Effects of Flood Duration on the Development of Phytophthora Root and Crown Rots of Apple

G. T. Browne and S. M. Mircetich

Plant pathologists, Agricultural Research Service, U. S. Department of Agriculture, Department of Plant Pathology, University of California, Davis 95616.

Accepted for publication 25 January 1988 (submitted for electronic processing).

ABSTRACT

Browne, G. T., and Mircetich, S. M. 1988. Effects of flood duration on the development of Phytophthora root and crown rots of apple. Phytopathology 78:846-851.

Delicious apple seedlings were grown for 10–12 wk in artificially infested or noninfested U.C. mix soil on tension plates. A −20 mbar soil water matric potential (ψ) was maintained constantly or interrupted once every 2 wk by 4-, 12-, 24-, or 48-hr flood intervals ($\psi_m = 0$). At constant $\psi_m = -20$ mbar, *Phytophthora cactorum* caused relatively mild (mean 18% root rot) and *P. cambivora* caused relatively severe (mean 60% root rot) disease, but *P. cryptogea* caused no disease. As the duration of flood intervals lengthened from 4 to 48 hr, severity of disease caused by *P. cryptogea* increased progressively from mild (mean 1% root rot) to severe (mean 52% root rot), but severity of disease caused by *P. cactorum* and *P. cambivora* was moderate (mean 13–46% root rot) and severe (mean 80–89% root rot), respectively, and was not highly correlated with flood duration. When apple leaf disks colonized by *P. cactorum*, *P. cambivora*, or *P. cryptogea* were held for 2 days at $\psi_m = -20$ mbar, subsequent flooding was required for the production and release of significant numbers of zoospores. When host tissue pieces were used as bait for zoospores in the experiments on effects of flood duration on disease severity, the mean incidences of bait infection by *P. cactorum* (70, 15, and 13%) and *P. cambivora* (20, 7, and 8%) decreased over the intervals of baiting that were, respectively, 0–4 hr, 20–24 hr, and 44–48 hr after flooding onset, whereas the mean incidences of infection by *P. cryptogea* over the same baiting intervals increased (36, 44, and 62%, respectively).

Additional keywords: collar rot, *Malus pumila*, soil saturation.

Phytophthora root and crown rot is a serious problem for apple production in California; losses have occasionally been extensive and involved the decline and death of high percentages of trees in affected orchards (15). Isolations from affected trees and greenhouse pathogenicity tests have implicated *P. cactorum* (Leb. & Cohen) Schroet., *P. drechsleri* Tucker, *P. megasperma* Drechsler, *P. cambivora* (Petri) Buisman, *P. cryptogea* Pethyb. & Laff., and six additional but unidentified *Phytophthora* spp. as causes of root and crown rots of apple in California (16,18). Orchard surveys in the state have shown that root and crown rot is most frequent and severe where soil is subject to prolonged periods of water saturation associated with poor surface or subsoil drainage and/or improper irrigation management (16).

Flooded soil conditions can influence development of Phytophthora root and crown rots by affecting both the activity of the fungus and the susceptibility of the host. Water saturation stimulates optimum release of zoospores of *Phytophthora* spp. (12) and is required for effective movement of zoospores in or on soil (6). In addition, relatively wet soil conditions can stimulate germination of oospores, chlamydospores, and sporangia in the vicinity of host roots (10,19,20). Soil anaerobiosis caused by flooding can disrupt the function and structure of roots and increase root exudations that stimulate zoospore chemotaxis (4). Low levels of oxygen tension, likely to occur during prolonged flooding, disposed inoculated cherry roots and crowns to infection and colonization by *P. cryptogea* (24).

The influence of the duration of flooded periods on disease severity often differs with the host and *Phytophthora* sp. involved. For example, soil flooding before inoculation with zoospores predisposed cultivars of alfalfa and rhododendron to root disease caused by *P. megasperma* f. sp. *medicaginis* and *P. cinnamomi*, respectively (2,9), but did not predispose cherries to root disease caused by *P. cryptogea* (24) or Fraser fir seedlings to root rot caused by *P. cinnamomi* (8). In experiments with different cherry and walnut rootstocks, the level of disease that developed as flood duration increased depended on the particular combination of rootstock and *Phytophthora* sp. involved; some combinations required no flooding for severe root and crown rot to develop, whereas other combinations required prolonged flooding for severe disease to occur (14,23,24).

Little is known about specific influences of the duration of flooded periods in soil on severity of Phytophthora root and crown rot of apple. We, therefore, studied the effects of various durations of soil flooding on development and severity of apple root and crown rots caused by *P. cactorum*, *P. cryptogea*, and *P. cambivora*. We focused on the relationship of severity of the root and crown rots to production and dispersal of inoculum by the three *Phytophthora* spp. A portion of this work has been reported previously (3).

MATERIALS AND METHODS

Effects of duration of soil flooding on disease severity. Isolates of *P. cactorum* (isolate P321), *P. cryptogea* (P1426), and *P. cambivora* (P325) used in experiments originated from root or crown tissue of diseased apple trees in California. Inocula were prepared by growing the *Phytophthora* spp. for 4–5 wk at 18–22 C in 0.5-L jars of sterile V-8 juice-vermiculite medium (22). Before use, inocula were removed from the jars, placed in cheesecloth-lined funnels, and washed several times with tap water to remove unassimilated nutrients. Inoculum was mixed with steam-pasteurized U.C. mix (1) at a rate of 25 cm^3 of vermiculite inoculum per liter of U.C. mix. Control soil consisted of U.C. mix that contained vermiculite medium alone at the same rate.

