Temporal Changes in Activity and Dormant Spore Populations of *Phytophthora cactorum* in New York Apple Orchard Soils

I. J. Horner and W. F. Wilcox

First author: Horticulture and Food Research Institute of New Zealand, Private Bag 92-169, Auckland; and second author: Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456.
Accepted for publication 19 June 1996.

ABSTRACT

Horner, I. J., and Wilcox, W. F. 1996. Temporal changes in activity and dormant spore populations of *Phytophthora cactorum* in New York apple orchard soils. Phytopathology 86:1133-1139.

Dormant spore populations of *Phytophthora cactorum* in three New York apple orchard soils were measured over a 2-year period using the SADAMCAP (soil air-dried and moistened chilled and plated) technique. At all three sites, populations were highest in early spring, declined steadily throughout the summer and fall, and increased again the following spring. Activity, defined as the presence of germinable sporangia or zoospores in the soil, was assessed and quantified using the "brief baiting" and SCAP (soil chilled and plated) techniques. For brief baiting, soil samples were flooded and baited with apple cotyledons for 4 h, and then baits were removed and further incubated in distilled water to determine colonization. For SCAP, soil samples were flooded and chilled, the drained water was plated on selective agar, and *P. cactorum* colonies were enumerated. There was no activity in field samples collected from late November to March. The first activity in spring coincided with the time that soil temperatures exceeded 10°C. In laboratory studies, there was no colonization of cotyledon baits when air-dried soil naturally infested with *P. cactorum* was remoistened, flooded, and baited at temperatures below 10°C. However, following incubation of soil at 22°C to allow oospore germination and sporangial formation, cotyledons were infected at temperatures down to 7°C. Once cotyledons were colonized, the fungus sporulated on leaf margins down to 6°C. In apple orchards, there was no correlation between measured *P. cactorum* activity and soil temperature when only periods above 10°C were considered. Neither activity nor oospore populations were closely associated with rainfall over the previous 5 days.

Additional keywords: chlamydospore, epidemiology.

Phytophthora cactorum (Lebert and Cohn) J. Schröt. causes root, crown, and collar rots of apple trees throughout the world's production regions (7,16). Oospores, capable of surviving freezing and air-drying, are the major survival structure for *P. cactorum* in the soil (21,26). However, infection generally occurs via zoospores released from sporangia, which are formed either directly from mycelium in host tissue or from germinated oospores (16). Thus, investigation of seasonal fluctuations in both the populations of *P. cactorum* oospores and the activity of sporangia and zoospores in apple orchard soils may contribute to elucidation of the biology and ecology of the fungus and the diseases it causes.

Braun and Schwinn (2) were unable to isolate *P. cactorum* from apple orchard soil in Germany during early winter, although the fungus was detected at other times. In contrast, Sewell et al. (23,24) were able to isolate *P. cactorum* from English apple orchard soils throughout the year, albeit with lowest frequency in the winter and only when soils were baited at laboratory temperatures. When soil was baited outside at ambient air temperatures, *P. cactorum* was isolated only from April or May until October, when mean daily air temperatures were above 8°C, suggesting that sporangia were produced only during this period. These early investigations of *P. cactorum* population dynamics were limited by a lack of accurate and reliable quantification techniques. Furthermore, the technique used (i.e., baiting with apple fruit) is relatively insensitive and would not have distinguished between different types of propagules.

Corresponding author: I. J. Horner; E-mail address: ihorner@hort.cri.nz

Publication no. P-1996-0830-03R

Direct plating procedures used successfully in population studies of other species of *Phytophthora* (18,27,28) have been attempted only rarely with *P. cactorum* (8,20) and generally have been confounded by the sensitivity of this fungus to hymexazol (15), a key ingredient in selective agar media used to control fungal contaminants. The recent development of the SADAMCAP (soil air-dried and moistened chilled and plated) technique (11) has provided a tool for more detailed and quantitative investigations of changes in soilborne *P. cactorum* populations, although it only measures dormant spore populations (11). Thus, the aim of this work was to i) develop techniques for assessing and quantifying the active stages of *P. cactorum*; ii) use these techniques and SADAMCAP to investigate seasonal fluctuations in dormant spore populations and activity of the fungus in apple orchard soils; and iii) relate these fluctuations to environmental parameters such as temperature and rainfall. Throughout this work, "activity" is defined as the presence of active propagules such as sporangia and zoospores; the level of activity is indicated by the enumeration of these propagules. A summary of this work has been published previously (10).

MATERIALS AND METHODS

Orchard soils. Studies were conducted in three apple orchard soils naturally infested with *P. cactorum*. Orchard 1 was a block of 'Cortland' on MM106 rootstock at the New York State Agricultural Experiment Station in Ontario County, NY. In orchard 2 (adjacent to orchard 1), trees were 'McIntosh' on seedling rootstock. Orchard 3 was a block of 'Rome' grown on seedling rootstock in a commercial orchard in Wayne County, NY, approximately 1 km south of Lake Ontario. All trees were mature, ranging in age from 25 to 50 years. Soils were described as Ovid, Odessa, and Lima

silt loams for orchards 1, 2, and 3, respectively. A more detailed description of these soils is given elsewhere (12).

Quantification of dormant spores. *P. cactorum* dormant spore populations were determined using the SADAMCAP technique (11). Briefly, this involved sieving and air-drying soil, moist incubation under lights for 4 days, and then flooding and chilling for 2 h. Next, the floodwater was drained, collected, vigorously mixed, incubated at room temperature for 10 min, and plated on PARP selective agar medium (15) (5 ml of agar/9-cm-diameter plate). *P. cactorum* colonies were marked after 20, 28, and 44 to 48 h, and data were recorded as numbers of colonies per plate to give a relative estimate of population under a standard set of conditions. Numbers were not converted to propagules per gram of soil because of the arbitrary nature and numerous assumptions required for such calculations (11).

