Spatial Distribution of *Phytophthora cactorum* in New York Apple Orchard Soils

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Accepted for publication 19 June 1996.

ABSTRACT

Horner, I. J., and Wilcox, W. F. 1996. Spatial distribution of *Phytophthora cactorum* in New York apple orchard soils. Phytopathology 86: 1122-1132.

Population assessments of dormant spores of *Phytophthora cactorum* in apple orchard soils yielded three clear distribution gradients. Populations at the bottoms of slopes were relatively high, declined with increasing distance up slopes, and strongly correlated with soil moisture content. Populations decreased with increasing distance from the tree trunk, becoming close to nil outside the tree-row herbicide strip. There was also a sharp decline in *P. cactorum* populations with increasing depth with approximately 50 and 70% of propagules in the top 3 and 6 cm of soil, respectively. In the absence of organic substrates, propagule numbers declined significantly after 18 months at or near the soil surface, but remained constant at 7- to 10-cm depth, indicating continual renewal of surface populations to maintain the steep depth gradient. Fallen apple leaves, fruit, and petals were all naturally colonized by *P. cactorum* in the field. Surface amendments with inoculated leaves in the fall resulted in a substantial increase in soil populations measured the following spring, both in microplots and directly beneath mature apple trees. Large quantities of earthworm castings (1.45 kg/m^2 from May to September) were collected from the soil surface beneath apple trees. These contained relatively high populations of *P. cactorum* at densities comparable with those in the surface layers of soil and were likely to have contributed to the steep vertical gradient observed.

Additional keywords: epidemiology, oospores, quantification, SADAMCAP.

Phytophthora cactorum (Lebert and Cohn) J. Schröt. causes crown, root, and collar rots of trees in virtually all apple-growing regions of the world (22). However, the spatial distribution of this pathogen in orchards, particularly as it relates to apple trees, has rarely been studied. Most studies of the spatial distribution of soilborne fungi have been carried out in pasture or annual crop systems (6,8,29,31), with few in perennial tree crops (2,26,34,35) and even fewer on an individual tree level. The endemic pathogen/host relationship in mature orchards with root systems established over many years can be very different from that in annual crop systems in which epidemics of soilborne plant pathogens develop anew each year from overwintering foci of inoculum (35).

Determination of spatial distribution patterns of *P. cactorum* as they relate to apple trees may give some insight into the biology of the fungus, help in efforts to develop effective disease control measures, and aid in the design of sampling strategies for assessment of pathogen populations. Results of past studies of spatial distribution of *P. cactorum* in apple orchards have been inconsistent; examples range from no evidence that distribution was influenced by trees (33) to cases in which the pathogen was readily detected only within a few centimeters of diseased trunks (26) and other examples between these two extremes (1,14). However, previous studies were limited by a lack of accurate and reliable quantification techniques. With the recent development of the soil air-dried and moistened chilled and plated (SADAMCAP) technique to quantify relative population densities of dormant *P. cactorum* propagules (17), a more detailed investigation of the spatial distribution of soilborne populations has become possible. *P. cactorum* has a relatively wide host range (28), but in modern apple orchards with tree-row herbicide strips, the fungus is primarily reliant on apple tree tissues for sustenance and reproduction (12). However, the importance of the various apple tissues in determining fungal distribution is unknown. Thus, the initial objective of this work was to investigate and describe the spatial patterns of soilborne *P. cactorum* populations in apple orchards, in particular relating to distance from tree trunk, depth in soil, and orchard topography. After distribution gradients were observed, additional experiments were conducted to investigate some of the factors (e.g., apple-tree litter, root distribution, and earthworm activity) that may account for the gradients and relate these findings to the biology of the pathogen. Preliminary results have been published (16).

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Publication no. P-1996-0830-02R

MATERIALS AND METHODS

Most studies were carried out during 1993 and 1994 in one or more of the five New York orchard sites described in Table 1. Samples were taken under randomly selected trees showing no obvious above-ground symptoms of root disease. Unless otherwise stated, a standard soil-sampling procedure was used: litter was removed and a 10-cm-deep soil sample was taken with a 20-mm-diameter steel corer, and *P. cactorum* populations were determined using the SADAMCAP technique (17). Briefly, this involved sieving and air-drying the soil, followed by moist incubation of a 10-g (air-dry weight) subsample under lights at 22°C for 4 days, and then flooding and chilling at 6°C for 2 h. Next, the floodwater was drained, collected, vigorously mixed for 30 s, incubated at room temperature for 10 min, and plated on PARP selective agar medium (21) (5 ml of agar/9-cm-diameter plate). *P. cactorum* colonies were marked after 20, 28, and 48 h. Throughout this work, *P. cactorum* populations are reported as "colony numbers per plate" using the standard SADAMCAP technique; these values were not converted to propagules per gram of soil, because of

the arbitrary nature and numerous assumptions required for such calculations (17).

Distribution around individual trees. *Distance.* During summers 1993 and 1994, core samples were taken on transects running perpendicularly from the tree trunk to the center of the interrow grass alley at distances of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 1.9, 2.4, 3.0, 3.8, and 4.8 m. Each replicate transect originated from a different tree, with five transects taken in each of orchards 1 (two occasions), 2, and 4, and 10 transects in orchard 3. To determine the relationship between *P. cactorum* populations and distance from trunks, *P. cactorum* colony numbers were transformed using natural logarithms and analyzed separately for each orchard using the General Linear Model (Minitab Release 9; Minitab Inc., State College, PA) and regression analysis. Additionally, in orchard 1, five trees were selected randomly for a detailed study of spatial distribution around individual trees. A total of 254 core samples were taken at 0.3-m intervals in a grid pattern centered around each tree within a quadrat that extended 2.4 m from the trunk in both directions perpendicular to the row (i.e., 0.2 m into the grassed alley beyond the herbicide strip) and 2.1 m in both directions parallel to the row (i.e., 50% of the distance to the adjacent trees within the row).

Microsampling. To investigate the spatial distribution and randomness of *P. cactorum* propagules on a magnified scale, microsampling of soil was carried out in mid-summer 1994. The surface 1 cm of soil was scraped away and discarded. A 5-mm-diameter cork borer was then used to sample a series of 12 adjacent 4-mm-deep cores 0.6 m from and parallel to the trunk. One line of 12 cores was taken beneath each of 15 trees in both orchards 1 and 2. Each sample was placed in a plastic cell well (each cell 22 mm in diameter) and air-dried. The average air-dried weight of individual samples was 0.1 g. After air-drying, samples in cell wells were moistened with 45 µl of distilled water and incubated in a humid chamber at 22°C under lights for 4 days, and then flooded with 2 ml of distilled water and baited with apple seedling cotyledons (20). (Baiting was considered more practical than SADAMCAP for detection of *P. cactorum* in very small soil volumes.) Five days after flooding, baits were scored for the presence or absence of *P. cactorum* sporangia growing from the cotyledon margins. Results from 1994 indicated a clumped distribution of *P. cactorum*; so, in August 1995, lines of 48 microcores beneath each of two trees in both orchards 1 and 2 were collected and baited in a similar manner. Data from 1995 were analyzed using "runs analysis" (Minitab Inc.) to determine whether propagule distribution was clumped, random, or even.

Depth. To determine the distribution profile of *P. cactorum* with depth in soil, from 10 to 54 cores were sampled approximately 0.8 m from the trunk of mature apple trees in each of four orchards, and each core was divided into depth categories: 0 to 1, 1 to 3, 3 to 6, 6 to 10, 10 to 15, 15 to 20, 20 to 30, and 30 to 40 cm. For the upper two depth categories (only 1- and 2-cm thick), a 30-mm-diameter cylinder of soil was sampled to obtain sufficient volume for analysis; for all other depth classes, the standard 20-mm-diameter corer was used. Samples were air-dried, and 10-g subsamples from each depth category from each sample core were analyzed using SADAMCAP. Data were analyzed separately for each orchard using the General Linear Model and regression analysis to determine the relationship between depth and *P. cactorum* population. In September 1993, nine 20-cm-deep cores 0.9 m from the trunk were sampled at even intervals around each of six trees in orchard 1. In August/September 1994, single cores 40-cm deep were sampled 0.8 m from the trunk of 10 different trees in each of orchards 1, 2, 4, and 5.