Experiments were conducted in a greenhouse where supplemental incandescent lighting was used to maintain a 15-hr photoperiod. Soil water matric potential (ψ_m) was controlled between periods of flooding with fritted glass tension plates in 600-ml Büchner funnels as described by Duniway (5,6). The funnels were placed in racks, and each funnel was connected by rubber tubing to a 1-L reservoir of 0.5-strength Hoagland's solution. Aluminum foil was wrapped around funnels and reservoirs to exclude light and prevent growth of algae. Flood and inoculum treatments were randomized in a complete block design. About 300 ml of infested or noninfested U.C. mix was added in several layers of 1.5–2 cm in thickness to partially fill each funnel. After we added each layer of U.C. mix, the soil was tamped moderately and then stirred slightly at the surface before the next

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1988.

layer was added. Delicious apple seedlings (*Malus pumila* Mill.) were previously grown in U.C. mix for 5–6 wk in 30-ml plastic cups that had drainage holes. At the end of the growing period, the root sytems of the seedlings had permeated the volume of the soil within the cups, and most of the roots were less than 2 mm in diameter. Just before the seedlings were transplanted, the shoots of the seedlings were trimmed to three or four leaves, and the portions of any roots that protruded through the drainage holes of the growing cups were removed. The seedlings and the 30 ml of U.C. mix around the roots were transplanted into the funnels (one seedling per funnel). Depending on the treatment, additional infested or noninfested soil was added so that each funnel finally contained 500 ml of soil at a bulk density of approximately 1.1 g/cm . About 150 ml of distilled water was then added to the soil in each funnel to establish moisture continuity between the soil and the tension plates. Liquid column length between the tension plates and reservoir fluid level was adjusted so that ψ_m of soil at middepth in the funnels (used as the reference point) was −20 mbar. Soil ψ_m at the top and bottom of the funnels was therefore −23.5 and −16.5 mbar, respectively; however, since no attempt was made to reduce transpiration of the seedlings by covering them with plastic bags, the ψ_m of the surface soil probably dropped below −23.5 mbar on occasion. Distilled water was added into the 1-L reservoirs of nutrient solution as needed to maintain proper reservoir fluid levels during the experiment.

Apple seedlings grown in noninfested soil or soil infested with *P. cactorum*, *P. cryptogea*, or *P. cambivora* were subjected to five separate soil moisture treatments: a constant soil ψ_m of −20 mbar, or a soil ψ_m of −20 mbar interrupted by 4, 12, 24, or 48 hr of flooding (ψ = 0 mbar) once every 2 wk. One week after transplanting, the flood treatments were initiated by clamping the rubber tubing between tension plates and fluid reservoirs and adding distilled water at 20–21 C to the soil until a 0.5–1.0 cm depth of water covered the surface of soil in funnels. Floods were ended by removing the clamps, and the surface water drained within 15 min.

At the end of experiments, shoot fresh weights were recorded. After being washed free from soil and blotted for excess moisture, root systems were weighed and visually evaluated for extent of root and crown rot. Roots were considered rotted if dark red to brown decay extended into the stele, and crown rot was identified by decay that occurred on the lower stem and/or main root axis. A crown rot score was assigned to each root system based on a visual estimate of the proportion of stem circumference girdled with crown rot; 0 was for no crown rot, 1 was for > 0 to 1/4 girdled, 2 was for > 1/4 to 1/2 girdled, 3 was for > 1/2 to < completely girdled, and 4 was for completely girdled. Root isolations were made on PVP selective medium (16) to confirm infection by each of the *Phytophthora* sp. from infested soil and the absence of infection from noninfested soil.

The experiment was done three times. In the first and second experiments, treatments were replicated five and four times, respectively, and U.C. mix infested with *P. cambivora* was subjected only to the 0-, 4-, and 48-hr flood treatments. Soil temperature ranged from 18 to 25 C in the first two experiments, and the plants were harvested and rated for disease severity 12 wk after transplanting into the Büchner funnels. In the third experiment, the complete set of flood (0, 4, 12, 24, and 48 hr) and inoculum (*P. cactorum*, *P. cambivora*, *P. cryptogea*, and control) treatments was replicated five times, soil temperature ranged from 18 to 30 C, and plants were harvested and rated for severity of disease 10 wk after transplanting.

Results were similar in all three experiments on effects of flood duration on severity of disease; therefore, results are shown only for the third experiment. To meet the normality requirements for analysis of variance, the root and shoot fresh weight data values were transformed to $\log_{10}$ values after being multiplied by 10. The multiplication was done, as recommended by Little and Hills (11), to avoid negative logarithms; the $\log_{10}$ transformation was used because standard deviations of the treatment means of the untransformed data increased with the magnitude of the means. The transformed root and shoot weights were subjected to a series of orthogonal contrasts to study the effects of flooding and inoculum treatments.

Trapping of zoospores in flood water. Zoospore traps were constructed and employed to investigate, in situ, the relationship of zoospore activity to disease severity in the experiments that used Büchner funnels to control flood duration. The trap containers were 89-ml cups with 3.5-cm-diameter openings cut out in the bottoms. The bottom openings in the cups were covered either with a nylon-mesh screen (openings 1.3 mm) that supported a 5–10 mm layer of builders sand or, alternatively, with a nylon cloth (openings 10 μm) and no sand. Both coverings were designed to prevent structures of *Phytophthora* other than motile zoospores from entering the trap cups during trapping episodes.