Development of activity assays. Two different procedures were developed for assaying and quantifying the activity of *P. cactorum* (i.e., germinable and infectious sporangia or zoospores in the soil). The first, which we termed "brief baiting", involved flooding soil samples within 1 h of collection from the field and baiting with cotyledons from 2- to 4-week-old 'McIntosh' apple seedlings (14). In preliminary experiments designed to determine the time required for zoospore release and infection of the cotyledons, soil naturally infested with *P. cactorum* was collected from the top 10 cm in orchard 2, sieved, and air-dried. Five replicate 10-g samples were then placed in 9-cm-diameter petri dishes, remoistened with 3.8 ml of distilled water (providing a matric potential of approximately –3.0 kPa), and incubated at 22°C under lights for 96 h to promote oospore germination and sporangial formation (11,14). Samples were then flooded and baited. After periods of either 1, 10, 15, 20, 30 min, 1, 2, 4, 8, or 24 h, four cotyledons were removed from each replicate petri dish and distributed singly to each of four rinsing treatments: (i) no rinse; (ii) double rinse in distilled water; (iii) 30 s in 50% ethanol followed by double rinse in distilled water; or (iv) 30 s in 1% sodium hypochlorite followed by double rinse in distilled water. Following rinsing, cotyledons were placed individually in 22-mm-diameter cell wells containing distilled water and incubated at 22°C under lights for 4 days. The incidence of colonized cotyledons was determined microscopically by checking for characteristic *P. cactorum* sporangia growing from the cotyledon margins (14). Results were similar in a repeat experiment using soil from orchard 1, so data were pooled for analysis.

Additional preliminary experiments were conducted to determine the minimum time for dormant spores to produce infectious propagules (presumably zoospores) in flooded soil. Soil collected from orchard 2 was air-dried for 5 days to kill sporangia (21,26), and then five replicate 10-g samples in glass petri plates were each flooded with 60 ml of water at 22°C and baited with 36 apple cotyledons. At intervals of 1, 12, 24, 32, 36, 40, 44, 48, 60, 72, 84, and 96 h, three cotyledons were removed from each plate, thoroughly rinsed in distilled water, incubated individually in distilled water in cell wells as described above, and observed microscopically for the presence of sporangia. Results were similar in a repeat experiment using soil from orchard 1, so data were pooled for analysis.

Based on the results from these experiments with air-dried soils, the brief baiting assay consisted of placing freshly collected soil (10 g on a dry weight basis) in a 9-cm-diameter glass petri dish and flooding with approximately 60 ml of distilled water. (The dry/fresh weight ratio was determined by oven-drying a 3- to 4-cm^3 subsample of soil for 30 min immediately before initiating the assay.) Cotyledon baits were floated on the floodwater for 4 h, vigorously rinsed in two changes of distilled water, and placed in cell wells containing distilled water. The cotyledons were then incubated at 22°C, and the colonization incidence was determined microscopically as described above.

The second procedure, SCAP (soil chilled and plated), was a reduced version of SADAMCAP in which the air-drying of soil and subsequent 4-day moist incubation period were deleted. Thus, within 1 h of collection from the field, soil samples were sieved and transferred to 9-cm-diameter glass petri dishes, flooded with distilled water, and incubated at 6°C for 2 h. The quantities of soil and water added to each plate were determined as described for the brief baiting assay above to give a total of 10 g of soil and 16 ml of water after flooding. After flooding, subsequent water collection and plating steps were similar to those described for SADAMCAP, except that drained water samples were not diluted fivefold before plating. Data were recorded as numbers of *P. cactorum* colonies per plate.

Orchard assessments. Temporal changes in dormant spore populations and activity were assessed in the three apple orchards described above. Four trial trees were selected in both orchards 1 and 2, and eight trial trees were selected in orchard 3. Only apparently healthy trees were selected for the trial to avoid problems with tree mortality and population changes caused by host decline during the trial period. Within the herbicide-treated strip beneath each tree, four sampling quadrats (80 × 20 cm) were marked out, with the center of the nearest long edge of each quadrat 0.6 m from the trunk. Quadrats were arranged approximately at cardinal points around each tree. At approximately monthly intervals from July 1993 to August 1995 (except for January to mid-March each year), one sample core (2-cm diameter × 10-cm deep) was taken at a randomly predetermined grid point within each quadrat. Within 1 h of collection, samples were sieved through a 2.36-mm mesh screen and thoroughly mixed. Half of each soil sample was air-dried, and the dormant spore population was assessed using the SADAMCAP technique. The remainder of each sample was pooled with the other three samples from the same tree for analysis of *P. cactorum* activity. From the pooled sample, an 11- to 13-g subsample (equivalent to 10 g of soil on a dry weight basis) was immediately assayed using the SCAP process. Two additional 10-g subsamples were assayed using the brief baiting process, with one subsample subjected to the 4-h incubation at ambient temperature in the field and the other at 22°C in an incubator. Under the ambient field conditions, samples were buried beneath apple trees in 8-cm-deep plastic cups so that the orchard soil surface was just below the top of the cup. Distilled water at the current field temperature (soil temperature measured at 3-cm depth beneath apple trees at 10:00 a.m. on the day of sampling) was used to flood samples. Cups were covered with a 5-cm-thick sheet of polystyrene to shield baits from direct solar radiation during incubation. Samples in the 22°C chamber were baited in 2-cm-deep petri plates flooded with distilled water at 22°C. After both bait incubation regimes, the eight cotyledons per subsample were removed, incubated in distilled water for 4 days at 22°C, and the colonization incidence determined as described above. During spring and early summer in 1994, additional samples to determine activity were taken midway between regular sample dates using this same protocol.

At each regular monthly sample time, a 10-cm-deep core was taken 1 m from the trunk of each sample tree and the gravimetric water content determined. These data were averaged to give a mean soil water content at each orchard at each sample time. Previous experiments indicated that soil-water matric potentials at given water contents were similar for the three soils (I. J. Horner and W. F. Wilcox, *unpublished data*). Daily precipitation and maximum, minimum, and mean soil temperatures at 5-cm depth under turf were recorded at the Climatological Reference Station (no. 3031840) at Geneva, NY, approximately 1.5 km from orchards 1 and 2 and 50 km from orchard 3. These data were used to calculate total precipitation and mean temperature over the 5 days before each soil sampling date. During spring 1994, temperature probes placed at 5-cm depth adjacent to the sample plots in orchard 1 indicated that soil temperatures were generally within 1.5°C of those measured at the Climatological Station.