To determine the relationship between soil depth and survival of *P. cactorum*, naturally infested soil from orchard 2 was sieved, bulked, thoroughly mixed, and then 25-g samples were placed into 22-µm mesh nylon bags (Tetko Inc., Briarcliff Manor, NY). Bags were then sewn closed with nylon thread and either placed on the soil surface or buried at depths of 5 or 10 cm, 0.8 m from the trunk of apple trees in this same orchard in mid-November 1994. One bag was buried at each depth at each of eight trees. In early September 1995, buried bags were retrieved. All were intact, with no sign of damage or holes. Soil from these bags was then air-dried and analyzed using SADAMCAP, and *P. cactorum* colony counts were compared with those similarly determined from eight 10-g samples of the bulked soil at the start of the experiment.

Distribution on an orchard scale. The relationship between *P. cactorum* population and orchard contour, in particular tree position on a slope, was investigated on two hilly sites. A series of seven- or eight-tree transects (12 and 6 replicate transects in orchards 3 and 5, respectively) were selected, running from the bottom to the top of the slopes. In both orchards, the angle of slope was between 0 and 4 degrees at the bottom and at the top of transects. The angle in the steepest part of transects (i.e., near the center) ranged from 8 to 15 and 9 to 14 degrees in orchards 3 and 5, respectively. Cores were taken 0.6 m from the trunk at cardinal points around each tree. All sampling for a given orchard was completed on the same day. Data from individual cores were transformed using natural logarithms, and an average population value was obtained for each tree. At each tree, relative water content of a single soil core was assessed gravimetrically. To simplify analysis, when individual transects from the gullies to the ridge crests were eight trees long, they were standardized to seven trees by arbitrarily eliminating data from one of the middle two trees (a

TABLE 1. Tree genotype, soil type, chemical analyses, and locations of orchards used for determination of spatial distribution patterns of *Phytophthora cactorum*

Orchard	Cultivar/ rootstock[x]	Soil type/ classification	Locality/ county	Soil chemical analyses[y]										
				pH	Organic matter (%)	P	K	Mg	Ca	Fe	Al	Mn	Zn	Cu
1	'Cortland'/ MM106	Ovid silt loam/ aeric ochraqualf	Geneva[z]/ Ontario	6.86	6.90	17.0	315	305	3450	1.9	10.1	34.0	2.31	0.8
2	'McIntosh'/ seedling	Odessa silt loam/ aeric ochraqualf	Geneva[z]/ Ontario	6.79	7.30	11.3	184	271	2391	0.5	6.5	16.8	2.04	1.2
3	'Rome'/ seedling	Canandaigua silt loam/ mollic haplaquept	Williamson/ Wayne	5.68	6.72	21.9	359	184	1088	4.5	44.9	11.4	3.22	0.6
4	'McIntosh'/ MM106	Lima silt loam/ glossoboric hapludalf	Seneca Castle/ Ontario	6.34	4.49	14.5	149	197	1521	1.2	16.8	26.5	1.85	0.5
5	'McIntosh'/ seedling	Palmyra gravely loam/ glossoboric hapludalf	Lyons/ Wayne	6.37	6.50	10.3	145	245	1694	3.8	27.1	23.1	7.24	0.3

[x] All trees were mature, approximately 20- to 50-years old.
[y] Available nutrients (mg/kg of soil). Samples were collected from the top 10 cm of soil beneath apple trees. Analyses were carried out by Cornell Nutrient Analysis Laboratories.
[z] New York State Agricultural Experiment Station.

total of four and one instances for orchards 3 and 5, respectively). To determine the relationship between *P. cactorum* populations and position on slope, population data were transformed using natural logarithms and analyzed separately for each orchard using the General Linear Model. Population data were also regressed against soil water content using simple transformations (natural logarithms and square roots) and second degree polynomials, and the best fit was reported.

In a separate study to further examine the relationship between soil water content and *P. cactorum* population, core samples were taken from around the same selected trees on 22 occasions at approximately monthly intervals over a 2-year period. On each occasion, four cores were extracted approximately 0.7 m from the trunk around each of four trees in each of orchards 1 and 2 and eight trees in orchard 3, and the *P. cactorum* colony count was determined using SADAMCAP. The gravimetric soil water content was determined for a single core at each tree on each occasion. For the purposes of the present work, the mean *P. cactorum* colony count was determined for each tree over all sample times, and then this value was regressed against the mean soil water content for each tree over all sample times. Other aspects of this experiment are described elsewhere by Horner and Wilcox (18).

To gauge the regional distribution of *P. cactorum* in a range of soils, two sample cores were taken 0.5 m from each of four to eight mature trees at each of 17 apple orchards in Wayne, Ontario, and Tompkins counties of New York State in September 1994. Each core was tested using SADAMCAP.

Factors contributing to observed distribution gradients. *Roots.* In 1994, root distribution in the top 10 cm of soil in orchards 1 and 2 was assessed and related to distance from trunk and *P. cactorum* population. For each of four sample trees in both orchards, cubes of soil (16 × 16 × 10-cm deep) were sampled at distances of 0.4, 0.8, 1.2, 1.8, 2.4, and 3.4 m (orchard 2 only) from the edge of the tree trunk in transects perpendicular to the tree row. Soil was sieved, and all apple roots were collected, rinsed, sorted into five arbitrary classes based on root diameter (<0.8, 0.8 to 2.0, 2 to 4, 4 to 10, and >10 mm, respectively), oven-dried, and weighed. The soil from each sample was thoroughly mixed, air-dried, and subsamples quantified for *P. cactorum* using SADAMCAP. *P. cactorum* colony numbers were regressed separately against root weight and distance from tree, and root weight was also regressed against distance from tree. Colony number and root weight data underwent simple transformation (natural logarithms and square roots), and second degree polynomials were calculated; the best fits were reported.

Separate columns of soil (16 × 16-cm surface area) were sampled 0.8 m from the trunk under the same eight trees as above to examine possible relationships between the distribution of apple roots and *P. cactorum* as a function of soil depth. Each column was divided into depth classes (0 to 1, 1 to 3, 3 to 6, 6 to 10, 10 to 15, and 15 to 20 cm) and soil sieved, roots weighed, and *P. cactorum* population assessed as above. Root weight and *P. cactorum* colony numbers were regressed against each other and depth class midpoint. Simple transformations (natural logarithms, square roots) and second degree polynomials were calculated, and the best fits were reported.

Fallen apple-tree tissues. To determine whether or not *P. cactorum* colonized fallen apple tree tissues in the field, isolations were made from naturally fallen petals, leaves, and fruit. Thirty mature fruit and 20 leaves were sampled beneath apple trees in both orchards 1 and 4 in early autumn 1994, and 30 immature windfall fruit in each of orchards 1, 2, and 4 in July 1995. Twenty petals were sampled in both orchards 1 and 3 in May 1995. All tissues were collected within 1.5 m of the trunk of five to six randomly selected trees. The fruit were rinsed with water and swabbed with 50% ethanol. The skin was peeled back and samples, preferably from the margin between rotten and healthy tissue, were plated onto PARP agar. Leaves were washed thoroughly with water, immersed in 50% ethanol for 1 min, rinsed in sterile water, and then five 5 × 5-mm samples per leaf were plated onto PARP agar. Petals were treated in a similar manner to that for leaves, except the entire petal was plated. Cultures resembling *Phytophthora* were subcultured onto corn meal agar for identification.

Litter microplots. To investigate the effect of various forms of leaf and fruit litter on subsequent levels of *P. cactorum* in the soil, microplots were established in late September 1993. The top 10 cm of soil naturally infested with *P. cactorum* was collected beneath apple trees in orchard 2 and sieved through a 5.5-mm mesh screen. Roots were then removed, fine vermiculite was added at a rate of 1:10 to reduce compaction and improve friability, and soil was thoroughly mixed by hand and in a cement mixer. The mixture was then placed in 18-cm-diameter × 18-cm-deep clay pots. Ten-gram samples were taken from 20% of the pots, and *P. cactorum* populations were assessed using the SADAMCAP technique. Pots were placed 40- to 50-cm apart in a gap (missing tree) within a row of mature apple trees and buried leaving the top 2 to 3 cm of the pot exposed. Straw mulch was applied between the pots to prevent rainsplash of soil and spores into the pots. Six replicates of each of the following treatments were applied in a randomized complete block design: treatment A, bare soil, no treatment; treatment B, shredded plastic mulch; treatment C, green apple leaves; treatment D, green apple leaves inoculated with *P. cactorum*; treatment E, abscised apple leaves; treatment F, mature apple fruit; treatment G, mature fruit inoculated with *P. cactorum*; and treatment H, alfalfa/grass hay mulch.