One trap cup was placed next to an apple seedling at the beginning of a 48-hr flood treatment for each *Phytophthora* sp. The cups were placed so that the trap bottom openings contacted the soil surface in the Büchner funnels. Ten to 12 6-mm-diameter leaf disks that were freshly cut from fully expanded apple leaves or, alternatively, 20–30 1-cm × 1-mm excised apple roots were used in each trap cup as zoospore bait. After we flooded the U.C. mix, successive sets of bait pieces were placed to remain in the surface water of the trap cups for 4 hr at the intervals of 0–4, 20–24, and 44–48 hr after flood initiation. At the end of each of the 4-hr baiting intervals, all of the bait pieces were removed from the trap cups, and 10 of the bait pieces from each of the traps were briefly rinsed in tap water and plated in PVP selective medium. The trap cups were removed from the soil surface when each 48-hr baiting experiment (run) ended. The selective medium plates were observed daily for 1 wk to determine the number of tissue pieces colonized by *P. cactorum*, *P. cambivora*, and *P. cryptogea*, and the percent colonized was calculated. The percentages of infection shown in the results section are means from 11 runs of the baiting treatments. The 11 runs were distributed over the three experiments on effects of flood duration on disease severity. The design of trap cups and type of bait tissue used were identical within runs but varied between runs as described above. The data were subjected to analysis of variance, which included a set of orthogonal contrasts used to investigate effects of *Phytophthora* spp. and interval of baiting on percent of bait pieces infected.

Effects of soil matric potential on production and indirect germination of sporangia. The effects of maintaining soil ψ_m at −20 mbar and of soil flooding (ψ_m = 0 mbar) on production and indirect germination of sporangia were investigated in a growth chamber where soil temperature was 19–20 C. Leaf disks (6 mm in diameter) were cut from Delicious apple seedlings, surface sterilized, and then colonized for 48 hr by *P. cactorum*, *P. cryptogea*, or *P. cambivora* after placement at the margin of V-8 agar cultures as described by Sugar (21). A 5-mm layer of U.C. mix that was previously passed through a 2-mm sieve was lightly packed onto Büchner funnel tension plates, and a small amount of tap water was added to establish moisture continuity between the soil and tension plate. Twenty to 25 colonized leaf disks were placed on the soil surface. An additional 10 mm of the sieved U.C. mix was placed and lightly packed on top of the leaf disks, then an additional small amount of tap water was added to establish moisture continuity between soil, leaf disks, and tension plates. Water column length below the tension plates was adjusted so that soil ψ_m at the level of leaf disks was −20 mbar, and funnels were covered with loosely fitting polyethylene bags to reduce evaporation.

After a 2-day incubation period in soil at ψ_m = −20 mbar, leaf disks were subjected to one of two soil moisture treatments for an additional 48 hr. Soil in three of the funnels (each funnel contained leaf disks colonized by only one of the three *Phytophthora* spp.) was flooded (ψ_m = 0) so that 2–3 mm of water stood on the soil surface, and soil in three other funnels, each containing leaf disks colonized by one of the three *Phytophthora* spp., remained at ψ_m = −20 mbar. Immediately before the flooding started, four leaf disks for each *Phytophthora* sp. were removed from soil in funnels. Four leaf disks for each *Phytophthora* sp. were also removed from both flooded (ψ_m = 0) and nonflooded (ψ_m = −20 mbar) soil 4, 12, 24, and 48 hr after initiation of the differential treatments. Leaf disks

were removed carefully with a small spatula to avoid disturbing other disks that were recovered subsequently.

After removal from soil, leaf disks were briefly and gently rinsed in tap water and stained in lacto-fuchsin (100 mg of acid-fuchsin per 100 ml of 85% lactic acid). Full and empty sporangia extending from edges of the leaf disks were counted under a microscope at 125×, and it was assumed that empty sporangia had germinated indirectly. The experimental treatments were run three times; therefore, the numbers of sporangia reported in the results section are means of the numbers present on 12 leaf disks.

RESULTS

Effects of duration of soil flooding on disease severity. The main effects as well as the interaction of flooding and inoculum treatments had highly significant ($P \leq 0.01$) effects on root and shoot fresh weights (Fig. 1A and B; Table 1). Flooding ($\psi_m = 0$, for 4–48 hr) caused greater reduction of root and shoot fresh weights compared with the nonflooded treatments ($\psi_m = -20$ mbar) when soil was infested with *Phytophthora* than when soil was noninfested (Contrast 1, Table 1). When reductions of root and shoot fresh weight caused by flooded as compared with nonflooded treatments were contrasted between *Phytophthora* inoculum treatments, the contrast was not significant between *P. cryptogea* vs. *P. cactorum* and *P. cambivora* (Contrast 2, Table 1), but was significant for shoot fresh weights between *P. cactorum* vs. *P. cambivora* (Contrast 3, Table 1); apparently the occurrence of soil flooding favored a greater increment of disease increase with *P. cambivora* than with *P. cactorum* (Fig. 1A and B).

