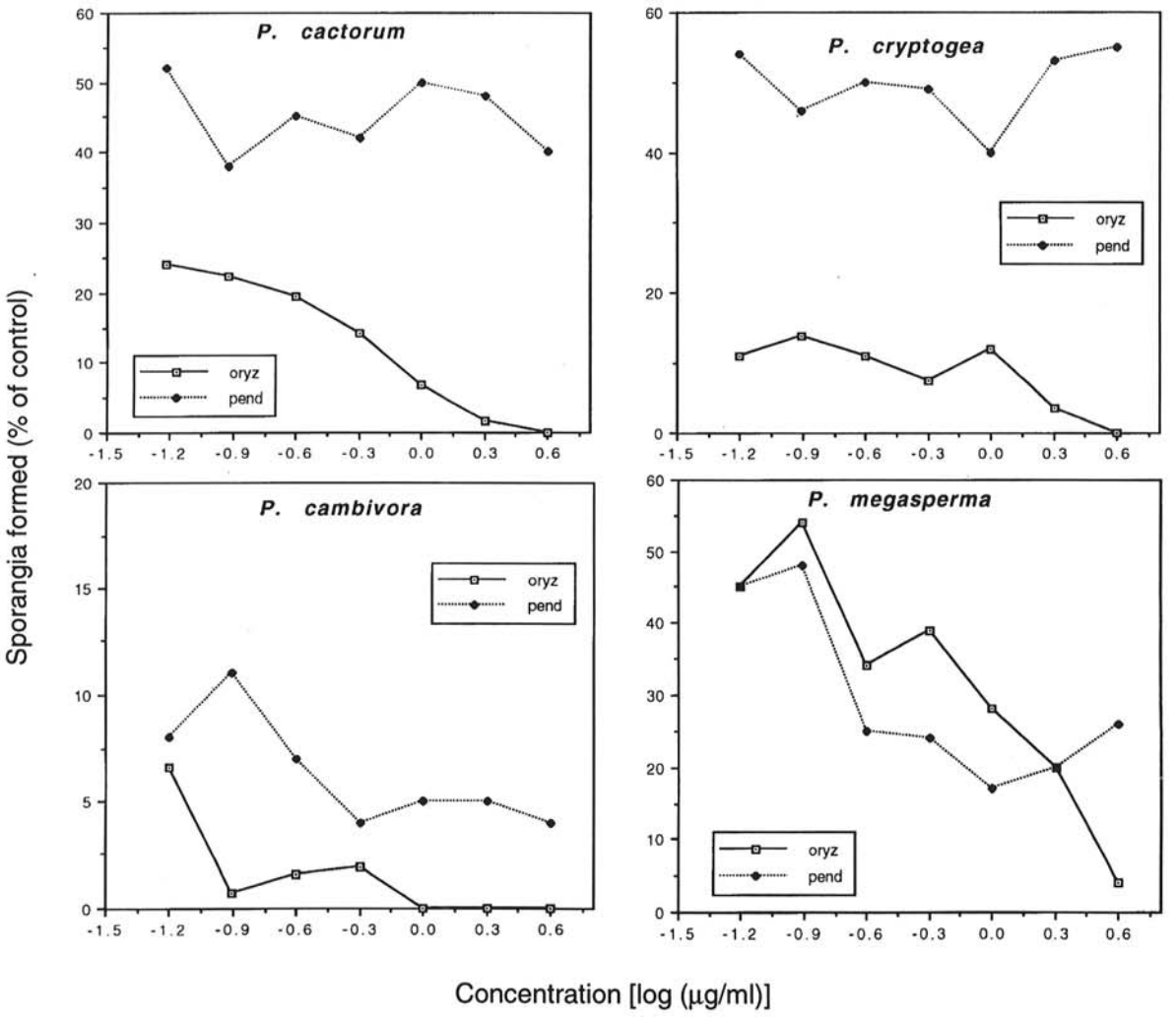

Fig. 2. Number of sporangia produced by four *Phytophthora* spp. after 24 h in soil extract amended with oryzalin or pendimethalin at concentrations of 0.0625 to 4.0 µg/ml, expressed as a percentage of the number of sporangia formed in control medium (soil extract amended with 0.5% ethanol).

pots treated with pendimethalin; and 0 to 2% for pots treated with oryzalin.