Healthy mature fruit and green leaves were harvested from trees of 'McIntosh', 'Cortland', and 'Empire', rinsed with water, and then either applied to pots directly (treatments C and F) or inoculated with *P. cactorum* (treatments D and G) before placement in pots. To inoculate, fruit (punctured multiple times using pins) and leaves were submerged for 6 h in a mixed zoospore suspension obtained from six different *P. cactorum* cultures, and then drained and incubated in a humid container at 22°C for 7 days. Immediately before placement in the field, sample leaves and fruit were surface-sterilized and portions plated on PARP agar plates to confirm colonization. Other sample leaves and fruit were frozen for 48 h to kill sporangia and mycelia, and then plated on PARP to determine the presence of germinable resting spores at this time.

For treatment E, abscised leaves were collected during autumn in mesh cloths suspended beneath apple trees, and then were applied to appropriate plots. For treatment B, sheets of clear plastic were shredded into 3- to 6-mm strips. For treatment H, fresh cut hay was obtained from a field of mixed alfalfa, ryegrass, and fescue.

All leaf, plastic, or hay mulch treatments were applied as a 2- to 3-cm-deep layer over the entire pot. Fruit were applied one layer thick. Following treatment application, all pots were covered with 2.5-cm wire mesh to keep materials in place, and plastic mesh shade cloth was laid on the ground over the entire plot to reduce interference from animals, plot desiccation, rainsplash between treatments, and unwanted addition of leaves and other plant parts. In September/October 1994, treatments C through H were reapplied.

In May 1995, remains of sample fruit and leaves were surface-sterilized and portions plated on PARP. All pots were removed from the field, broken open, and soil divided into depth classes: 0 to 1, 1 to 2, 2 to 4, 4 to 7, and 7 to 10 cm. Soil in each depth class was sieved, thoroughly mixed, and the *P. cactorum* populations in 10-g subsamples were assessed using SADAMCAP. A mean colony count per plate was obtained for each plot by weighting the data from each depth class (according to its thickness), and these values were compared among treatments using analysis of variance and Tukey's test (Minitab Release 9). Individual data from each depth class were transformed using natural logarithms, and then regressed against the midpoint depth value for that class.

Litter tree plots. To further investigate the role of litter, six apple trees in orchard 1 (soil naturally infested with *P. cactorum*) were selected for a trial beginning in late September 1993. Nine plots, measuring 50 × 50 cm with the closest side 70 cm from the trunk, were established within the herbicide strip beneath each tree. Treatments were randomly assigned to the plots, with each tree representing one complete block. Treatments were the same as in the microplot experiment above, with an added treatment (I) that was left undisturbed. All litter and weeds were removed from the remaining plots before treatment application; soil was otherwise undisturbed. Thereafter, all plots were covered with plastic mesh except treatment I, which was left uncovered throughout the experiment. All treatments were reapplied in September/October 1994.

In spring 1995, 20-mm-diameter core samples were taken to a depth of 15 cm in the center of each plot 95 cm from the trunk. Cores were subdivided into depth classes (0 to 1, 1 to 3, 3 to 6, 6 to 10, and 10 to 15 cm), and each subsample was assessed using SADAMCAP. Individual data from each depth class were transformed using natural logarithms, and then regressed against the midpoint depth value for that class. A mean colony count per plate for each plot (down to 10 cm) was obtained by weighting the data from each depth class, and these values were compared using analysis of variance and Tukey's test.

Earthworms. Large volumes of earthworm castings were noticed beneath apple trees, and preliminary studies using SADAMCAP indicated that they contained high numbers of *P. cactorum* spores. Castings within a 30-cm quadrat were collected. Four core samples were taken from the same area, divided into depth classes (0 to 1, 1 to 3, 3 to 6, 6 to 10, 10 to 15, and 15 to 20 cm), and *P. cactorum* populations determined using SADAMCAP to compare populations in castings with those found in soil. Colony counts from the four cores were averaged to give a single depth profile for each quadrat. Five quadrats were sampled in orchard 2 in September 1994.

In a separate experiment to determine the volume of earthworm castings deposited on the soil surface in the course of a growing season, a 30-cm quadrat was marked out 0.7 to 1.0 m from the trunk of each of four apple trees in orchard 1. Every week from May 1 to September 30, 1995, earthworm castings were removed, oven-dried, and weighed.

RESULTS

Distribution around individual trees. *Distance.* In transects taken from tree trunks into the grass interrows, populations of *P. cactorum* were highest within a 1- to 2-m radius of the trunk, with a steep decline beyond this distance, and almost nil beyond the edge of the herbicide strip (Fig. 1). Regression analyses of log-transformed *P. cactorum* colony numbers showed that although populations varied considerably between orchards, as indicated by the range of intercepts, the trends of the gradients were similar (Fig. 1F). All slopes were highly significant ($P < 0.001$) and r^2 values ranged from 37.4 to 71.3%. Additional trials in orchards 1 and 2 gave similar results (data not shown). Comparable results were obtained with intensive sampling around five trees in orchard 1 (Fig. 2). Although there was some variability, every tree showed a similar overall trend, with a steep decline in population with increasing distance from the trunk.

Microsampling. In both 1994 and 1995, *P. cactorum* was detected in 65 to 92% of the 0.1-g microcores (Table 2). Comparison of the observed number of runs (i.e., uninterrupted sequences of microcores yielding *P. cactorum* in consecutive samples) with the number expected if the distribution was random ("runs analysis") indicated a significant clumped distribution in all four microcore transects sampled in 1995.

Depth. There was a steep decline in *P. cactorum* population with increasing depth at all sample sites, with approximately 50% of the total population in the top 3 cm and 70% in the top 6 cm of soil (Fig. 3). Below a depth of 20 cm, either nil or very low numbers of *P. cactorum* propagules were recovered. Regression analyses showed highly significant ($P < 0.001$) linear relationships between depth and natural log of the *P. cactorum* colony counts, with r^2 values of 26.4, 61.9, 74.5, 52.5, and 49.7% for orchards 1 (September 1993 and August 1994), 2, 4, and 5, respectively (Fig. 3).

When a naturally infested soil was enclosed for 10 months in 22-µm mesh nylon bags in an apple orchard, *P. cactorum* was retrieved less frequently from bags placed on the soil surface than from those buried 5- and 10-cm deep, with mean colony counts of 21.8, 88.8, and 80.6 per plate, respectively. The mean *P. cactorum* colony count of the soil before bagging was 71.0.

Distribution on an orchard scale. On both undulating hill sites studied, *P. cactorum* populations were relatively high at the bottom parts of slopes and very low at the top (Fig. 4). This trend was strongly associated with soil water content ($r^2 = 54.6\%$, $P < 0.001$ and $r^2 = 27.3\%$, $P = 0.002$ for orchards 3 and 5, respectively), i.e., in the wetter sites at the bottom of slopes, populations were much higher than at the drier sites at the top of slopes.