The linear component of root and shoot fresh weight reductions as flood duration was lengthened from 4 to 48 hr did not differ significantly between the plants grown in noninfested soil versus the plants grown in the treatments artificially infested with *Phytophthora* (Contrast 4, Table 1). However, the linear trend of reduction in root and shoot fresh weight as flood duration was lengthened from 4 to 48 hr was significantly greater with *P. cryptogea* than with *P. cactorum* and *P. cambivora* (Contrast 5, Table 1, Fig. 1A and B); severity of disease caused by *P. cryptogea* increased progressively as flood duration was lengthened from 4 to 48 hr, whereas severity of disease caused by *P. cactorum* and *P. cambivora* was not closely correlated with the duration of flooding from 4 to 48 hr. *P. cambivora* reduced root and shoot fresh weights more than *P. cactorum* did (Fig. 1A and B), but there was no significant difference between *P. cactorum* and *P. cambivora* when comparing the linear trends of fresh weight reduction in the flooded treatments (Contrast 6, Table 1).

Root fresh weights were negatively correlated ($r = -0.77$) with percent root rot when untransformed data values were used. Only negligible root rot (means less than 5%) occurred in the control treatments (Fig. 1C). In the soil infested with *P. cryptogea*, less than 4% root rot developed in the 0-, 4-, and 12-hr flood treatments, but as flood duration was lengthened to 24 and 48 hr, root rot increased to a mean of 40 and 52%, respectively (Fig. 1C). *P. cactorum* caused mild to moderate (means 13–46%) root rot, and *P. cambivora* caused severe (means 60–89%) root rot. Severity of root rot caused by these species was not as closely related to the duration of flooding as was severity of root rot caused by *P. cryptogea* (Fig. 1C).

Crown rot scores were less correlated ($r = -0.65$) with root fresh weights than was percent root rot. Nevertheless, *P. cryptogea* caused no crown rot in the nonflooded, the 4-, or the 12-hr flood treatments but caused moderate crown rot (mean crown rot score of 2.0) in the 24- and 48-hr flood treatments. Crown rot scores in soil infested with *P. cactorum* and *P. cambivora* ranged from 0 to 1.6 and 0.8 to 3.8, respectively. The severity of crown rot caused by *P. cactorum* and *P. cambivora* was not closely related to the duration of flooding but generally tended to be less in the absence of flooding.

Trapping of zoospores in flood water. When the zoospore traps were used in situ during the three experiments on effects of flood duration on disease severity, there was a highly significant ($P \leq 0.01$) interaction of interval of baiting × *Phytophthora* sp. and a significant ($P \leq 0.05$) interaction of run × *Phytophthora* sp. (Table 2). Orthoganol contrasts revealed highly significant ($P \leq 0.01$) differences in the linear trends of bait infection over the successive intervals of baiting between *P. cryptogea* vs. *P. cactorum* and *P. cambivora* (Contrast 1, Table 2) and between *P. cactorum* vs. *P. cambivora* (Contrast 2, Table 2). While the mean frequency of infection of *P. cryptogea* increased from 36 to 44 to 62% over the successive 4-hr intervals of baiting that were, respectively, 0–4, 20–24, and 44–48 hr after the onset of flooding, mean percent