In the first experiment, treatment with the labeled rate of oryzalin at 3.36 kg/ha reduced the mean root weight of uninoculated seedlings by 38% relative to that of uninoculated seedlings in herbicide-free soil (significant at $P = 0.01$, t test). In subsequent experiments, when application rates of oryzalin and pendimethalin were reduced to 1.12 or 2.24 kg/ha to compensate for the high porosity of the soil mix, neither herbicide caused significant ($P = 0.05$) reductions in root weight nor any other symptoms of phytotoxicity.

DISCUSSION

Oryzalin consistently and significantly reduced the incidence of crown rot on Mahaleb cherry seedlings when soil mixes infested with *P. cambivora*, *P. cryptogea*, or *P. megasperma* were treated with nonphytotoxic herbicide rates. This effect was associated with a 92 to 100% reduction (relative to the untreated control) in the frequency with which the pathogens were baited during repeated flooding episodes, suggesting that it resulted from a corresponding reduction in the production and motility of their zoospores. Concentrations of oryzalin in the soil solution were not measured, but this value has been reported as 0.23 µg/ml in field soil at registered rates of application (22). When examined in vitro at a similar concentration (0.25 µg/ml), sporangium production was reduced by 98, 89, and 66% for the three *Phytophthora* spp., respectively, whereas zoospore motility was reduced by only 10 to 46% after 120 to 210 min of exposure. Similarly, although pendimethalin generally was more inhibitory than oryzalin to zoospore motility, it generally was less inhibitory to sporangium formation, disease development, and pathogen detection during flooding episodes. Thus, the reductions in disease incidence and baiting frequencies effected by these herbicides most likely were due to a reduction in sporangium (hence zoospore) production, although reduced zoospore motility also may have played a secondary role.

These results generally agree with those reported by others for the effects of dinitroanaline herbicides on common root rot of pea caused by *A. euteiches*. Although reduced disease severity in treated soils was originally attributed to an effect of the herbicides on the host rather than the pathogen (11), Grau (8) later showed that disease suppression was strongly associated with a concomitant reduction in the number of motile zoospores of the pathogen when soil solutions contained 0.03 to 0.25 µg of dinitramine or trifluralin per ml. Similarly, Teasdale et al. (22) found that four dinitroanaline herbicides, including oryzalin, completely inhibited the differentiation of primary spores into motile zoospores at a concentration of 0.10 µg/ml and concluded that the demonstrated disease suppression in soils treated with these herbicides was best explained by an inhibition of zoospore production. At similar concentrations, dinitroanaline herbicides have shown little suppression of mycelial growth of either *A. euteiches* (8,11) or the four *Phytophthora* spp. examined in the current study. Thus, the inhibition of mycelial growth obtained in the current study with higher concentrations of oryzalin is probably irrelevant to disease development under field conditions.