When mean population data collected on 22 occasions over a 2-year period beneath given trees (18) were regressed against mean soil water content at the same trees, there was a significant positive linear relationship in two orchards, with r^2 values of 91.6 and

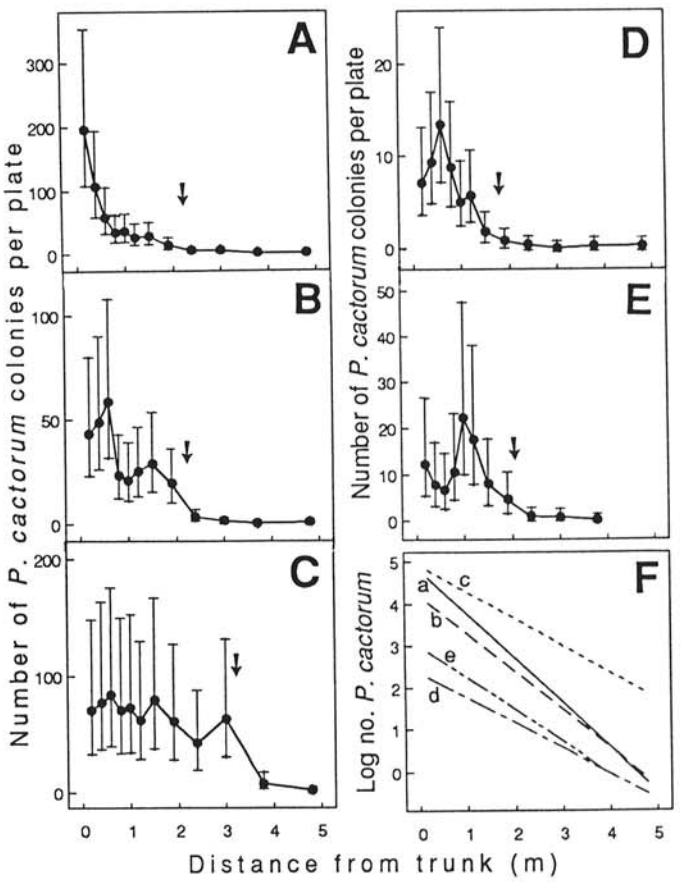

Fig. 1. Soil populations of *Phytophthora cactorum* at various distances from apple tree trunks. Core samples were taken on transects running from the trunk to the center of the interrow grass alley. Each replicate transect was at a different tree, with five transects taken in each of orchards 1 (sampled twice), 2, and 4, and 10 transects in orchard 3. Populations were assessed using SADAMCAP. Data, expressed as *P. cactorum* colony counts per plate, were transformed using natural logarithms and analyzed separately for each orchard. Points represent means for each distance on each orchard. The arrows indicate the edge of the herbicide strip. **A,** Orchard 1, August 1993. **B to E,** Orchards 1 to 4, respectively, sampled September 1994. **F,** Natural logarithm of *P. cactorum* colony counts per plate regressed against distance from tree. Lines a to e correspond to data in plots **A to E.**

77.3% (P values of 0.045 and 0.003) for orchards 1 and 3, respectively, i.e., wetter sites had higher populations (data not shown). In orchard 2, soil water content was very similar at each tree, which precluded regression analysis.

P. cactorum was detected in all 17 orchards surveyed and was present in at least 50% of samples from each site. Overall, out of a total of 194 10-g samples tested, 160 yielded one or more *P. cactorum* colonies. There was a wide within-orchard and between-orchard range in detected populations. In the orchards with lowest and highest *P. cactorum* populations, colony counts per plate in undiluted SADAMCAP samples (17) ranged from 0 to 9 and 110 to 1,040, respectively. SADAMCAP was an effective tool for assessing *P. cactorum* at every site, and contamination by other fungi on agar plates was a major problem in only four of the 194 samples.

Factors contributing to distribution gradients. *Roots.* In both orchards where root distribution was surveyed, both fibrous root (<2.0 mm in diameter) weight and *P. cactorum* colony count were significantly ($P < 0.05$) negatively correlated with distance from trunk (Fig. 5), although the analyses were biased by the very low values at greatest distance from trunk. Root weight and *P. cactorum* colony count were positively correlated in orchard 1, but were not significantly correlated on orchard 2 ($r^2 = 23.0\%$, $P = 0.019$ and $r^2 = 8.9\%$, $P = 0.086$, respectively). When roots greater than 2 mm in diameter were included in the analysis, the relationships between root weight and *P. cactorum* population were either similar or weaker.

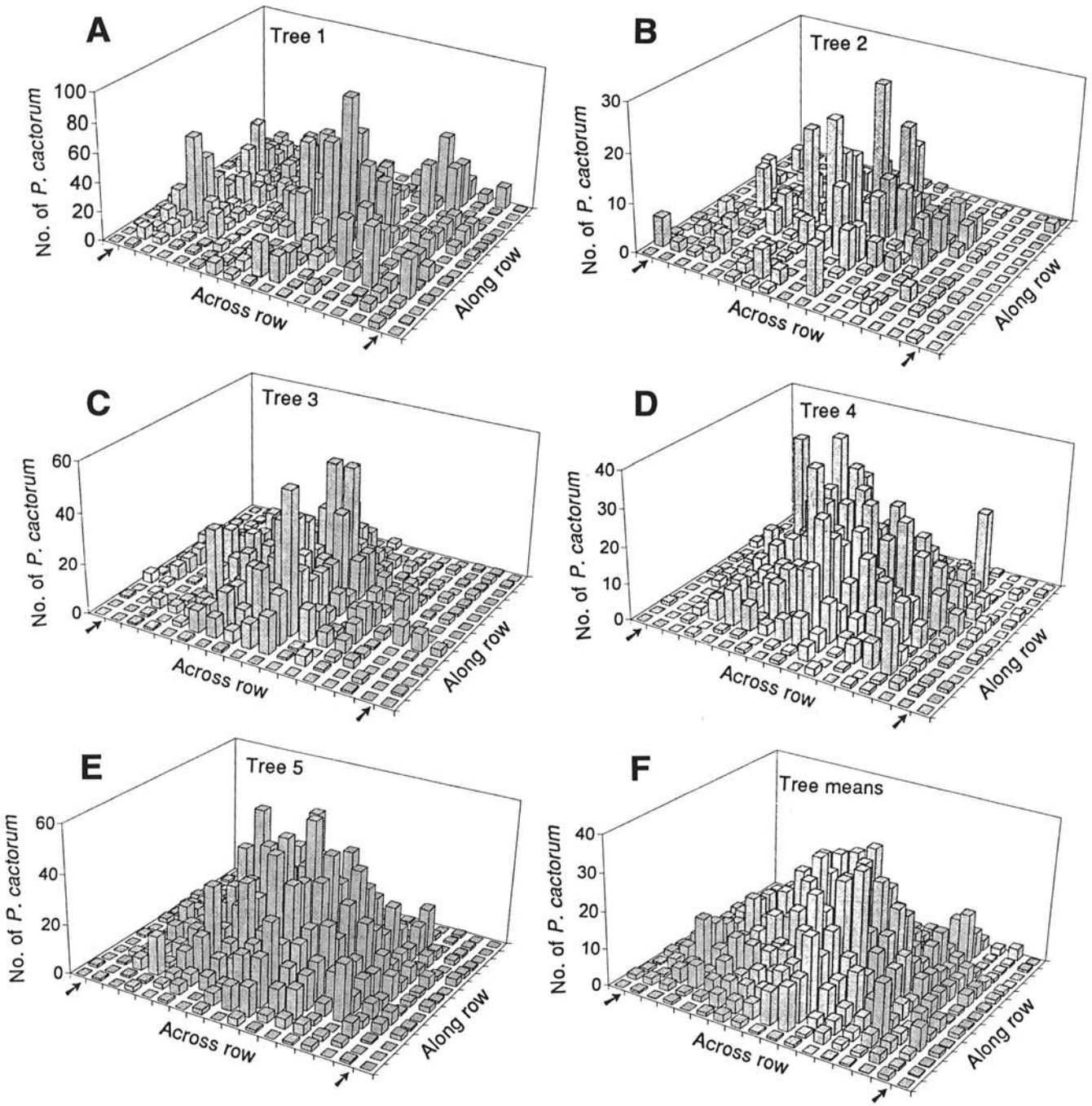

Fig. 2. Soil populations of *Phytophthora cactorum* sampled around apple trees. Five randomly selected trees were sampled in orchard 1. Two hundred and fifty-four 10-cm-deep core samples were taken at 30-cm intervals in a grid pattern within a quadrat that extended 2.4 m from the trunk in both directions perpendicular to the row (i.e., 20 cm into the grassed alley beyond the herbicide strip) and 2.1 m in both directions parallel to the row (i.e., 50% of the distance to the adjacent tree within the row). Populations were assessed using SADAMCAP, and data were expressed as *P. cactorum* colony counts per plate. **A to E,** Individual data for trees 1 to 5, respectively. **F,** Means of all five trees. Arrows indicate the edge of the herbicide strip. The tree trunk was located at the center of each plot.