P. cactorum colony count data from SADAMCAP and SCAP tests were transformed by natural logarithms and, for each orchard, these data and the cotyledon-colonization incidences from brief baiting tests were analyzed using the General Linear Model (Minitab Release 9; Minitab Inc., State College, PA). Additionally, mean values for *P. cactorum* colony count and cotyledon-colonization incidences were determined for each orchard at each sample time and regressed against either total rainfall or mean soil temperature for the 5-day period before each sampling or against the mean orchard soil water content at the time of sampling.

Effect of temperature on infection developing from dormant spore inoculum. To determine the minimum temperature for dormant spores to germinate and produce infectious propagules, soil from orchard 2 was baited under three different incubation regimes at temperatures of 4, 6, 7, 8, 9, 10, 11, 12, and 22°C. For each temperature and incubation regime, two replicate 9-cm-diameter petri plates were filled with 20 g of soil previously air-dried for 3 days. Soil was subsequently remoistened with 7.5 ml of distilled water (to give soil-water matric potential ≈ –3.0 kPa) and incubated for 4 to 5 days. Each soil plate was flooded and baited with five apple cotyledons. The presence of *P. cactorum* sporangia growing from the margin of baits was determined microscopically daily up to 8 days, and after 10, 14, and 21 days. Results were similar when the experiment was repeated using soil from orchard 1, so data were pooled.

Incubation regime 1. Air-dried soil was frozen for 24 h to ensure that no sporangia or zoospores survived (19,21,26). Next, soil was held dry at the test temperature for 2 days, remoistened with distilled water (preadjusted to the respective test temperatures), and incubated for 5 days at the test temperature. Samples were then flooded with preadjusted distilled water, baited, and further incubated at the test temperature before determining the incidence of bait colonization by *P. cactorum*.

Incubation regime 2. Following air-drying, soil samples were remoistened with distilled water preadjusted to 22°C, and incubated at 22°C under lights for 4 days to promote oospore germination and sporangial formation (11,14). Samples were then placed in an incubator at the test temperature, flooded with water (preadjusted to the appropriate temperature), baited, and further incubated.

Incubation regime 3. Following air-drying and moist incubation at 22°C under lights for 4 days, soil samples were flooded with water at 22°C, baits were added, and plates were incubated at 22°C for 8 h to ensure infection of baits. Cotyledons were then rinsed in distilled water, placed in cell wells containing sterile distilled water, and incubated at the test temperature. Additional cotyledons were maintained at 22°C and, at hourly intervals from 24 to 36 h from the initiation of baiting, they were microscopically checked for the presence of *P. cactorum* sporangia growing from the leaf margin.

TABLE 1. Effect of flooding duration/bait exposure time and bait rinse treatment on infection of apple cotyledons by *Phytophthora cactorum*

	Duration of flooding and bait exposure (min) [x]									
Rinse treatment	1	10	15	20	30	60	120	240	480	1,440
No rinse	0[y]	3	10	10	10	10	10	10	10	10
Distilled water, 2 times	0	0	7	8	10	10	10	10	10	10
50% ethanol, 30 s[z]	0	0	0	0	2	4	10	10	10	10
1% sodium hypochlorite, 30 s[z]	0	0	0	0	0	2	3	6	9	10

[x] Air-dried soil from apple orchards naturally infested with *P. cactorum* was remoistened and incubated at 22°C under lights for 96 h to promote oospore germination and sporangial formation. Soil was then flooded and immediately baited with apple cotyledons, which were removed and rinsed after the indicated exposure times.

[y] Data are the numbers of cotyledons (out of 10) infected by *P. cactorum,* recorded after incubation in distilled water for 4 days following baiting. Data were pooled from two experiments, in both of which a single cotyledon was sampled from five replicate petri plates after each exposure period.

[z] Ethanol and sodium hypochlorite treatments were followed by a double rinse in distilled water.

RESULTS

Dynamics of zoospore release and infection. When samples of air-dried soil from apple orchards naturally infested with *P. cactorum* were remoistened and incubated to promote oospore germination and sporangial formation, 30% of unrinsed cotyledon baits removed after 10 min of subsequent flooding were infected, and 100% were infected when removed after 15 min (Table 1). Only slightly longer periods of exposure to the flood water were required for these infection incidences to develop when baits were rinsed in distilled water following removal. However, when baits were subsequently rinsed in solutions of ethanol or sodium hypochlorite, no infection was detected before 30- or 60-min exposure periods, and 100% infection incidences required 120- and 1,440-min exposures, respectively (Table 1).

In contrast, when air-dried soil was immediately flooded and baited without a prior moist incubation period, there was no infection of baits removed (and subsequently rinsed in distilled water) within the first 32 h. The first infections by *P. cactorum* were noted after flooding for 36 h, and the number of baits infected increased steadily from this time to a peak after 84 h (Fig. 1). Repeated experiments with emphasis on incubation times between 20 and 40 h confirmed that the first infection occurred between 32 and 36 h (data not shown).

Orchard assessments. *Dormant spore populations.* In all three orchards studied, the number of *P. cactorum* propagules detected by the SADAMCAP technique was highest in the spring and declined steadily throughout the summer to relatively low numbers by autumn (Fig. 2). In 1994 and 1995 in all three orchards, populations had returned to relatively high levels by the time the first samples were taken in early spring (late March/early April). When the mean orchard *P. cactorum* colony count for each sampling