Oryzalin and other dinitroanaline herbicides profoundly interfere with the microtubules (MTs) of higher plants (2,19), moss (23), algae (20), protozoans (7), and fungi (15); indeed, they have been referred to as "the colchicines of the plant kingdom" (3). This effect appears to be caused by a binding of the chemicals to susceptible tubulin (12,20), which results in depolymerization of existing MTs and prevents polymerization of new ones in a variety of organisms at oryzalin concentrations relevant to field conditions, i.e., 0.035 to 0.35 µg/ml (7,15,19,23). The anti-MT activity of oryzalin directly disrupts the development of the flagellar stages of different microorganisms, e.g., it inhibits the differentiation of the protozoan *Leishmania mexicana* into its flagellated motile state (7), and it profoundly interferes with several steps in the differentiation and cleavage of zoospores within sporangia of *P. cinnamomi* (15). These effects have been attributed to the varied roles of MTs in the cytoskeleton of the affected cells. There have been no mechanistic studies that directly explain the suppression exerted by dinitroanaline herbicides on the initial formation of *Phytophthora* sporangia, as was observed in the current experiments. However, the anti-MT activity of oryzalin does impair the transport of nuclei in moss (23) and tobacco (2) and probably retards the transport of mitochondria in *P. cinnamomi* (15). Either or both mechanisms could suppress sporangium formation and, as a result, merit further study. Furthermore, because the flagella of *Phytophthora* zoospores are composed primarily of an array of MTs (10), anti-MT

TABLE 2. Analysis of variance for sporangium formation (percentage of control treatment) by four *Phytophthora* spp. in nonsterile soil extract amended with oryzalin or pendimethalin

Source	df	F value	$P > F$
All data[z]			
Replicate	4	1.97	0.10
Herbicide	1	18.29	0.0001
Species	3	45.27	0.0001
Herbicide × species	3	30.48	0.0001
Concentration	7	35.55	0.0001
Herbicide × concentration	7	5.63	0.0001
Species × concentration	21	0.70	0.83
Herbicide × species × concentration	21	0.77	0.76
P. cactorum			
Replicate	4	1.64	0.17
Herbicide	1	22.09	0.0001
Concentration	7	5.06	0.0002
Herbicide × concentration	7	1.82	0.10
P. cambivora			
Replicate	4	9.86	0.0001
Herbicide	1	5.29	0.03
Concentration	7	21.57	0.0001
Herbicide × concentration	7	0.72	0.66
P. cryptogea			
Replicate	4	7.02	0.0001
Herbicide	1	63.95	0.0001
Concentration	7	17.80	0.0001
Herbicide × concentration	7	5.26	0.0001
P. megasperma			
Replicate	4	7.64	0.0001
Herbicide	1	37.33	0.0001
Concentration	7	13.74	0.0001
Herbicide × concentration	7	2.96	0.01

[z] All four *Phytophthora* spp.

TABLE 3. Repeated measures analysis of variance for the persistence of motility by zoospores of three *Phytophthora* spp. in nonsterile soil extract 30 to 210 min after addition of oryzalin or pendimethalin

Source	df	F value	$P > F$
P. cactorum			
Replicate	3	1.4	0.24
Herbicide	1	2,233.0	0.0001
Concentration	7	109.7	0.0001
Herbicide × concentration	7	127.6	0.0001
P. cryptogea			
Replicate	3	52.08	0.0001
Herbicide	1	99.56	0.0001
Concentration	7	49.92	0.0001
Herbicide × concentration	7	8.59	0.0001
P. megasperma			
Replicate	3	54.82	0.0001
Herbicide	1	2.07	0.15
Concentration	7	128.39	0.0001
Herbicide × concentration	7	19.18	0.0001