The relationships between soil depth, root weight, and *P. cactorum* soil population differed only slightly for orchards 1 and 2 (Fig. 6). In orchard 1, *P. cactorum* population density was strongly negatively correlated with depth (r^2 = 44%, $P < 0.001$), whereas fibrous root weight (diameter <2.0 mm) and depth were very

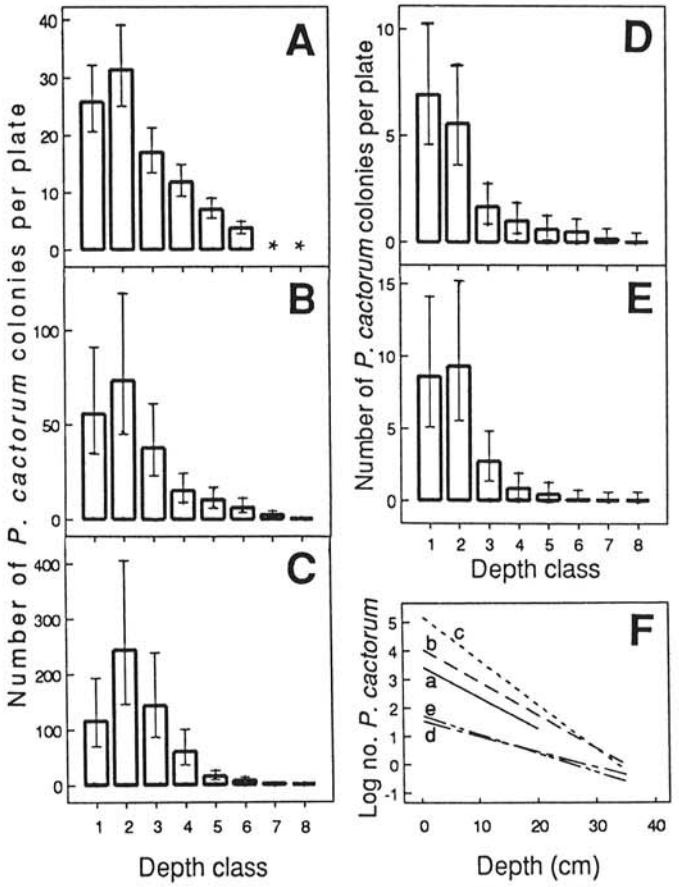

Fig. 3. Populations of *Phytophthora cactorum* at different depths in apple orchard soils. Soil depth classes 1 to 8 correspond to depths of 0 to 1, 1 to 3, 3 to 6, 6 to 10, 10 to 15, 15 to 20, 20 to 30, and 30 to 40 cm, respectively. SADAMCAP was used to determine *P. cactorum* populations, expressed as colony counts per plate. Data were transformed using natural logarithms prior to analysis. Error bars represent 95% confidence intervals. **A,** Orchard 1, means of 54 cores sampled in September 1993 (* = no data collected for depth classes 7 and 8). **B to E,** Orchards 1, 2, 4, and 5, respectively, means of 10 cores sampled at each orchard during August/September 1994. **F,** Predicted relationship between *P. cactorum* populations and soil depth, obtained by regressing the natural logarithm of *P. cactorum* colony count against the midpoint of the depth class. Lines a to e correspond to data in plots **A to E**.

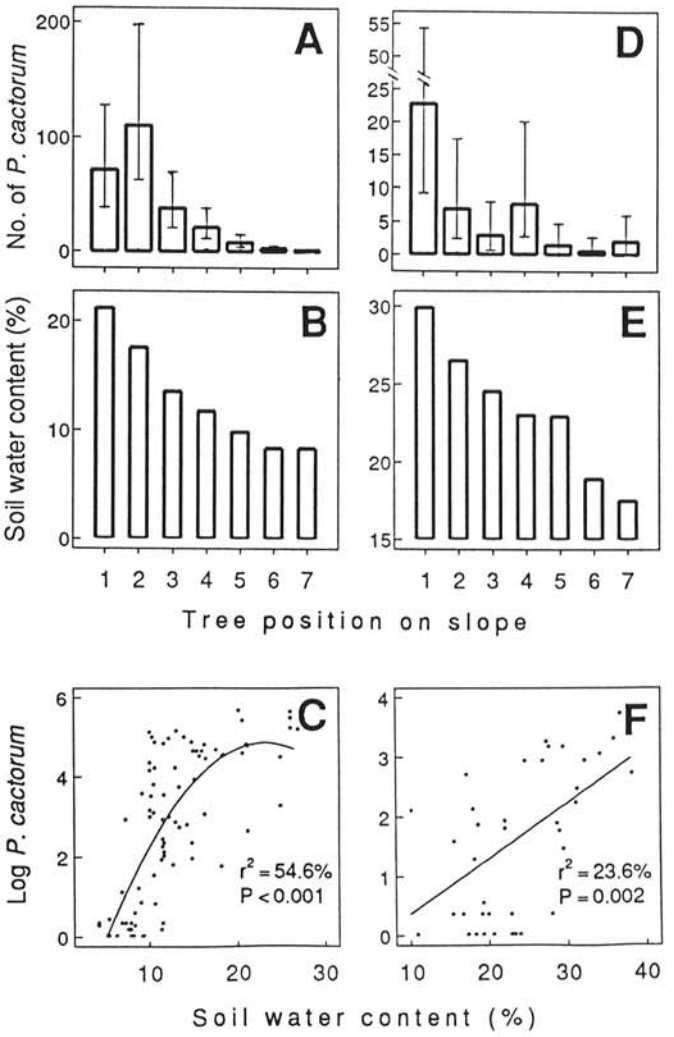

Fig. 4. Effect of slope position on soil water content and *Phytophthora cactorum* population beneath apple trees. Five 10-cm cores were taken at each tree on a series of seven-tree transects running from the bottom (tree 1) to the top (tree 7) of slopes. SADAMCAP was used to estimate *P. cactorum* populations in four cores, and data were averaged to obtain a mean colony count per plate at each tree. The fifth core was used to measure gravimetric water content. There were 12 and 6 transects in orchards 3 and 5, respectively. **A, B, and C,** Orchard 3. **D, E, and F,** Orchard 5. **A and D,** Mean number of *P. cactorum* colony counts per plate. Data were transformed using natural logarithms before analysis. Error bars are 95% confidence intervals. **B and E,** Mean percentage of soil water content. **C and F,** Regression analyses of mean natural logarithm of *P. cactorum* colony count at each tree as a function of gravimetric water content at the same tree.

TABLE 2. Presence of *Phytophthora cactorum* in microcores collected beneath apple trees

Year	Orchard[t]	Tree[u]	No. of cores[u]	Cores yielding *P. cactorum* (%)[v]	Expected no. of runs[w]	Observed no. of runs[x]	*P* value[y]
1994	1	1–15	180	76.7	…[z]	…	…
	2	1–15	180	91.7	…	…	…
1995	1	1	48	64.6	23	16	0.0264
		2	48	77.1	18	11	0.0038
	2	1	48	77.1	18	13	0.0391
		2	48	75.0	19	13	0.0189

[t] Orchards as described in Table 1.
[u] In 1994, lines of 12 consecutive microcores (5-mm diameter × 4-mm deep) were sampled beneath each of 15 trees in both orchards. In 1995, lines of 48 consecutive microcores were sampled beneath each of two trees in both orchards, and data from each line were analyzed separately.
[v] Soil samples were air-dried, moistened with 0.045 ml of distilled water, incubated at 22°C for 4 days, and then flooded and baited with a single apple cotyledon. Data refer to the frequency of cotyledons infected with *P. cactorum* as determined microscopically after 5 days of flood incubation at 22°C.
[w] Expected number of runs (i.e., uninterrupted sequences of microcores yielding *P. cactorum* in consecutive samples if distribution was random). Data were analyzed using "runs analysis" (Minitab Inc.).
[x] An observed number of runs lower than expected indicates a clumped distribution.
[y] Probability that distribution was random.
[z] "…" indicates "runs analyses" were not attempted.

weakly positively correlated (r^2 = 14.2%, P = 0.039), primarily because of low root weights in the top 3 cm of soil. For instance, when the SADAMCAP technique was applied to 10-g soil samples from the 1- to 3-cm- versus the 15- to 20-cm-depth profile, *P. cactorum* colony numbers decreased from 8.7 to 1.4, respectively. In contrast, fibrous root weights increased from approximately 200 to 700 mg per liter of soil within these same respective profiles. Correspondingly, there was a strong negative relationship between *P. cactorum* colony count and root weight (r^2 = 49.9%, P < 0.001).