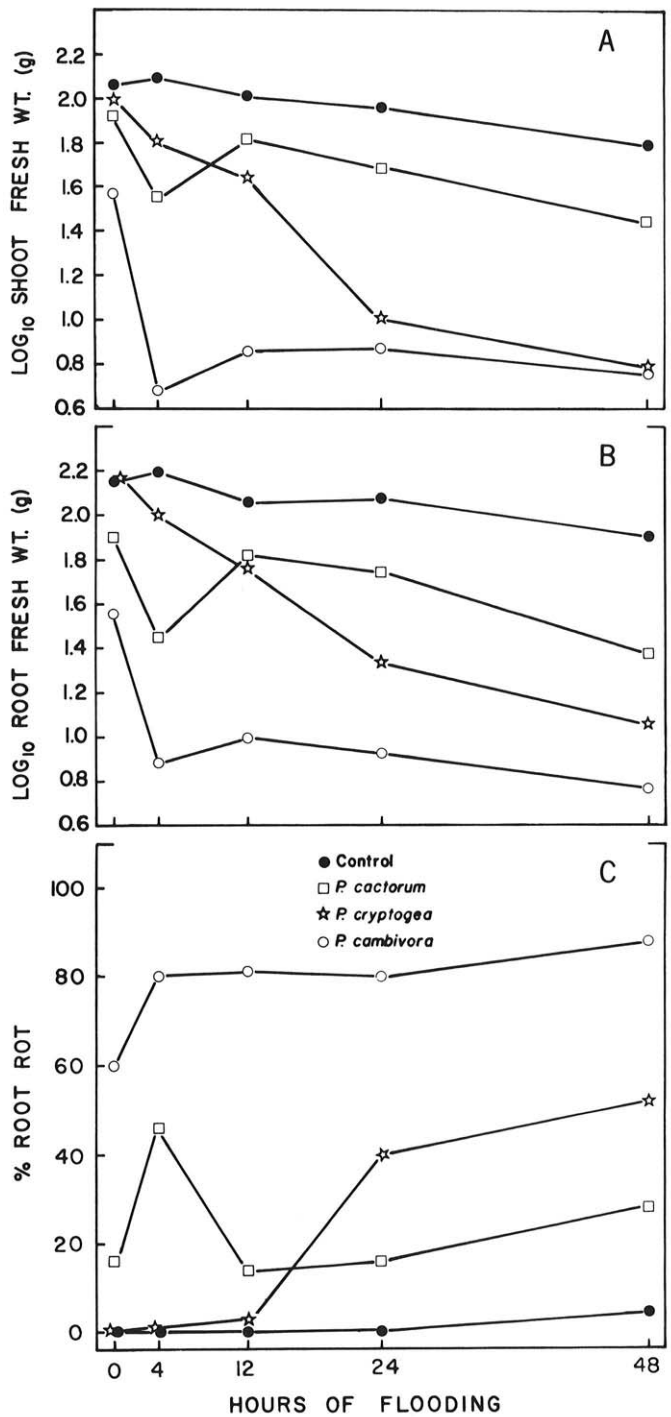

Fig. 1. Effects of flood duration on: **A,** $\log_{10}$ shoot fresh weight; **B,** $\log_{10}$ root fresh weight; and **C,** percent root rot of Delicious apple seedlings grown for 10 wk in soil artificially infested with *Phytophthora cactorum*, *P. cambivora*, or *P. cryptogea*. Soil water matric potential (ψ_m) was maintained at −20 mbar except when interrupted by the biweekly intervals of flooding indicated. Before the logarithms were calculated, the data values were multiplied by 10. Data points are means of five replicates per treatment.

infection over the respective intervals decressed in treatments with *P. cactorum* from 70 to 15 to 13%, and in treatments with *P. cambivora* from 20 to 7 to 8% (Fig. 2). The magnitude of decrease in percent bait pieces infected over the successive intervals of baiting was greater for *P. cactorum* than for *P. cambivora* since percent infection by *P. cactorum* was relatively high from the first baiting interval (Fig. 2). There was no signficant interaction of quadratic trend over intervals of baiting × *P. cryptogea* vs. *P. cactorum* and *P. cambivora* (Contrast 3, Table 2) or quadratic trend over intervals of baiting × *P. cactorum* vs. *P. cambivora* (Contrast 4, Table 2).

Effects of soil water matric potential on production and indirect germination of sporangia. The numbers of sporangia formed per leaf disk colonized by *P. cactorum* remained relatively constant (mean 62–145) during the 48-hr period of differential soil moisture treatments of $\psi_m = 0$ and $\psi_m = -20$ mbar (Fig. 3A). *P. cactorum* produced fewer sporangia than either *P. cryptogea* or *P. cambivora* when soil was flooded ($\psi_m = 0$) after the initial 2-day incubation at $\psi_m = -20$ mbar (Fig. 3A–C). *P. cactorum* sporangia did not germinate indirectly in soil held constantly at $\psi_m = -20$ mbar, but about 5% of the sporangia germinated indirectly in soil within 4, 12, 24, and 48 hr under flooded conditions (Fig. 3A).

P. cryptogea formed relatively large numbers of sporangia (mean 317) per leaf disk within the initial 2 days at $\psi_m = -20$ mbar. Over the following 24 hr, the numbers of sporangia of *P. cryptogea* present on leaf disks increased to a mean of 661 and 525 sporangia per leaf disk in the $\psi_m = 0$ and $\psi_m = -20$ mbar treatments, respectively. Less than 0.5% of the sporangia of *P. cryptogea* were germinated indirectly in the soil held continuously at $\psi_m = -20$

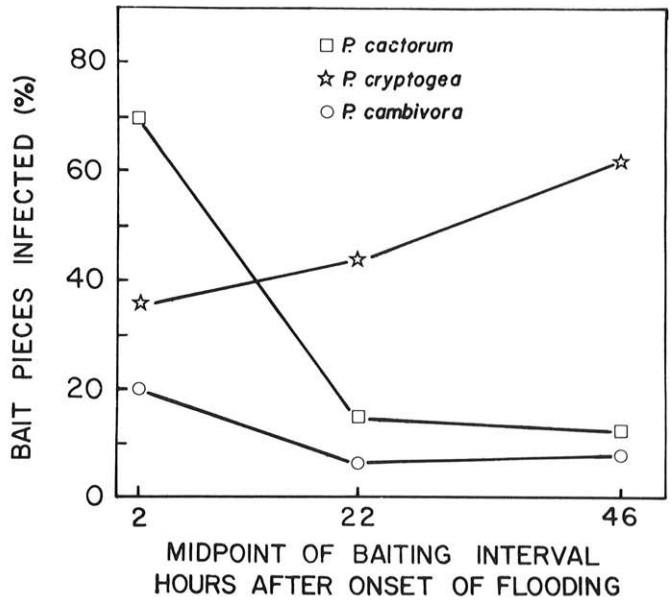

Fig. 2. Percent infection of apple leaf disks and root pieces used as bait for zoospores of three *Phytophthora* spp. in experiments on effects of duration of soil flooding on disease severity. Successive sets of the bait pieces were exposed for 4-hr intervals that were 0–4, 20–24, and 44–48 hr after the onset of flooding. The percentages are means from 11 runs of the baiting treatments and are based on the number of bait pieces from which the appropriate *Phytophthora* sp. was isolated on selective medium.

TABLE 1. Analysis of variance of $\log_{10}$ values[a] of root and shoot fresh weight data from a representative experiment on effects of flood duration on severity of root and crown rot of apple caused by three *Phytophthora* spp.