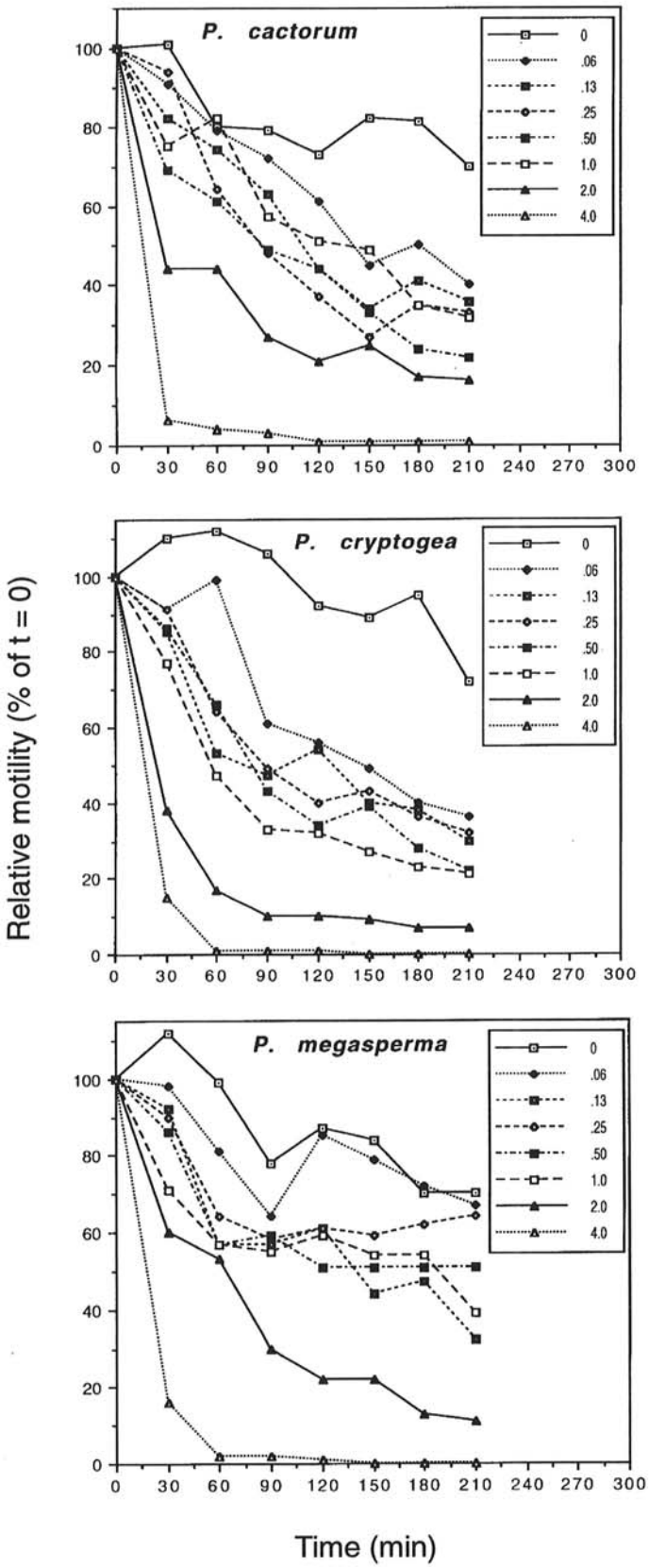

Fig. 3. Motility of zoospores of three *Phytophthora* spp. in soil extract amended with pendimethalin at concentrations of 0.0625 to 4.0 µg/ml, expressed as a percentage of the number of motile zoospores observed after various lengths of time after addition of the herbicide (or 0.5% ethanol for the 0 concentration) relative to the number observed immediately prior to treatment ($t = 0$).