In orchard 2, there also was a negative correlation (r^2 = 39.1%, P = 0.001) between *P. cactorum* population and soil depth (117.6 versus 9.9 colonies in the top 1- to 3-cm versus the 15- to 20-cm profile, respectively), and a positive correlation between root weight and depth (r^2 = 18.5%, P = 0.021), primarily because of very low root densities in the top 1 cm. However, there was no significant correlation between *P. cactorum* colony count and root weight (P = 0.171).

Fallen apple tree tissues. Wind-fall apple fruit, leaves, and petals were all naturally colonized by *P. cactorum* in the field (Table 3). Fruit, in particular, showed a high level of colonization, especially those sampled in July 1995.

Litter microplots. In microplots containing soil naturally infested with *P. cactorum*, those amended with inoculated leaves developed populations that were >240% higher than those in the bare soil control plots (Table 4). No other treatment differed sig-

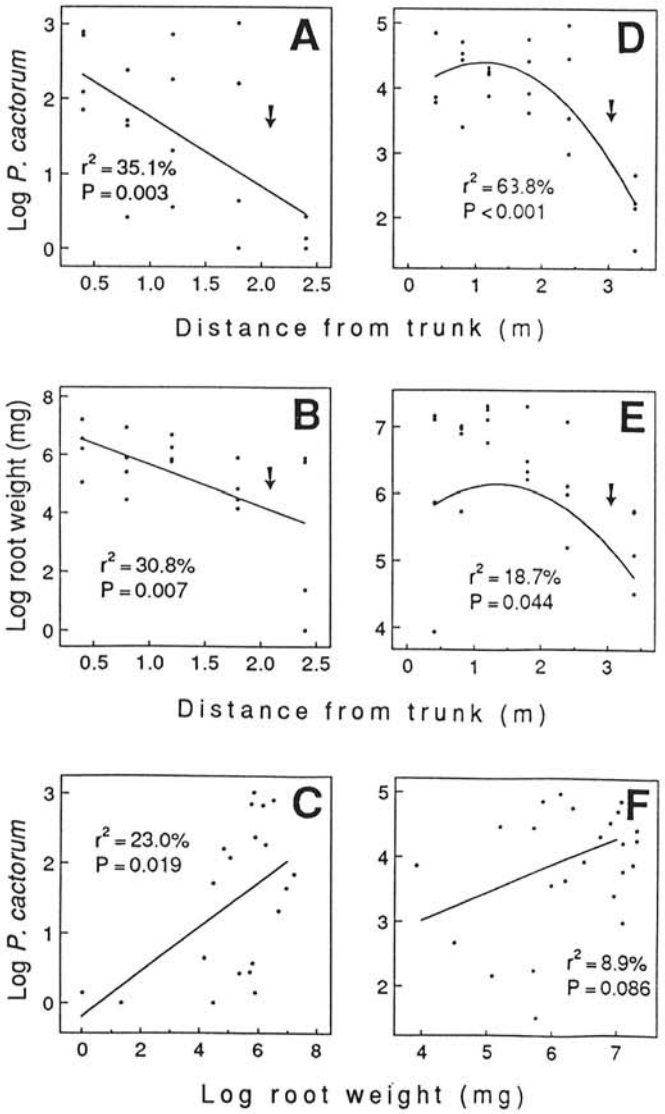

Fig. 5. Relationship between distance from trunk, fibrous root weight, and *Phytophthora cactorum* population beneath apple trees. **A, B, and C,** Orchard 1. **D, E, and F,** Orchard 2. At each of four trees in both orchards, 16 × 16-cm squares of soil, 10-cm deep, were sampled at intervals from the edge of the tree trunk to the grassed interrow. Apple roots were collected, rinsed, oven-dried, and weighed (only data from roots less than 2 mm in diameter are presented). Soil from each sample was thoroughly mixed, *P. cactorum* population was measured in five subsamples per cube of soil using SADAMCAP, and data were averaged to give a mean colony count per plate for each sample. Mean colony counts were regressed against root weight in the same cube of soil and against distance from trunk. Arrows indicate the edge of the herbicide strip.

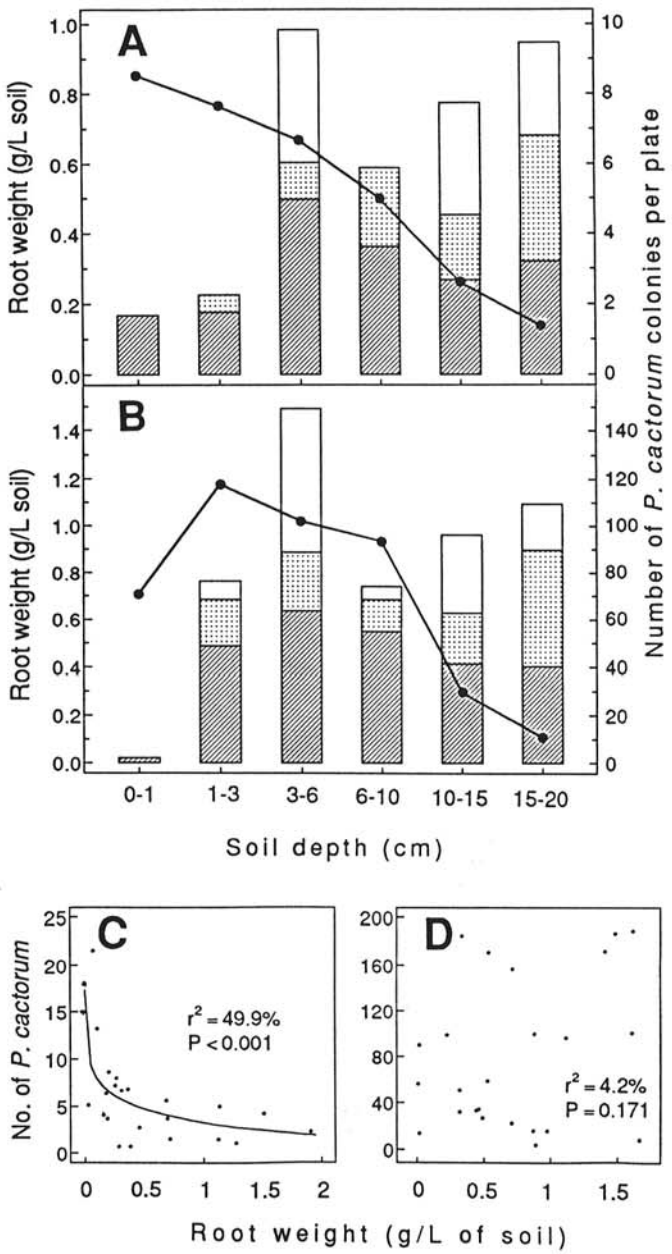

Fig. 6. Relationship between soil depth, root weight, and *Phytophthora cactorum* population beneath apple trees. **A and C,** Orchard 1. **B and D,** Orchard 2. **A and B,** At each of four trees in each orchard, 16 × 16-cm quadrats of soil were sampled and divided into depth classes. Apple roots in each depth class were collected, rinsed, sorted into size classes, oven-dried, and weighed. "Hatched", "stipled", and "open" columns indicate mean weight of root size classes <0.8, 0.8 to 2, and 2 to 4 mm in diameter, respectively. Soil from each sample was thoroughly mixed, and *P. cactorum* populations were measured in five subsamples per depth class per square of soil using SADAMCAP. Data were transformed using natural logarithms and averaged to give a mean colony count per plate (—●—). **C and D,** Mean *P. cactorum* colony count obtained for each depth class within each quadrat regressed against fibrous-root dry weight (total of roots <2 mm in diameter) for the same depth class and quadrat.

nificantly from the control. In all treatments except that with inoculated leaves and in contrast with the results from undisturbed orchards summarized in Figures 3 and 6, measured *P. cactorum* populations were lowest near the surface, as indicated by the positive slope of the depth gradient.