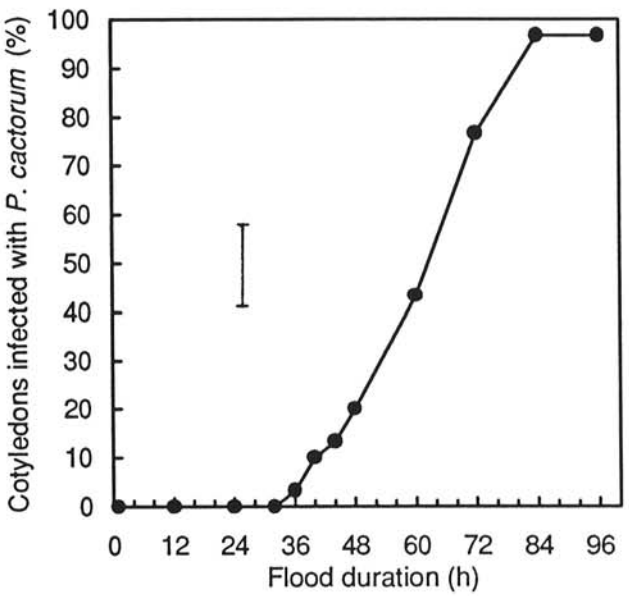

Fig. 1. Effect of flood duration of previously air-dried soils on the incidence of infection of apple cotyledon baits by *Phytophthora cactorum*. Five replicate 20-g samples of apple soil naturally infested with *P. cactorum* were air-dried for 5 days, flooded at 22°C in glass petri plates, and baited with 36 apple cotyledons each. At the indicated intervals, three cotyledons were removed from each plate, thoroughly rinsed in distilled water, incubated individually for 4 days in cell wells containing distilled water, and observed microscopically for the presence of sporangia to indicate infection. Data from two such experiments with similar results were pooled. Data points are the percentage of cotyledons infected (out of 30 total) at each time interval. The error bar represents a 95% confidence interval.

date was regressed against mean gravimetric soil water content for the same date and against total rainfall or mean soil temperature in the previous 5 days, no significant correlations were found.

Activity. Correlation analyses comparing results from the three activity assessment techniques (SCAP, brief baiting in the field, and brief baiting at 22°C) over the 2-year sample period showed that the respective colony count data and cotyledon-infection incidences were closely correlated ($P < 0.001$) for all combinations. There was no significant difference ($P = 0.67$) between the percentage of cotyledons infected when baited at ambient field temperatures or at 22°C. In limited microscopic examinations 24 h after plating with the SCAP technique, colonies could be traced to zoospore cysts in all cases in which the colony origin was not obscured by soil debris (approximately 50% of colonies).

A similar pattern of activity was observed in both years in all three orchards investigated, although the level of activity in orchard 3 was considerably lower than in orchards 1 and 2 (Fig. 3), which did not differ significantly from each other in analyses using the Generalized Linear Model. There was no activity before late March/early April, fluctuating levels of activity throughout the spring and summer, and a cessation of activity by November (Fig. 3). When activity data from orchards 1 and 2, either individually or combined, were regressed against soil temperature, there was a significant positive correlation ($P < 0.001$). However, there were very few infectious propagules detected when soil temperatures were below 10°C (Fig. 4). Thus, when data were considered only from samples collected when soil temperatures were above 10°C (averaged over the previous 5 days), there was no significant relationship between soil temperature and activity measurement, with r^2 values less than 3.0% and P values greater than 0.23 for all combinations of orchard and activity measurement methods. Soil temperature (and rainfall) data were not considered relevant to orchard 3, 50 km from the weather station.

When activity data were regressed against rainfall over the 5 days before sampling, there was a weak positive relationship in orchard 1, with r^2 values of 13.7, 15.6, and 9.5% and P values of 0.025, 0.019, and 0.057 for SCAP, field baiting, and baiting at 22°C, respectively. In orchard 2, the relationship was only slightly stronger, with r^2 values of 8.4, 22.1, and 15.8% and P values of 0.066, 0.006, and 0.019 for the same measurement techniques,