Source of variation	df	Root fresh weight MS	Root fresh weight F[b]	Shoot fresh weight MS	Shoot fresh weight F[b]
Block	4	0.1087	1.27	0.1074	2.28
Flooding	4	1.1318	13.27**	1.2567	26.69**
Inoculum	3	4.6435	54.44**	4.7484	100.86**
Flooding × inoculum	12	0.2276	2.67**	0.2890	6.14**
Contrasts					
1) Nonflooded[c] vs. flooded[d] × Control vs. *Phytophthora*	(1)	0.5340	6.26*	0.6627	14.08**
2) Nonflooded vs. flooded × *P. cryptogea* vs. *P. cambivora* and *P. cactorum*	(1)	0.0617	0.72	0.0330	0.70
3) Nonflooded vs. flooded × *P. cactorum* vs. *P. cambivora*	(1)	0.2792	3.27	0.4738	10.06**
4) Linear trend in flooded × Control vs. *Phytophthora*	(1)	0.0573	0.67	0.0275	0.58
5) Linear trend in flooded × *P. cryptogea* vs. *P. cambivora* and *P. cactorum*	(1)	1.0460	12.26**	1.7410	36.98**
6) Linear trend in flooded × *P. cactorum* vs. *P. cambivora*	(1)	0.0021	0.02	0.0744	1.58
Error	74	0.0853		0.04708	

[a] Root and shoot fresh weights were multiplied by 10 before transformation to $\log_{10}$ values to avoid negative logarithms.
[b] (*) follows F values significant at $P \leq 0.05$, (**) follows F values significant at $P \leq 0.01$.
[c] ψ_m held constantly at −20 mbar.
[d] $\psi_m = -20$ mbar interrupted once every 2 wk with 4–48-hr flooded periods.

TABLE 2. Analysis of variance of percent infection of apple leaf disks and root pieces used as bait for zoospores of three *Phytophthora* spp. in experiments on effects of duration of soil flooding on disease severity

Source of variation	df	MS	F[b]
Run[a]	10	1,798.42	2.92*
Interval of baiting	2	3,614.15	5.88**
Phytophthora sp.	2	10,652.87	17.33**
Run × *Phytophthora* sp.	20	1,398.98	2.28*
Run × interval of baiting	20	678.01	1.10
Interval of baiting × *Phytophthora* sp.	4	5,236.16	8.52**
Contrasts			
1) Linear trend over intervals of baiting × *P. cryptogea* vs. *P. cactorum* and *P. cambivora*	(1)	12,924.0	21.02**
2) Linear trend over intervals of baiting × *P. cactorum* vs. *P. cambivora*	(1)	5,381.4	8.75**
3) Quadratic trend over intervals of baiting × *P. cryptogea* vs. *P. cactorum* and *P. cambivora*	(1)	1,002.4	1.63
4) Quadratic trend over intervals of baiting × *P. cactorum* vs. *P. cambivora*	(1)	1,636.8	2.66
Error	30	614.67	

[a] A run consisted of one replication of the nine treatment combinations of intervals of baiting and *Phytophthora* sp.
[b] (*) follows F values significant at $P \leq 0.05$, (**) follows F significant at $P \leq 0.01$.

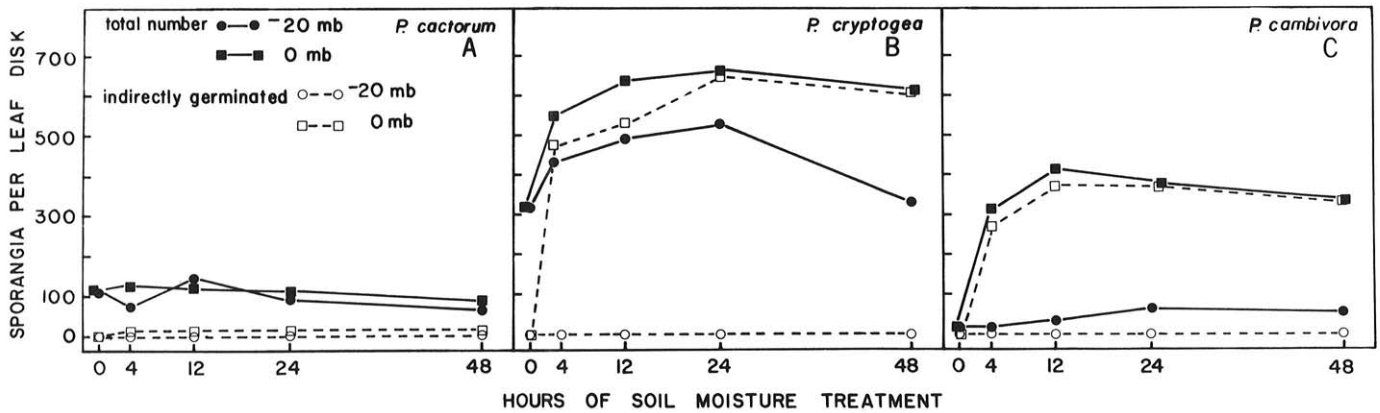

Fig. 3A–C. Effects of soil moisture level on production and indirect germination of sporangia by three *Phytophthora* spp. Leaf disks colonized by mycelium were initially held in U.C. mix at $\psi_m = -20$ mbar for 2 days, after which half of the leaf disks were subjected to flooding ($\psi_m = 0$) and the other half remained in soil at $\psi_m = -20$ mbar. Total numbers (full and empty) of sporangia per leaf disk are plotted as a function of the time after the final soil moisture conditions were imposed. Full sporangia had not germinated and it was assumed that empty sporangia had germinated indirectly. Data points are means of the numbers of sporangia present on 12 leaf disks.

mbar, but 86% of the sporangia were germinated indirectly at 4 hr after flooding started, and nearly all of them were germinated indirectly within 24 hr after flooding ($\psi_m = 0$) started (Fig. 3B).