In samples taken from pots at the start of the experiment in September 1993, SADAMCAP analysis yielded a mean of 117.0 *P. cactorum* colonies per plate. In May 1995, populations in the top 1 cm of the bare soil and plastic mulch treatments had declined to 14.5 and 30.3 colonies per plate, respectively. In contrast, colony counts in the bottom depth class (7 to 10 cm) in the same respective treatments were 104.0 and 117.3, comparable with the initial population.

Isolations from inoculated leaves and fruit before placement in the field confirmed that these tissues were colonized by *P. cactorum*; the fungus was recovered from more than 90% of plated tissue pieces. When tissues were frozen for 48 h before attempts at isolation, *P. cactorum* could still be isolated readily from leaves (15 out of 20 tissue pieces), but it was more difficult to isolate from fruit (2 out of 20 tissue pieces). In the spring following fall treatment applications, *P. cactorum* was successfully isolated from 2 of 30 leaf fragments in treatment D (inoculated leaves), but it could not be isolated from debris in any other leaf or fruit treatment. Tissues in all treatments were dried out and fragmented by this time.

Litter tree plots. The responses to treatments in litter amendment experiments beneath apple trees were similar to those in the microplots. Again, the only treatment with significantly higher *P. cactorum* counts than the control was the inoculated leaf amendment (Table 4), with an almost fourfold increase in average colony counts in the top 10 cm of soil. Below the 6-cm depth, measured populations were similar for all treatments (data not shown). However, in contrast to the microplot experiments, *P. cactorum* populations decreased with depth in all treatments. The final depth gradient in the inoculated leaf treatment was steeper than in all other treatments (Table 4).

Earthworms. P. cactorum population densities in earthworm castings on the soil surface were comparable with those found in the surface 1 to 3 cm of soil and much higher than populations found at greater depths (Fig. 7). In plots where earthworm castings were regularly removed and weighed, an average of 1.45 kg/m^2 were collected between May 1 and September 30, 1995. During heavy rainfall, castings were pulverized and could not be collected. Thus, the above figure is almost certainly an underestimate of the true mass of castings.

DISCUSSION

Three clear gradients in the distribution of *P. cactorum* propagules in apple orchards were identified: populations decreased i) from the bottom to the top of a slope; ii) with increasing distance from a tree trunk; and iii) with increasing depth in the soil.

The correlation of propagule number with downward position on slope and related increasing soil moisture content is consistent with a wealth of reports concerning the distribution of soilborne *Phytophthora* diseases. In apple orchards, the most serious outbreaks of *Phytophthora* root, crown, and collar rots are usually in areas receiving seepage or runoff from higher ground (25,36) or in wet areas at the foot of slopes or in hollows or gullies (37). The observed trend in our population data supports the premise that where conditions are favorable for disease, high soil populations will result. Similarly, Marks and Mitchell (23) found high populations of *P. megasperma* in poorly drained areas with a history of alfalfa root rot, but the fungus was not detected on contiguous, well-drained hills and slopes.

The consistent decline in *P. cactorum* population with increasing distance from tree contrasts with the findings of Sewell et al. (33), who found no difference in the frequency of isolation from soil proximal to or distant from apple trees infected with this fungus. However, in other studies, detection of *P. cactorum* with safflower baits was greatest near the tree trunk (1). Similarly, McIntosh (26) found the highest number of *P. cactorum* propagules very close to diseased trunks, very few at 15 cm distant, and almost nil 30 cm away. These early studies were probably limited by relatively insensitive detection techniques. When our *P. cactorum* colony counts were converted to propagules per gram of soil, numbers were considerably larger than earlier reports (13,26), and all of our studies were under apparently healthy trees.

The decline in *P. cactorum* population with increasing depth was similar to, but much steeper than, that noted in studies of other *Phytophthora* spp. (8,34,38). Interestingly, however, it appears that persistence of *P. cactorum* dormant spores is less near the soil surface than at greater depths. For instance, over an 18-month period in control treatments of the litter microplot experiments, populations declined substantially near the soil surface, but remained high at a 7- to 10-cm depth. Similarly, populations in soil samples contained in nylon mesh bags declined over a 10-month period when bags were maintained on the soil surface, but not when they were buried at a 5- or 10-cm depth. Possible reasons for such declines near the surface include germination in response to light (2,27) and lysis due to environmental extremes. These results indicate that without continual replenishment, surface populations of *P. cactorum* would decline relative to those at

TABLE 3. Proportion of fallen fruit, leaves, and petals naturally colonized by *Phytophthora cactorum* in apple orchards

		Frequency of *P. cactorum* isolation[z]				
Tissue	Date	Orchard 1	Orchard 2	Orchard 3	Orchard 4	Cumulative isolation frequency (%)
Fruit	July '95	21/30	25/30	...	26/30	80.0
Fruit	Sept. '94	5/30	...	...	17/30	36.7
Leaves	Sept. '94	4/20	...	...	6/20	25.0
Petals	May '95	3/20	...	4/20	...	17.5

[z] Samples from naturally fallen apple tissues collected from the soil surface beneath trees were plated onto PARP after surface sterilization. Data are the number of apple tissues from which *P. cactorum* was successfully isolated out of the total number plated.

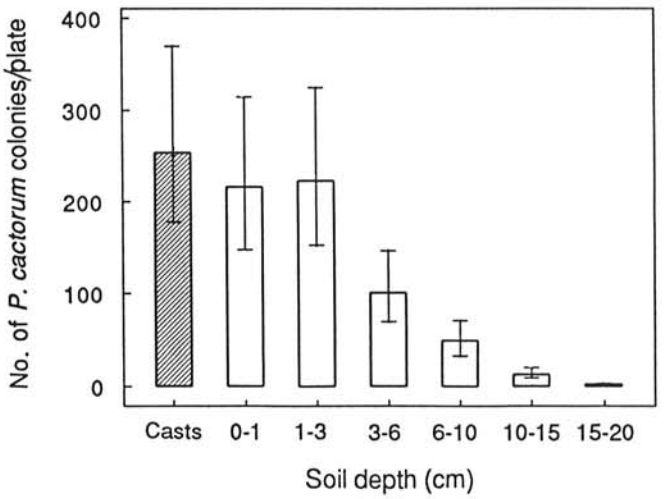

Fig. 7. *Phytophthora cactorum* population densities in earthworm castings and surface layers of apple orchard soil. Earthworm castings collected in five 30-cm square quadrats beneath apple trees in orchard 2 were analyzed using SADAMCAP to determine *P. cactorum* populations (shaded column). Four sample cores taken within each quadrat were divided into the indicated depth classes, and subsamples were analyzed using SADAMCAP. Data from the four cores were averaged to give a mean *P. cactorum* colony count per plate for each depth class in each quadrat, and these means, together with casting data, were analyzed using the General Linear Model. Bars indicate 95% confidence intervals.

greater depths, and the steep depth gradient observed in orchards (Fig. 3) would not be maintained.

The well-documented stimulation of *P. cactorum* oospore germination in response to light (2,17) assumes ecological significance when oospore accumulation near the soil surface is considered. At the soil surface, there is a ready supply of leaf, fruit, and other tree debris most of the year that can be colonized readily by *P. cactorum*. Surface accumulation of oospores also facilitates dispersal in surface water (7). Apple cotyledon baits floated in puddles that formed briefly beneath apple trees during heavy rain were colonized consistently by *P. cactorum* (I. J. Horner and W. F. Wilcox, *unpublished data*), presumably the result of zoospore infections. The negative geotaxis of *P. cactorum* zoospores (7) is also consistent with a surface-oriented existence.

Roots, litter, and earthworms were all investigated as possible contributors to the observed depth and distance gradients that were consistent over a number of orchards. In studies related to depth of sample, there was no significant relationship between root and *P. cactorum* population densities at one orchard and a significant negative correlation at the other. At both sites, there were few roots in the top 1 to 3 cm of soil, where *P. cactorum* populations were at their highest. Thus, root infection and subsequent release of oospores apparently does not contribute to the steep depth gradient in *P. cactorum* distribution, and it is likely that roots and *P. cactorum* are responding independently to depth or some related factor.