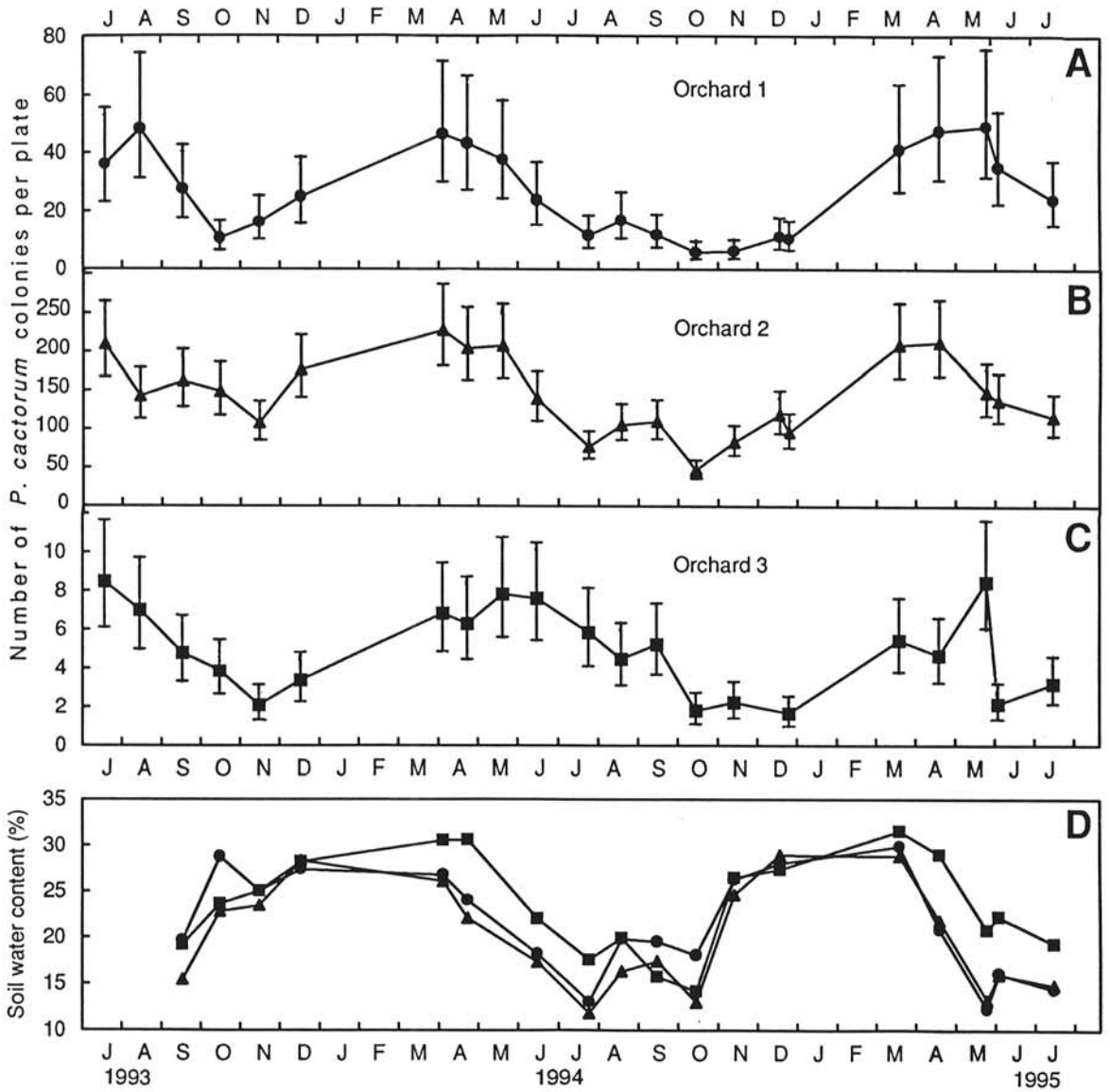

Fig. 2. Relative dormant spore populations of *Phytophthora cactorum* and gravimetric water content in three apple orchard soils over a 2-year period. **A, B, and C,** Number of *P. cactorum* colonies per plate for samples collected in orchards 1, 2, and 3, respectively, measured using SADAMCAP. For each sample time, data are means of single 10-cm-deep cores collected at randomly selected points within each of four quadrats around each of four, four, or eight trees in orchards 1, 2, or 3, respectively. Error bars represent 95% confidence intervals. Data were transformed using natural logarithms prior to analysis. **D,** Mean percentage of soil water content [(weight of water/weight of soil) × 100] in a single 10-cm core collected at each tree at each sample time. ● = orchard 1, ▲ = orchard 2, and ■ = orchard 3.

respectively. When activity data from sample dates with mean soil temperature less than 10°C were omitted from the analysis, similar results were obtained. When activity data at each sample date was regressed (separately for each orchard) against mean gravimetric soil water content for the same date, relationships were either nil or slightly negative. When data from sample dates with soil temperatures less than 10°C were omitted, no significant relationship between activity and soil water content was found.

The level of activity detected in 1994 was much higher than that in 1995, especially over the spring period (Fig. 3). Total rainfall from April through June was 233.2 and 88.9 mm for 1994 and 1995, respectively. Soil temperatures during the preceding winters differed greatly in the 2 years, with relatively constant temperatures near 0°C from January to March 1994 (due to continuous snow cover) and widely fluctuating temperatures (extremes from –6.0 to +15°C) over the same period in 1995.

Effect of temperature on dormant spore germination and infection. When air-dried soil was moistened, flooded, and baited at various constant temperatures (incubation regime 1), infection occurred only at temperatures above 10°C (Table 2). However, when sporangia were preformed in the soil (after 4 days of moist incubation at 22°C) before flooding and baiting at the test temperatures (incubation regime 2), infection and subsequent sporulation on baits occurred at temperatures down to 7°C. Similarly, when baits were exposed to *P. cactorum* zoospores at 22°C for 8 h to allow infections to establish and then incubated at lower temperatures (incubation regime 3), subsequent colonization and sporulation from the baits occurred down to 6°C. Incubation temperature following initial infection by zoospores affected both the dynamics and quality of subsequent sporulation from the cotyledon baits. This was most clearly shown in incubation regime 3, in which baits initially exposed to zoospores for 8 h at 22°C first sporulated 10 days later when subsequently incubated at 6 or 7°C