In contrast to results with *P. cryptogea*, very few sporangia (mean 15, Fig. 3C) were formed per leaf disk colonized by *P. cambivora* during the initial 2-day incubation in soil at $\psi_m = -20$ mbar, and only small numbers of sporangia (mean 17–63, Fig. 3C) were formed by *P. cambivora* during an additional 48 hr at $\psi_m = -20$ mbar. However, flooding the soil after the initial 2 days in soil at $\psi_m = -20$ mbar caused a rapid and large increase to a mean of over 400 sporangia formed by *P. cambivora* per leaf disk within 12 hr. Furthermore, most sporangia of *P. cambivora* germinated indirectly within a few hours after formation in flooded soil (Fig. 3C).

DISCUSSION

Our results showed that soil moisture conditions that stimulate the release and infective activity of zoospores are essential for development of apple root and crown rot caused by *P. cryptogea*. When soil was maintained at $\psi_m = -20$ mbar, less than 0.5% of sporangia of *P. cryptogea* released zoospores (Fig. 3B), and no root or crown rot or significant fresh weight reductions resulted (Fig. 1A–C); only the treatments subjected to flooding developed disease in soil infested with *P. cryptogea*.

With *P. cactorum* and *P. cambivora*, flooded soil conditions that stimulated the release of zoospores favored disease development, but, in contrast to results with *P. cryptogea*, were not essential for development of root and crown rot. *P. cactorum* induced mild and *P. cambivora* induced severe fresh weight reductions and root and crown rot under nonsaturated conditions ($\psi_m = -20$ mbar) that prevented the release of large numbers of zoospores (Fig. 1A–C and 3A and C). It may be possible that other structures in addition to zoospores are important in initiating infection caused by *P. cactorum* and *P. cambivora* in apple. It also seems likely that since the apple seedlings were highly susceptible to *P. cambivora*, a relatively small number of infections were required to induce severe disease caused by this *Phytophthora* sp.

The influence of soil flooding on severity of disease caused by *P. cryptogea* and *P. cambivora* in this study was similar to that in previous studies with cherries (23,24) and walnuts (14). In highly susceptible Mahaleb cherry seedlings, *P. cambivora* incited significant root and crown rot in soil at constant $\psi_m = -10$ or -25 mbar and severe disease with relatively short durations of flooding. In walnuts and cherries, severity of disease caused by *P. cryptogea* increased to the duration of soil flooding episodes.

In soil infested with *P. cactorum*, the seemingly inordinate decline in root and shoot fresh weights and increase in percentage of root rot in plants flooded for 4 hr compared with the nonflooded, the 12-, and the 24-hr flood treatments (Fig. 1A–C) occurred in two of the three experiments. Results of the baiting study suggested that in the flood water above the soil surface, infective zoospore activity of *P. cactorum* was greater during the first 4 hr of flooding than during the intervals 20–24 or 44–48 hr after initiation of flooding. Perhaps draining of the flood water after the 4 hr of flooding resulted in a passive movement of motile zoospores which favored distribution around and infection of apple roots, but this was not investigated.

The results of the zoospore trapping experiments provide some evidence for a relationship between the duration of infective zoospore activity in flood water and the influence of flood duration on severity of disease caused by *P. cactorum*, *P. cryptogea*, and *P. cambivora*. It can be interpreted from results in Figure 2 that infective activity of zoospores of *P. cactorum* and *P. cambivora* was maximum during the initial hours of flooded periods, and prolonging floods beyond periods of maximum zoospore activity did not increase severity of disease caused by these two species (Fig. 1A–G). In contrast, infective activity of zoospores of *P. cryptogea* in flood water apparently increased during the 48 hr when zoospore trapping was done (Fig. 2), and the severe root rot and fresh weight reductions caused by *P. cryptogea* with long flooded periods (Fig. 1A–C) may have resulted from prolonged activity or continuous production of zoospores of *P. cryptogea* and their movement to root and crown tissue.

It seems likely that prolonged recovery of zoospores of *P. cryptogea* in the surface flood water was not entirely dictated by prolonged motility of individual zoospores in the flooded soil profile; MacDonald (13) reported that most zoospores of *P. cryptogea* did not remain motile in flooded soil for more than a few hours, although zoospores can remain motile for longer periods in liquid systems in the absence of soil particles (7).

The results from experiments on the effects of soil ψ_m on the production and indirect germination of sporangia (Fig. 3), and the results from experiments where zoospores were trapped (Fig. 2) provided measures of the production of inoculum and the infective activity of inoculum, respectively. Flooding of the soil ($\psi_m = 0$) after incubation at 20 mbar stimulated the production and indirect germination of sporangia by *P. cambivora* and *P. cryptogea* (Fig. 3) and, likewise, facilitated infective activity of zoospores in ways that were characteristic for each of these two species (Fig. 2). Compared with *P. cryptogea* and *P. cambivora*, *P. cactorum* produced relatively low numbers of sporangia in flooded soil ($\psi_m = 0$) from colonized leaf disks (Fig. 3A–C). However, in the baiting experiment (Fig. 2). *P. cactorum* infected a relatively high percentage of bait pieces after the first 4-hr interval of baiting compared with the other two species. This apparent paradox suggests that conditions such as temperature and substrate for inoculum production, which differed between the experiments that used colonized leaf disks (Fig. 3) and the baiting experiments (Fig. 2), may have interacted differently with *P. cactorum* than with *P.*

cryptogea and *P. cambivora*.