Colonization of apple tree litter on the soil surface and subsequent breakdown of tissue to release oospores could contribute to a steep vertical gradient in distribution. A number of authors have previously noted colonization of fallen fruit by *P. cactorum* (3,4, 14,33), and this phenomenon was confirmed in our studies. We also observed colonization of naturally fallen leaves (as found previously with *P. syringae* [9,10]) and petals. Oospores can form in all of these tissues, though more so in leaves (11). However, in experiments amending the soil surface with apple leaf and fruit litter, there appeared to be very little natural colonization by *P. cactorum*. Since plots were not irrigated, it is possible that tissues, especially leaves, dried out before they could be colonized. Harris (10) noted that as leaves senesced and dried out, the ability of *P. syringae* to colonize declined rapidly. Nevertheless, the large increase in *P. cactorum* population in the top few centimeters of soil following amendment with inoculated leaves in our litter experiments demonstrated the potential contribution of leaf colonization to maintaining soil populations in the upper strata. Although the lack of increase in soil populations following amendment with inoculated fruit is surprising, this may be because of the production of *P. cactorum* oospores per gram of tissue being considerably lower in apple fruit compared with leaves (11). A crude calculation, multiplying the weight of tissue added to plots times Harris' (11) estimates of oospore production, indicates that four to five times as many oospores may have been added in inoculated leaf relative to inoculated fruit plots.

Earthworms bury and consume considerable volumes of apple leaves annually (30), and presumably also ingest other plant parts such as petals, fallen fruitlets, and decomposing roots, all of which can contain *P. cactorum* oospores. The quantity of earthworm castings collected from the soil surface from May to September (>1.4 kg/m^2) corresponds to previous reports of annual ingestion of soil by earthworms of 2.6 kg/m^2 (32). The high density of *P. cactorum* found in earthworm castings, comparable with that in the top centimeter of soil, suggests that the actions of earthworms can play a major role in the ecology of *P. cactorum* in apple orchards. By depositing castings and their cargo of oospores, earthworms concentrate these *P. cactorum* propagules on the soil surface, thus contributing to the steep depth gradient in propagule number detected with the SADAMCAP technique.

Reasons for the decline in population with distance from tree are less clear. Despite the lack of correlation between fibrous root and *P. cactorum* population densities in depth studies, fibrous root infection with subsequent decay and release of oospores remains a potential contributor to the distance gradient. Although *P. cactorum* is primarily considered a crown- and collar-rot pathogen of

TABLE 4. *Phytophthora cactorum* colony counts in naturally infested apple orchard soil following various surface amendments

Experiment	Treatment[u]	*P. cactorum* colony count[v]	Regression analysis[w]			
			Intercept	Slope	r^2 (%)	*P*
Microplots[x]						
	A, control (bare)	51.40 a	3.22	0.194	52.7	<0.001
	B, control (plastic mulch)	69.95 a	3.68	0.153	56.0	<0.001
	C, green apple leaves	64.63 a	3.66	0.137	36.3	<0.001
	D, inoculated apple leaves[y]	176.32 b	5.07	0.029	0.0	0.450
	E, abscised apple leaves	68.48 a	3.80	0.115	42.4	<0.001
	F, apple fruit	63.20 a	3.83	0.088	48.9	<0.001
	G, inoculated apple fruit[y]	60.62 a	3.42	0.183	58.6	<0.001
	H, hay mulch	78.84 a	4.00	0.099	44.2	<0.001
Tree plots[z]						
	A, control (bare)	40.43 a	4.08	–0.111	39.0	<0.001
	B, control (plastic mulch)	38.62 a	4.37	–0.203	56.7	<0.001
	C, green apple leaves	28.94 a	3.87	–0.164	44.9	<0.001
	D, inoculated apple leaves[y]	151.64 b	5.75	–0.245	49.5	<0.001
	E, abscised apple leaves	34.48 a	4.21	–0.160	31.6	<0.001
	F, apple fruit	12.41 a	2.76	–0.105	28.2	0.001
	G, inoculated apple fruit[y]	27.88 a	3.18	–0.052	2.0	0.217
	H, hay mulch	20.11 a	3.18	–0.083	7.2	0.082
	I, natural (no treatment)	31.69 a	3.86	–0.136	36.4	<0.001

[u] Treatments were applied in September/October 1993 and reapplied 12 months later (as described in text).

[v] Mean *P. cactorum* colony counts (weighted average, obtained from SADAMCAP analysis of soil depth classes down to 10 cm), six replicates per treatment, measured in May 1995. Letters indicate significance classes ($P = 0.05$) determined using Tukey's test (Minitab Release 9). Microplot and tree-plot experiments were analyzed separately.

[w] Regression analyses of the natural logarithm of *P. cactorum* colony count versus soil depth (depth midpoint used for each depth class). Samples were taken from 0 to 1, 1 to 2, 2 to 4, 4 to 7, and 7 to 10 cm in microplots and from 0 to 1, 1 to 3, 3 to 6, 6 to 10, and 10 to 15 cm in tree plots. Microplot and tree-plot data were analyzed separately.

[x] Microplots consisted of 18-cm diameter clay pots containing thoroughly mixed apple orchard soil naturally infested with *P. cactorum*. Pots were 18-cm deep and were buried in an apple orchard, leaving 2 to 3 cm above the ground. There were six replicates of each treatment in a randomized complete block design.

[y] Inoculated with a zoospore suspension from six isolates of *P. cactorum*.

[z] One plot of each treatment was established on the soil beneath each of six mature apple trees in a randomized complete block design.

apple trees (22) and published accounts of naturally occurring infections of fibrous roots are rare (12,24), the fungus can be isolated readily from fibrous roots of both diseased and apparently healthy trees (I. J. Horner and W. F. Wilcox, *unpublished data*). Similarly, there are numerous reports of infection of seedling roots in greenhouse studies (5,15), and oospores can form abundantly in such roots of apple seedlings after inoculation with *P. cactorum* (15). Although we did not find evidence of a strong relationship between fibrous root weight and *P. cactorum* population in samples taken at intervals out from apple trees (Fig. 5), it should be noted that oospores recorded in soil at a given time probably reflect infection and subsequent decay of plant tissues from the previous season and won't necessarily correspond well to the current distribution of available substrates. In citrus orchards, there was no correlation between root and *P. parasitica* densities when samples were collected from beneath apparently healthy trees, but the population became more closely related to root distribution where trees were mildly declining (34).

Differences in accumulation of litter and in activity of earthworms at different distances from the tree were not investigated, but could potentially contribute to the observed distance gradient. It is also possible that, close to the trunk where it is more shaded, fallen leaves are more readily colonized by *P. cactorum* because of reduced leaf desiccation and high water contents at the soil surface.

P. cactorum was found in all 17 orchards surveyed, a result similar to that of Jeffers (19). No attempts were made to assess various characteristics that may account for the wide variations in populations found at different sites that, superficially, appeared very similar. A study of such factors may be a worthy area of future research.

The observed distribution of *P. cactorum* propagules in the soil gives some insight into potential strategies for controlling disease. The fact that most *P. cactorum* propagules are in the top few centimeters of soil means that they are potentially vulnerable to chemical or biological amendments that may have limited abilities to infiltrate the soil. If apple tree litter is indeed important in the propagation of *P. cactorum*, then management or treatment of this substrate on problem sites could potentially reduce populations and ultimately disease. Because of the steep decline in population with increasing distance from tree, old tree sites should be avoided when orchards are replanted, to minimize primary inoculum density in soil around the new plantings.

Population studies using techniques such as SADAMCAP could potentially be useful in assessing the effectiveness of experimental measures to control *P. cactorum.* Using the assumption that, over time, soil populations will increase where conditions are favorable for disease and decrease under unfavorable conditions, experimental treatments could be compared over a much shorter time period than would normally be required in studies investigating tree mortality. However, because of the various gradients observed in apple orchards, strict sampling protocols should be used in studies of *P. cactorum* populations. Consistency in sample distance from the tree and, in particular, sample depth is important to avoid bias and unnecessary variability. Samples should always be collected using corers, rather than trowels or shovels. Variability from tree to tree should also be considered, particularly on undulating sites.

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