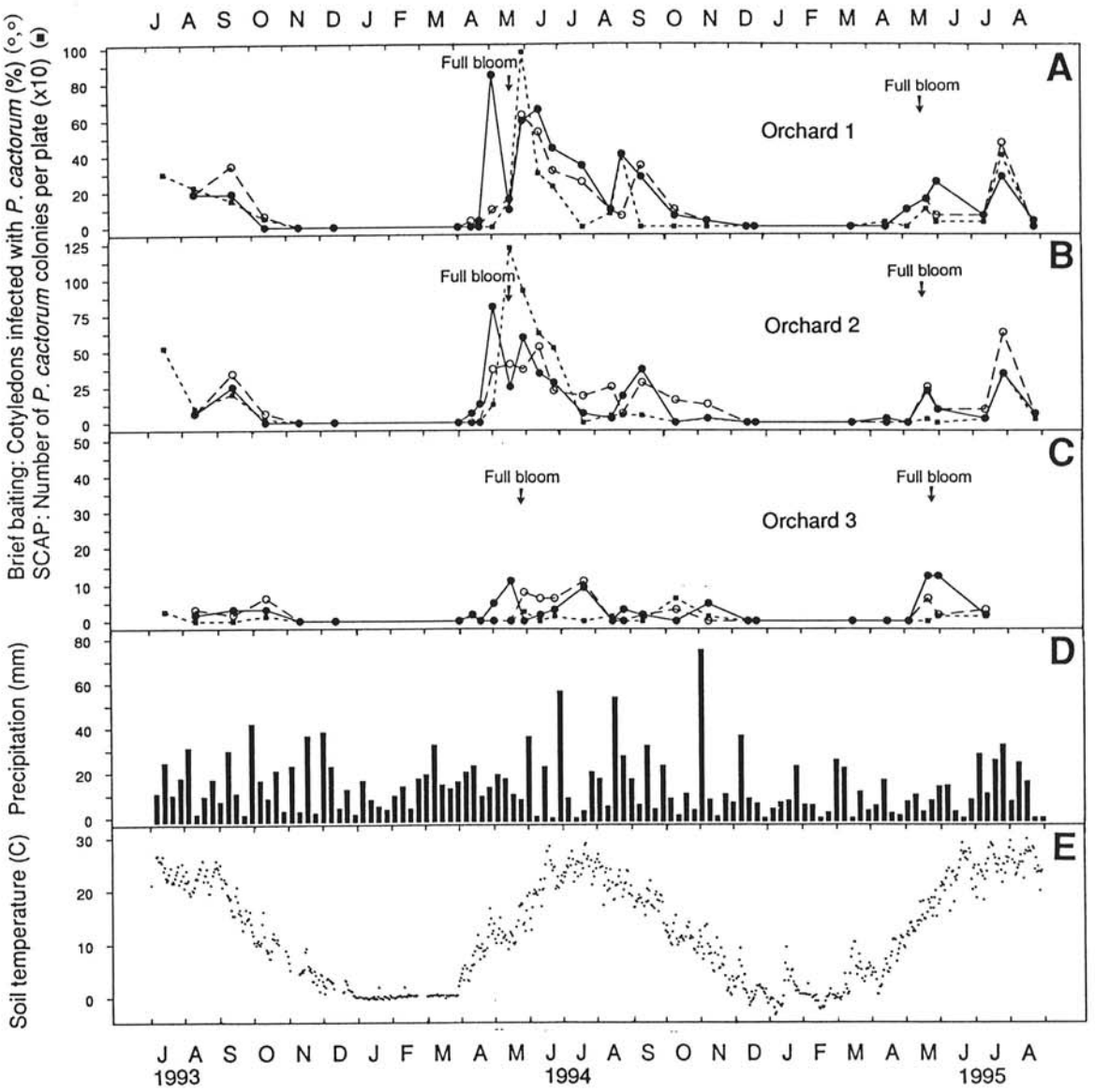

Fig. 3. Relative activity of *Phytophthora cactorum*, rainfall, and soil temperature recorded over a 2-year period in three apple orchards. **A, B, and C,** Activity measured three different ways in each of orchards 1, 2, and 3, respectively, from July 1993 to August 1995. Four 10-cm-deep cores were sampled at the indicated times beneath each of four, four, and eight trial trees in orchards 1, 2, and 3, respectively. Immediately after collection, the four cores from each tree were pooled, and subsamples from each tree were flooded and assessed with the brief baiting technique either in the field (—●—) or in a 22°C incubator (- -○- -). For each sampling date, data points represent the percentage of apple cotyledon baits infected out of a total of 32 for orchards 1 and 2, and 64 for orchard 3. Concurrently, a third subsample from each tree was immediately assessed using the SCAP technique (- -■- -), i.e., soil was flooded, chilled, drained, and the drained water plated on selective agar. Data are mean *P. cactorum* colony counts per plate (× 10) for each orchard at each sample time. **D and E,** Precipitation and soil temperature at 5-cm depth measured at a weather station approximately 1.5 km from orchards 1 and 2 and 50 km from orchard 3. Data are weekly totals and daily means for precipitation and temperature, respectively.

(Table 2). In contrast, when cotyledon baits were maintained continuously at 22°C, the first signs of *P. cactorum* sporangia growing from the cotyledon margin were observed 30 h after the initiation of baiting. After 33 h, these sporangia appeared mature and, when chilled at 6°C for 30 min, they released zoospores. At incubation temperatures below 10°C, *P. cactorum* formed chlamydospores from infected cotyledon margins. These chlamydospores were terminal, spherical, nonpapillate with diameters that ranged from 29 to 52 µm (mean 41.7 µm), and not associated with antheridia. Thus, they were readily distinguishable from oospores and sporangia. Chlamydospores became more frequent at lower temperatures and, at 6 to 8°C, chlamydospores were far more prevalent than sporangia.

DISCUSSION

The seasonal trend in dormant spore population detected by the SADAMCAP technique was very similar in all three trial orchards, even though population levels differed greatly among orchards. The steady decline in measured populations from the spring peak throughout the summer was likely because of the germination of many oospores that produce sporangia during this period, as reflected in the assayed flushes of activity over the summer. There was, presumably, also some biological and physical lysis of oospores as the soils became warm during the growing season (25). It cannot be determined whether the relatively high populations recorded at the start of the trial in July and August 1993 represent a peak in population for that year or the continuation of a decline in population from even higher densities in the spring. The 1992 growing season was extremely wet, which could have contributed to high levels of *P. cactorum* in the soil. Since SADAMCAP measures only populations of germinable oospores (and perhaps chlamydospores) in the soil (11), and oospores form predominantly within host tissue (21), many such spores would not be recovered until at least some months after formation. Recently formed spores would be either discarded with roots during the sieving process or protected within small tissue fragments (18,29), which could account for the low numbers observed in the fall despite activity and infection of plant tissues throughout the summer (12). The high numbers of dormant spores recorded in the spring probably reflect infections of tree litter and roots that occurred the previous summer or fall and the subsequent release of oospores into the soil as tissues were physically or biologically broken down (12). It is also possible that overwintering in the soil satisfies a chilling requirement for oospore germination (1,2); that freezing (and associated dehydration) may stimulate oospore germination, analogous to the air-drying procedure in SADAMCAP (11); or that winter simply provides a necessary period of oospore maturation (7). Thus, the lack of correlation between relative oospore population and current soil temperature or moisture level is not surprising, because of the presumed time lag between production of oospores and their detection.

Reports of *P. cactorum* oospores in soil and various apple tree tissues are common (6,7), and we consider oospores to be the predominant dormant spore type measured by the SADAMCAP technique. There are very few reports of naturally occurring *P. cactorum* chlamydospores (3,21). Nevertheless, Darmono and Parke (3) noted production of chlamydospores by each of 11 *P. cactorum* isolates incubated at 4°C. Similarly, we noted that chlamydospores were produced on apple-cotyledon-bait margins when incubated at temperatures less than 10°C. Thus, chlamydospores should not be discounted as possible contributors to colony counts obtained using SADAMCAP.