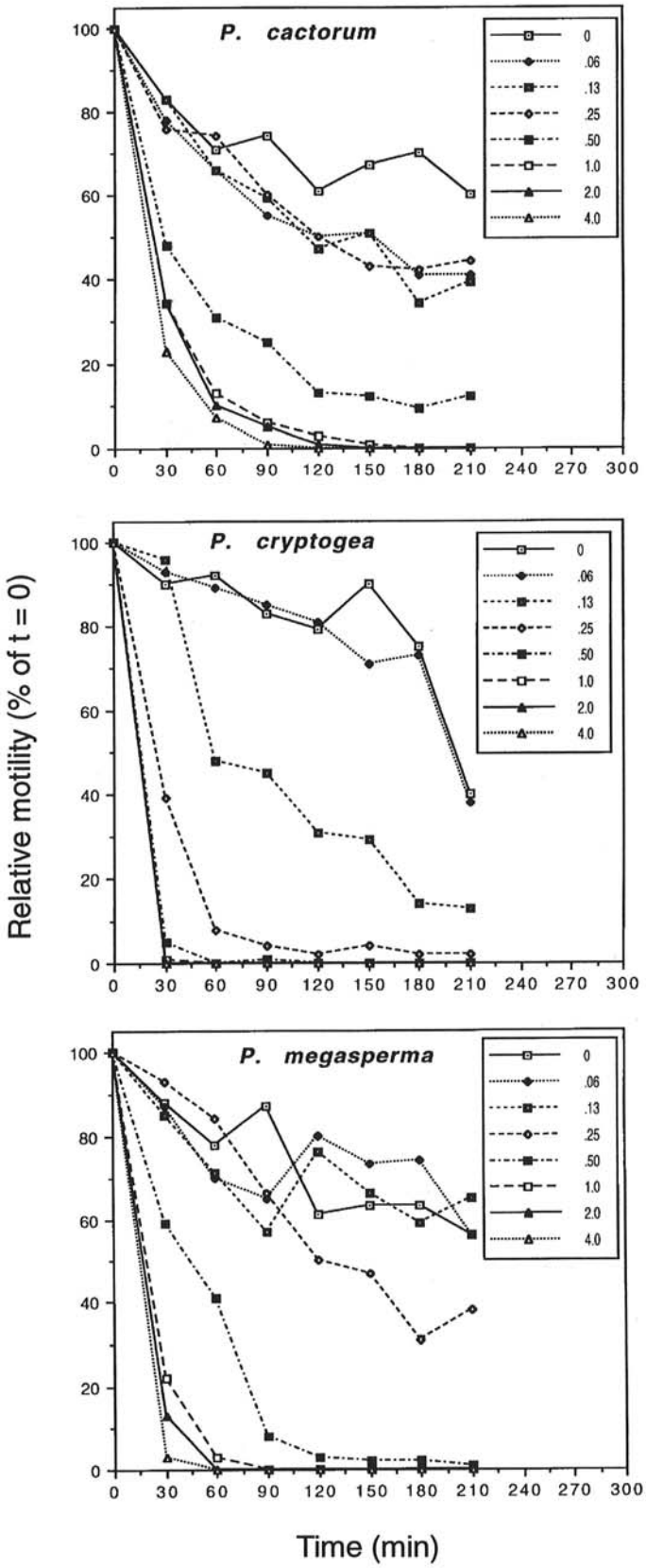

Fig. 4. Motility of zoospores of three *Phytophthora* spp. in soil extract amended with oryzalin at concentrations of 0.0625 to 4.0 µg/ml, expressed as a percentage of the number of motile zoospores observed after various lengths of time after addition of the herbicide (or 0.5% ethanol for the 0 concentration) relative to the number observed immediately prior to treatment ($t = 0$).

TABLE 4. Influence of dinitroanaline herbicides on the incidence of crown rot of Mahaleb cherry seedlings grown for 3 to 5 months in soil mix (1 volume of pasteurized sandy loam:2 volumes of fine vermiculite) artificially infested with three *Phytophthora* spp. and the frequency of pathogen baiting during biweekly flooding episodes

			Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Exp. 6	
Phytophthora sp.[v]	Herbicide[w]	Rate (kg/ha)	Crown rot[x]	% Baited[y]	Crown rot	% Baited	Crown rot	% Baited	Crown rot	% Baited	Crown rot	% Baited	Crown rot	% Baited
P. cryptogea	None	0.00	...[z]	...	...	...	6/6	97.2 a	6/6	96.2 a	6/6	97.8 a	3/6	98.9 a
	Oryzalin	1.12	...	...	...	...	0/6	3.3 b	...	...	0/6	5.6 c	0/6	7.2 c
		2.24	...	...	...	...	0/6	0.0 b	...	...	...	...	...	...
	Pendimethalin	1.12	...	...	...	...	...	...	6/6	86.1 a	...	...	...	...
		2.24	...	...	...	...	...	...	6/6	55.5 b	6/6	63.3 b	2/6	61.6 b
P. cambivora	None	0.00	5/6	58.1 a	5/6	62.4 a	5/6	76.1 a	5/6	95.3 a	6/6	86.0 a	3/6	48.9 a
	Oryzalin	1.12	...	...	0/6	0.0 b	1/6	1.6 b	...	...	0/6	0.0 c	0/6	0.0 c
		2.24	...	...	0/6	0.3 b	0/6	0.0 b	...	...	...	...	...	...
		3.36	0/6	0.0 b	...	...	...	...	...	...	...	...	...	...
	Pendimethalin	1.12	...	...	...	...	...	...	6/6	76.8 b	...	...	...	...
		2.24	...	...	...	...	...	...	0/6	50.0 c	0/6	20.0 b	0/6	11.1 b
P. megasperma	None	0.00	6/6	39.4 a	1/6	20.1 a	6/6	25.6 a	2/6	47.2 a	0/6	38.0 a	0/6	27.2 a
	Oryzalin	1.12	...	...	0/6	0.3 b	0/6	1.8 b	...	...	0/6	0.0 b	0/6	0.0 b
		2.24	...	...	0/6	0.0 b	0/6	0.0 b	...	...	...	...	...	...
		3.36	0/6	0.0 b	...	...	...	...	...	...	...	...	...	...
	Pendimethalin	1.12	...	...	...	...	...	...	1/6	23.2 ab	...	...	...	...
		2.24	...	...	...	...	...	...	0/6	35.2 b	0/6	0.0 b	0/6	6.1 b

[v] A single *Phytophthora* sp. was introduced into the soil of individual pots 1 day after herbicide applications, using leaf disks freshly colonized by the pathogen.
[w] Oryzalin and pendimethalin were sprayed onto the soil surface as formulated product (Surflan A.S. and Prowl 4E, respectively) suspended in tap water at a rate of 826 liters of spray solution per ha. No herbicide treatment caused phytotoxicity on check plants grown in uninfested soil, except for the 3.36 kg/ha rate of oryzalin in experiment 1.
[x] Incidence of crown rot on six replicate seedlings per treatment. Check plants grown in uninfested soil had no crown rot.
[y] Cumulative percentage of leaf-disk baits from which a *Phytophthora* sp. was isolated after each of five, nine, five, five, five, and six flooding episodes in experiments 1 through 6, respectively. Values represent the means from 36 individual leaf disks per treatment (6 disks per pot × 6 replicate pots) examined after each flooding episode. Data within a column (experiment) were analyzed separately for each *Phytophthora* sp.; means within these groups not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian k ratio t test. No *Phytophthora* spp. were recovered from disk baits in the uninoculated control treatments.
[z] Treatment not included in this experiment.

agents should have a deleterious structural effect on these organs; such an effect may account for the reductions in motility observed after both dinitroanalines were added to zoospore suspensions in the current investigation.

Although oryzalin (and to a lesser extent pendimethalin) had a pronounced effect on all examined *Phytophthora* spp., both in vitro and in greenhouse tests, the relevance of these findings to disease control under field conditions remains unknown. In fruit tree orchards, both herbicides are applied to the soil surface and leach only a short distance, e.g., 96% of the oryzalin applied to a sandy loam was retained in the top 4 cm (24). Thus, any disease control provided by these herbicides is likely to be restricted to a similar region near the soil surface. Although most of the tree root system lies more deeply and would be unaffected, it is within this narrow locus that fatal crown rot infections frequently occur on both pome (16) and stone fruit (5) trees. Perhaps relatedly, this is also the zone within which nearly half of the inoculum of *P. cactorum* has been detected within New York apple orchard soils (13,14). Thus, oryzalin in particular has the potential to provide a meaningful if limited contribution to an integrated program for control of *Phytophthora* crown rot of deciduous fruit trees under field conditions. The likelihood and magnitude of this potential can be determined only through appropriate orchard testing.

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