Several factors may have contributed to the statistically significant interaction of run × *Phytophthora* sp. in the baiting experiments where zoospores were trapped (Table 2). Factors such as temperature, type of baiting tissue (i.e., root pieces or leaf disks), and differential changes in inoculum density of *P. cactorum*, *P. cambivora*, and *P. cryptogea* over successive runs of baiting experiments may have contributed to the interaction, but the experimental design did not permit evaluation of these possible effects.

Our studies indicate that careful management of soil water to limit periods of soil saturation may be successful in minimizing losses of apples to root and crown rot caused by *P. cryptogea*. Soil water management may be less successful in limiting the disease in apple caused by *P. cactorum* and *P. cambivora*. However, because small deficits in soil water matric potential lessened disease caused by all three of these *Phytophthora* spp., using horticultural practices that limit the occurrence of prolonged and frequent periods of soil saturation, especially around tree crowns, such as strategically applying irrigation water, planting trees on raised berms, and providing for good surface and internal soil drainage, may reduce losses due to root and crown rot caused by *P. cactorum* and *P. cambivora*. The use of apple rootstocks that are relatively resistant to the disease is presently under investigation and may augment the benefits of careful soil water management.

LITERATURE CITED

1. Baker, K. F. 1972. The U.C. system for producing healthy container grown plants. Calif. Agric. Exp. Stn. Man. 23:68-86.
2. Blaker, N. S., and MacDonald, J. D. 1981. Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to Phythphthora root and crown rot. Phytopathology 71:831-834.
3. Browne, G. T., and Mircetich, S. M. 1984. Effects of flood duration on severity of apple root and crown rot caused by three *Phytophthora* spp. (Abstr.) Phytopathology 74:1136.
4. Drew, M. C., and Lynch, J. M. 1980. Soil anaerobiosis, microorganisms, and root function. Annu. Rev. Phytopathol. 18:37-66.
5. Duniway, J. M. 1975. Formation of sporangia by *Phytophthora drechsleri* in soil at high matric potentials. Can. J. Bot. 53:1270-1275.
6. Duniway, J. M. 1976. Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. Phytopathology 66:877-882.
7. Hickman, C. J. 1970. Biology of *Phytophthora* zoospores. Phytopathology 60:1128-1135.
8. Kenerly, C. M., Papke, K., and Bruck, R. I. 1984. Effect of flooding on development of Phytophthora root rot in Fraser fir seedlings. Phytopathology 74:401-404.
9. Kuan, T.-L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.
10. Kuan, T.-L., and Erwin, D. C. 1982. Effect of soil matric potential on Phytophthora root rot of alfalfa. Phytopathology 72:543-548.
11. Little, T. M., and Hills, F. J. 1978. Agricultural Experimentation. John Wiley & Sons, New York. 350 pp.
12. MacDonald, J. D., and Duniway, J. M. 1978. Influence of the matric and osmotic component of water potential on zoospore discharge in *Phytophthora*. Phytopathology 68:751-755.
13. MacDonald, J. D., and Duniway, J. M. 1978. Influence of soil texture and temperature on the motility of *Phytophthora cryptogea* and *P. megasperma* zoospores. Phytopathology 68:1627-1630.
14. Matheron, M. E., and Mircetich, S. M. 1985. Influence of flooding duration on development of Phytophthora root and crown rot of *Juglans hindsii* and Paradox walnut rootstocks. Phytopathology 75:973-976.
15. Mircetich, J. M. 1975. Phytophthora root and crown rot of orchard trees. Calif. Plant Pathol. 24:1-2.
16. Mircetich, S. M., and Browne, G. T. 1987. Phytophthora root and crown rot of deciduous fruit trees: Progress and problems in etiology, epidemiology and control. Pages 64-95 in: Proc. of the Summerland Res. Stn. Commemorative Symposium: Challenges and Opportunities in Fruit Production, Protection and Utilization Research. N. E. Looney, ed. The Summerland Res. Stn., Agriculture Canada, Summerland, B. C., Canada.
17. Mircetich, S. M., and Matheron, M. E. 1976. Phytophthora root and crown rot of cherry trees. Phytopathology 66:549-558.
18. Mircetich, S. M., and Matheron, M. E., and Tyler, R. H. 1976. Apple root and crown rot caused by *Phytophthora cambivora* and *Phytophthora* spp. (Abstr.) Proc. Am. Phytopathol. Soc. 3:228.
19. Sterne, R. E., Zentmyer, G. A., and Kaufman, M. R. 1977. The effect of matric and osmotic potential of soil on Phytophthora root disease of *Persea indica*. Phytopathology 67:1491-1494.
20. Sterne, R. E., Zentmyer, G. A., and Kaufman, M. R. 1977. The influence of matric potential soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi*. Phytopathology 67:1495-1500.
21. Sugar, D. 1977. The development of sporangia of *Phytophthora cambivora*, *P. megasperma*, and *P. drechsleri* and severity of root and crown rot in *Prunus mahaleb* as influenced by soil matric potential M.S. thesis. University of California, Davis. 56 pp.
22. Wilcox, W. F., and Mircetich, S. M. 1985. Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75:221-226.
23. Wilcox, W. F., and Mircetich, S. M. 1985. Influence of soil water matric potential on the development of Phytophthora root and crown rots of Mahaleb cherry. Phytopathology 75:648-653.
24. Wilcox, W. F., and Mircetich, S. M. 1985. Effects of flooding duration on the development of Phytophthora root and crown rots of cherry. Phytopathology 75:1451-1455.