We defined "activity" of *P. cactorum* as the presence of germinable sporangia, zoospores, or zoospore cysts in the soil, as these are the propagules that cause the majority of apple tree infections (16). To assay such activity, we flooded freshly collected soils for 2 to 4 h and either plated the resultant propagule suspensions (confirmed to be zoospores) onto selective agar or baited them. In our previous work (11) and in preliminary experiments with air-dried soil (Fig. 1), we have shown that, at the temperatures used, the time required for oospore/chlamydospore germination, sporangium formation, and subsequent release of zoospores exceeds 32 h. In contrast, sporangia that are present when flooding is initi-

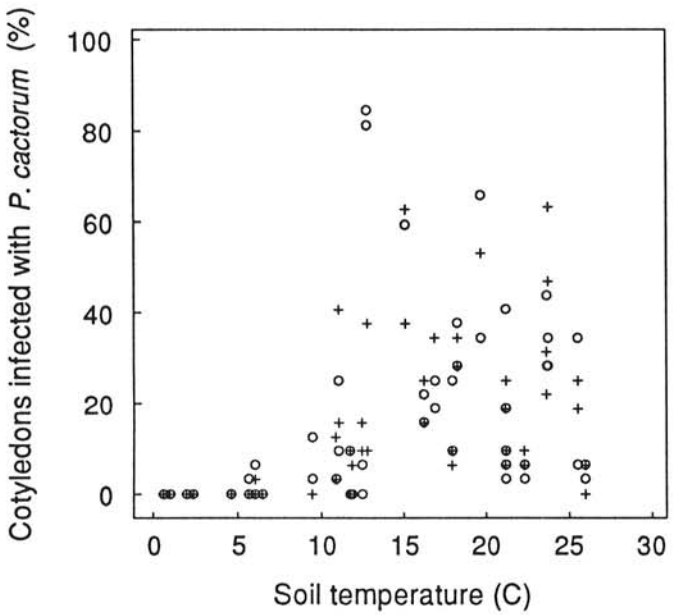

Fig. 4. Relationship between temperature and activity of *Phytophthora cactorum* in two apple orchard soils. Beneath each of four trial trees in both orchards 1 and 2, four 10-cm-deep cores were sampled at approximately monthly intervals from July 1993 to August 1995, excluding January to mid-March. Additional samples were taken midway between monthly samplings during spring 1994. Cores from each tree were pooled and, within 1 h of collection from the field, subsamples from each tree were flooded and baited with eight apple cotyledons either in the field (○) or in a 22°C incubator (+). After 4 h, baits were removed, rinsed, and incubated in the light at 22°C for 4 days, when infection by *P. cactorum* was determined microscopically. Data points are the mean percentage of cotyledons infected by *P. cactorum* at each sample time in both orchards plotted against the mean daily soil temperature (at a 5-cm depth) over the 5 days before sampling, as measured approximately 1.5 km from the sample orchards.

TABLE 2. Effect of incubation temperature regime on infection of apple cotyledon baits by *Phytophthora cactorum*

	Test temperature (°C)								
Incubation regime	4	6	7	8	9	10	11	12	22
1[v]	…[w]	…	…	…	…	…	8,10[x]	6,6	2,2
2[y]	…	…	14,14	7,8	7,8	6,6	5,6	4,4	2,3
3[z]	…	10,14	10,10	7,8	7,8	6,6	5,5	4,5	2,3

[v] Soil naturally infested with *P. cactorum* was collected from beneath apple trees, sieved, air-dried, and then incubated and baited with apple cotyledons under regime 1: at the test temperature, air-dried soil was remoistened with 7.5 ml of water/20 g of soil, incubated for 5 days, and then flooded and baited.

[w] … indicates no sporangia were observed after 21 days.

[x] Data (x,y) are days to first appearance of sporangia on the margin of baits and days to 50% of baits with sporangia, determined microscopically through 21 days from the commencement of flooding and baiting. There were four soil plates at each temperature and incubation regime, each baited with five apple cotyledons. Results from two experiments were similar, so data were combined.

[y] Soil naturally infested with *P. cactorum* was collected from beneath apple trees, sieved, air-dried, and then incubated and baited with apple cotyledons under regime 2: air-dried soil was remoistened and incubated at 22°C for 4 days, and then flooded and baited at the test temperature.

[z] Soil naturally infested with *P. cactorum* was collected from beneath apple trees, sieved, air-dried, and then incubated and baited with apple cotyledons under regime 3: air-dried soil was remoistened and incubated at 22°C for 4 days, flooded and baited at 22°C for 8 h to ensure infection by *P. cactorum*, and then baits were removed, rinsed, and incubated at test temperature.

ated release zoospores within minutes (Table 1). Thus, we are confident that the SCAP and brief baiting techniques did, in fact, provide a measure of activity as defined above that was not confounded by the detection of propagules developing from oospores or chlamydospores during the assay. It is likely that these techniques measure only the *P. cactorum* sporangia capable of releasing zoospores (i.e., those of greatest pathological importance), reportedly only about 15% of the total sporangia in the soil (4). Thus, the brief baiting and SCAP procedures are potentially useful tools for detailed studies of the environmental or cultural factors that affect the activity of *P. cactorum* and its ability to cause tree infection.

No activity was detected in orchard soils from late autumn until early spring. Sporangia and zoospore cysts are killed by freezing (21), and oospores do not commonly germinate at temperatures below 10°C (9) (Table 2). Thus, the first activity was detected in spring at about the time the mean daily soil temperatures reached 10°C or above. In the rare instances when active propagules were detected and the mean soil temperature for the previous 5 days was less than 10°C, temperatures had, because of daily fluctuations, periodically been above this threshold. The experiments baiting naturally infested soil at a range of temperatures (Table 2) indicated that, subsequent to oospore germination, zoospores could be released and infect apple cotyledons at temperatures as low as 6°C. This is consistent with the temperature limit for infection of apple fruits reported previously by Grove and Boal (5).

There was no correlation between measured *P. cactorum* activity in apple orchards and temperatures above 10°C. However, in laboratory experiments with naturally infested soil, the time elapsed from exposure of baits to flooded soil and production of sporangia from infected tissue was reduced from 6 days at 10°C to 33 h at 22°C. This short generation time, particularly at warmer temperatures, indicates the potential for rapid development of disease epidemics induced by *P. cactorum* when suitable conditions occur.

The correlation between field activity of *P. cactorum* and rainfall over the previous 5 days was weak, although our sampling regime, at either 2-week or monthly intervals, may not have been frequent enough to accurately determine responses to rainfall. Nevertheless, it is interesting to note that from April to June 1994, when total rainfall was 233 mm, measured activity of *P. cactorum* was considerably higher than that over the same period in 1995, when mean rainfall was only 89 mm. In studies of the spatial distribution of *P. cactorum* in apple orchards, the highest populations were noted beneath trees in the wettest sites, in particular at the bottom of the slopes (12).

Apple trees appear most susceptible to infection by *P. cactorum* during the spring (13,17,22). Sewell and Wilson (22) reported that apple trees were very susceptible to *P. cactorum* from bud break to early fruit development, with peak susceptibility coinciding with the "pink bud" stage. Our studies indicated that this period of peak susceptibility coincided with the period of highest detectable oospore or chlamydospore populations. *P. cactorum* was active in the soil throughout this period, with peak activity coinciding with late petal fall. Thus, disease development might be particularly favored in years when a warm spring coincides with periods of sufficient soil moisture to permit multiple cycles of infection.

LITERATURE CITED

1. Braun, H., and Nienhaus, F. 1959. Fortgeführte Untersuchungen über die Kragenfaüle des Apfels (*Phytophthora cactorum*). Phytopathol. Z. 36:169-208.
2. Braun, H., and Schwinn, F. J. 1963. Fortgeführte Untersuchungen über den Erreger der Kragenfäule des Apfelsbaumes (*Phytophthora cactorum*). II. Phytopathol. Z. 47:327-370.
3. Darmono, T. W., and Parke, J. L. 1990. Chlamydospores of *Phytophthora cactorum*: Their production, structure, and infectivity. Can. J. Bot. 68:640-645.
4. Gisi, U. 1975. Investigations on the soil phase of *Phytophthora cactorum* (Leb. et Cohn) Schroet. with fluorescent optical direct observation. Z. Pflanzenkr. Pflanzenschutz 82:355-377.
5. Grove, G. G., and Boal, R. J. 1991. Influence of temperature and wetness duration on infection of immature apple and pear fruit by *Phytophthora cactorum.* Phytopathology 81:1465-1471.
6. Harris, D. C. 1989. The significance of apple tree tissues for regeneration of *Phytophthora cactorum* in apple orchards. J. Hortic. Sci. 64:389-393.
7. Harris, D. C. 1991. The *Phytophthora* diseases of apple. J. Hortic. Sci. 66:513-544.
8. Harris, D. C., and Bielenin, A. 1986. Evaluation of selective media and bait methods for estimating *Phytophthora cactorum* in apple orchard soils. Plant Pathol. 35:565-574.
9. Harris, D. C., Cole, D. M., and Gibbs, J. A. 1981. Diseases caused by *Phytophthora* species. Page 81 in: Rep. East Malling Res. Stn. (Maidstone, Engl.) 1980.
10. Horner, I. J., and Wilcox, W. F. 1995. Spatial and temporal distribution of *Phytophthora cactorum* in apple orchard soils. (Abstr.) Phytopathology 85:1125.
11. Horner, I. J., and Wilcox, W. F. 1995. SADAMCAP, a technique for quantifying populations of *Phytophthora cactorum* in apple orchard soils. Phytopathology 85:1400-1408.
12. Horner, I. J., and Wilcox, W. F. 1996. Spatial distribution of *Phytophthora cactorum* in New York apple orchard soils. Phytopathology 86:1122-1132.
13. Jeffers, S. N., and Aldwinckle, H. S. 1986. Seasonal variation in extent of colonization of two apple rootstocks by five species of *Phytophthora.* Plant Dis. 70:941-945.
14. Jeffers, S. N., and Aldwinckle, H. S. 1987. Enhancing detection of *Phytophthora cactorum* in naturally infested soil. Phytopathology 77:1475-1482.
15. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.
16. Jeffers, S. N., and Wilcox, W. F. 1990. *Phytophthora* crown, collar, and root rots. Pages 43-45 in: Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. The American Phytopathological Society, St. Paul, MN.
17. Long, P. G. 1982. Apple tree resistance to collar rot disease. N.Z. J. Agric. Sci. 16:54-56.
18. Lutz, A. L., and Menge, J. A. 1991. Population fluctuations and the numbers and types of propagules of *Phytophthora parasitica* that occur in irrigated citrus groves. Plant Dis. 75:173-179.
19. McIntosh, D. L. 1972. Effects of soil water suction, soil temperature, carbon and nitrogen amendments, and host rootlets on survival in soil of zoospores of *Phytophthora cactorum.* Can. J. Bot. 50:269-272.
20. McIntosh, D. L. 1977. *Phytophthora cactorum* propagule density levels in orchard soil. Plant Dis. Rep. 61:528-532.
21. Meyer, D., and Schönbeck, F. 1975. Investigations on the development of *Phytophthora cactorum* (Leb. et Cohn) Schroet. in soil. Z. Pflanzenkr. Pflanzenschutz 82:337-354.
22. Sewell, G. W. F., and Wilson, J. F. 1973. *Phytophthora* collar rot of apple: Seasonal effects on infection and disease development. Ann. Appl. Biol. 74:149-158.
23. Sewell, G. W. F., and Wilson, J. F. 1974. Seasonal factors and *Phytophthora* collar rot. Pages 183-185 in: Rep. East Malling Res. Stn. (Maidstone, Engl.) 1973.
24. Sewell, G. W. F., Wilson, J. F., and Dakwa, J. T. 1974. Seasonal variations in the activity in soil of *Phytophthora cactorum, P. syringae,* and *P. citricola* in relation to collar rot disease of apple. Ann. Appl. Biol. 76:179-186.
25. Sneh, B., Humble, S. J., and Lockwood, J. L. 1977. Parasitism of oospores of *Phytophthora megasperma* var. *sojae, P. cactorum, Pythium* sp., and *Aphanomyces euteiches* in soil by oomycetes, chytridiomycetes, hyphomycetes, actinomycetes, and bacteria. Phytopathology 67:622-628.
26. Sneh, B., and McIntosh, D. L. 1974. Studies of the behaviour and survival of *Phytophthora cactorum* in soil. Can. J. Bot. 52:795-802.
27. Timmer, L. W., Sandler, H. A., Graham, J. H., and Zitko, S. E. 1988. Sampling citrus orchards in Florida to estimate populations of *Phytophthora parasitica.* Phytopathology 78:940-944.
28. Timmer, L. W., Zitko, S. E., Sandler, H. A., and Graham, J. H. 1989. Seasonal and spatial analysis of populations of *Phytophthora parasitica* in citrus orchards in Florida. Plant Dis. 73:810-813.
29. Weste, G. 1983. Population dynamics and survival of *Phytophthora.* Pages 237-257 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartniki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN.