Techniques

Enhancing Detection of *Phytophthora cactorum* in Naturally Infested Soil

S. N. Jeffers and H. S. Aldwinckle

Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456.
Present address of first author: Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706.

ABSTRACT

Jeffers, S. N., and Aldwinckle, H. S. 1987. Enhancing detection of *Phytophthora cactorum* in naturally infested soil. Phytopathology 77:1475-1482.

Reliable methods were needed to detect *Phytophthora cactorum*, one of the primary causal agents of Phytophthora crown rot of apple trees, in its natural soil environment. Apple or pear fruits, used in a baiting bioassay, were ineffective at detecting *P. cactorum* in naturally infested soil. Apple seedlings, cotyledons, and seedling leaf pieces were successful baits, but cotyledons were the most sensitive and efficient. Completely air drying soil subsamples and then remoistening them for several days before flooding and adding plant tissue baits (extended baiting procedure) greatly enhanced the detection of *P. cactorum* when compared to the standard, direct baiting procedure without prior manipulation of soil moisture. Bioassay incubation temperature, volume of water added to remoisten air-dried soil subsamples, and incubation period following remoistening all

affected detection, but the photoperiod during incubation did not. The advantages of an extended baiting bioassay with apple cotyledons were: greater sensitivity than with pear or apple fruits or by direct baiting, readily available and inexpensive baits, formation of sporangia of *P. cactorum* directly on necrotic cotyledons, and lack of interference by contaminating *Pythium* species. Cotyledons also were colonized by zoospores of *P. cambivora*, *P. citricola*, and *P. cryptogea*, but not by those of *P. megasperma*, *P. syringae*, *P. drechsleri*, or an unidentified *Phytophthora* species. The extended bioassay procedure routinely has provided a relatively rapid and efficient means of detecting *P. cactorum* in a diversity of soils within and around New York apple orchards.

Additional key words: *Malus pumila*, soilborne plant pathogens.

Phytophthora cactorum (Lebert & Cohn) Schroeter and other *Phytophthora* species cause crown rot of apple trees (*Malus pumila* Miller) in New York and worldwide (1,15). This species was recovered most consistently and frequently in our isolations from symptomatic trees throughout New York between 1978 and 1984 (S. N. Jeffers and H. S. Aldwinckle, *unpublished*). Reliable and efficient techniques are needed for detecting *P. cactorum* in naturally infested soil in order to study ecology of the fungus and epidemiology of crown rot. Two types of assay are possible: using selective media to assay soil directly for germinable propagules or using a bioassay to selectively bait or trap the pathogen. An excellent review of this topic has recently been published (29).

Selective media have been most effective for recovering heterothallic species of *Phytophthora*, those species that routinely do not produce oospores for survival in soil (Table 2 in [29]). The infrequent use of selective media to recover homothallic species that do survive in soil as oospores is presumably due to the poor or inconsistent germination of these propagules (4,27,31). Selective media for the specific recovery of *P. cactorum* from soil were developed using soil artificially infested with active propagules (20,25), but not with dormant oospores that occur in the orchard; these media have since been found unsuccessful for detecting *P. cactorum* in apple orchard soils (3). Furthermore, the

growth of mycelium of *P. cactorum* was inhibited by hymexazol, an amendment incorporated into selective media to limit the development of interfering colonies of *Pythium* species (16,29).

Baiting bioassays are the most commonly used methods for detecting both homothallic and heterothallic species of *Phytophthora* in soil (Table 1 in [29]). The main advantages over using selective media are: greater success with homothallic species, larger volumes of soil can be assayed with potentially greater sensitivity, bioassays are less complicated and less expensive, and they can be more specific, depending on bait susceptibility. Numerous baiting systems have been used with varying success for detecting *P. cactorum* in orchard soils (reviewed in [3,23,29]), including immature fruit (apple, apricot, peach, pear, and strawberry) incubated in moist, saturated, or flooded soil, or safflower seedlings planted in sterile soil overlaid with a layer of infested soil. Additional baits used for *P. cactorum* include apple leaf pieces and twigs (28), apple seedlings (18,19), and hemp or rape seeds (4,26). In forest soils, pine needle baits were successful, whereas lupine radicles were not (6). However, pear and apple fruit have been used most often (23,29) and, in comparisons, pears have been preferred (18,19,30). The sensitivity of pear fruits was questioned during preliminary experiments when we could not detect *P. cactorum* with this bait in soil collected around infected trees.

The safflower technique (3), developed to overcome the inherent problems with pear and apple fruit bioassays, was selective for *P.*

cactorum, but required growing plants for 4–5 wk in the greenhouse or growth chamber and took considerable space. In addition, safflower seedlings were not successful baits when floated over a flooded soil sample (3), which is the most widely used and successful type of baiting procedure for *Phytophthora* spp. (29).

We felt it was necessary to use a bait derived from apple tissue because the host-specificity of isolates of *P. cactorum* affected

TABLE 1. A comparison of apple seedling leaf piece and apple cotyledon baits for detecting *Phytophthora cactorum* in naturally infested soils[a]

Trial no.[d]	Successful replicates[b] (no.)		Successful baits[c] (no.)	
	Leaf pieces	Cotyledons	Leaf pieces	Cotyledons
1*	5/5	5/5	23/40	11/15
2	4/5	2/5	7/50	5/25
3	5/5	5/5	16/50	7/25
4	10/10	10/10	45/50	44/50
5	9/9	9/9	39/45	33/45
6	3/10	8/10	7/40	24/40
7*	3/5	3/5	9/75	15/25
8	2/5	2/5	6/50	9/25
Total	41/54	44/54	152/400	148/250
Proportion	0.76	0.81	0.38	0.59
$X^2(P)^e$	0.22 (0.66)		27.0 (<0.005)	

[a] Baits were from apple seedlings grown from open-pollinated seed (seed parent cultivar Grimes Golden). All soils were air dried and then remoistened before flooding and adding baits (extended baiting procedure). Soils and specific baiting protocols varied among the different trials.
[b] Number of replicates, petri dishes, or jars, each containing an equal volume of thoroughly mixed soil, that had at least one bait colonized by *P. cactorum* per total number of replicates used.
[c] Total number of baits colonized by *P. cactorum* in all replicates per total number of baits used.
[d] Eight independent trials in which leaf piece and cotyledon baits were incubated in the same vessels or were incubated in separate vessels (*).
[e] X^2 = Chi-square statistic, corrected for continuity, with 1 df; P = probability of a greater chi-square value occurring (24).

TABLE 2. A comparison of apple seedling and apple cotyledon baits for detecting *Phytophthora cactorum* in naturally infested soils[a]

Trial no.[d]	Successful replicates[b] (no.)		Successful baits[c] (no.)	
	Seedlings	Cotyledons	Seedlings	Cotyledons
1*	2/5	3/5	8/20	15/25
2	8/10	8/10	23/40	24/40
3	9/10	9/10	33/40	35/40
4	10/10	10/10	34/40	35/40
5	5/10	5/10	12/40	13/40
6	9/10	9/10	31/40	42/50
7*	10/10	10/10	34/40	40/40
8*	9/10	10/10	30/40	40/40
9	10/10	10/10	30/40	40/40
10	10/10	10/10	34/40	40/40
Total	82/95	84/95	269/380	324/395
Proportion	0.86	0.88	0.71	0.82
$X^2(P)^e$	0.05 (0.84)		13.02 (<0.005)	

[a] Baits were from apple seedlings grown from open-pollinated seed (seed parent cultivar Grimes Golden). All soils were air dried and then remoistened before flooding and adding baits (extended baiting procedure). Soils and specific baiting protocols varied among the different trials.
[b] Number of replicates, petri dishes, or jars, each containing an equal volume of thoroughly mixed soil, that had at least one bait colonized by *P. cactorum* per total number of replicates used.
[c] Total number of baits colonized by *P. cactorum* in all replicates per total number of baits used.
[d] Ten independent trials in which seedling and cotyledon baits were incubated in the same vessels or were incubated in separate vessels (*).
[e] X^2 = Chi-square statistic, corrected for continuity, with 1 df; P = probability of a greater chi-square value occurring (24).

bioassay detection previously (30). Seedlings of various plants have been successful, convenient baits for other *Phytophthora* species (29), and apple seedlings are known to be very susceptible to *P. cactorum* (1,15,18,19). Recently, it has been demonstrated that manipulation of soil moisture before flooding in a bioassay enhanced detection of several species of *Phytophthora* (5,12,27). In particular, Stack and Millar (27) found that complete air drying of soil subsamples followed by several days of incubation after remoistening was especially beneficial for detecting *P. megasperma* Drechsler f. sp. *medicaginis*.

Our goal in this project was to devise a system for the rapid, efficient detection of *P. cactorum* in naturally infested soils in and around New York apple orchards. We could then begin to investigate the ecology of this pathogen and the epidemiology of Phytophthora crown rot. Preliminary reports have been published (13,14).

MATERIALS AND METHODS

Soils. Several different naturally infested soils were collected in and around New York apple orchards between 1981 and 1983 and were stored in polyethylene bags at 1 to 2 C in the dark. Each soil was sieved through a 6-mm- and a 3-mm-mesh screen and thoroughly mixed before being assayed.

Production of apple seedlings. Apple seedlings and seedling pieces used as baits were grown from open-pollinated seeds, cultivar Grimes Golden or occasionally cultivar McIntosh. Seeds were removed from ripe apples, washed vigorously in tap water, disinfested in 1% NaOCl for 20 min, rinsed thoroughly, and allowed to air dry at room temperature for 4–7 days. Dry seeds were stored at 4 C in a loosely closed jar to avoid condensation. For after-ripening, seeds were treated with 0.5% NaOCl for 5 min, thoroughly rinsed, blotted dry, dusted with 10% captan, and then stratified in moistened, fine-textured vermiculite (2 parts vermiculite:1 part distilled water, v/v) in a beaker, enclosed in two polyethylene bags, at 4 C in the dark until radicles emerged (4–6 wk). Germinated seeds were planted in flats of autoclaved coarse-textured vermiculite, and flats were placed on a greenhouse bench and watered as needed. No fertilizer was used. Seedlings were ready for use in 12–14 days and remained usable up to 4–5 wk.

Baiting systems. Apple seedlings, seedling pieces (cotyledons, leaf pieces, and hypocotyl segments), and pear and apple fruits were tested as baits for *P. cactorum*. Preliminary tests showed that surface disinfestation of baits was unnecessary. Infection of baits, primarily apple and pear fruits, by *P. cactorum* was confirmed after a transfer made to pimaricin, ampicillin, and rifampicin selective medium (PAR) (16) produced characteristic growth and sporulation.

Intact apple seedlings were uprooted gently from flats and each was threaded through one of four 6-mm holes drilled in a plastic petri dish lid (100 × 15 mm); seedlings were supported by their cotyledons. Plastic lids were placed on disinfested 475-ml glass jars (90 mm in diameter × 90 mm high) containing a 60- or 30-ml subsample of test soil flooded with 350–400 ml of distilled water. Seedling roots did not touch the soil surface; excessively long roots were trimmed appropriately. A replicate consisted of one jar, and three to 10 replicates were used per trial. Jars were kept in a growth chamber at 20 C with a 16-hr photoperiod for up to 7 days. The hypocotyl and roots of seedlings were examined macroscopically for necrosis and microscopically (40–60×) for sporangia of *P. cactorum*. Necrosis and sporangium production were regarded as constituting successful colonization. When multiple baits were used to assay an individual soil subsample (e.g., four seedlings per jar or five cotyledons per dish), the number of replicate jars or dishes with at least one colonized bait and the total number of baits colonized in all replicates per treatment were counted.

Individual cotyledons, 5-mm hypocotyl segments, and 5- × 10-mm leaf strips or 5-mm leaf disks were excised and placed immediately in tap water to avoid desiccation. Four to six cotyledons, 10 hypocotyl segments, or four to 15 leaf pieces were floated over a 30-ml subsample of test soil in a sterile, deep glass petri dish (100 × 20 mm) flooded with 60 ml distilled water (Fig. 1).

Each dish was a replicate, and three to 10 replicates were used per trial. To compare bait sensitivity, cotyledons or leaf pieces also were placed in jars with intact seedlings or were placed in the same petri dish. Incubation conditions were the same as for seedlings. Partially or wholly necrotic baits were identified macroscopically and were examined for sporangia of *P. cactorum* as above.

Mature, slightly underripe apple, cultivar Grimes Golden, and pear, cultivar Bartlett, fruits were collected from orchards at the New York State Agricultural Experiment Station in Geneva, NY, and stored in a cold room (2 C). To remove any residual pesticides that may have adversely affected colonization by zoospores, fruits were washed in soap and water, rinsed, and swabbed with 95% ethanol. Individual fruits were placed in sterile 475-ml glass jars, each containing a 60-ml subsample of test soil. Soils were flooded with enough distilled water to bring the water level up to the broadest part of the fruit. Each jar was a replicate, and five or 10 replicates were used per trial. Jars were incubated in a growth chamber (20 C and 16-hr photoperiod) for 4–6 days, after which fruits were removed from flooded soils, rinsed in tap water, and patted dry. They were then placed in moist chambers and returned to the growth chamber for an additional 7 days. During this time, fruits were observed for characteristic firm, brown lesions (18), from which isolations were made. After removal of fruits from jars, the water was decanted and the soil was allowed to air dry for 7–10 days at room temperature. It was then subjected to extended baiting (see below) with cotyledons or leaf pieces.

Data on the effectiveness of various baits were compared in two-by-two contingency tables, and differences were analyzed by a chi-square test. The calculated value of chi-square was corrected for continuity as suggested by Snedecor and Cochran (25). The total proportions of either replicates or individual baits that successfully detected *P. cactorum* were combined for all trials and analyzed separately.

Extended vs. direct baiting. The conventional method of bioassay is to bait a subsample of test soil without any pretreatment other than sieving. We have termed this "direct baiting" (= Stack and Millar's "abbreviated bioassy" [27]). In the extended baiting procedure, soil subsamples are completely air dried and then remoistened and incubated for several days before they are flooded and baited (27). Specifically, 30- or 60-ml soil subsamples were placed in baiting vessels and allowed to air dry for 3–7 days at room temperature (25–27 C). Soils typically were air dry by the second day, when repeated weighings became constant. Subsamples were remoistened with distilled water, 10–12 ml for 30 ml of soil and 10–15 ml for 60 ml of soil. These were the minimum volumes that consistently allowed the soil to remain moist throughout the incubation period. Vessels were covered with lids and incubated for 3 days at 20 C with a 16-hr photoperiod. Baiting was then done as described above. To compare extended with direct baiting, soil subsamples were first baited directly and then were baited by the extended procedure. Less frequently, separate subsamples were tested simultaneously by the direct and extended bioassays.

Parameters affecting the extended baiting bioassay. In an attempt to optimize detection of *P. cactorum* by the extended baiting bioassay, four parameters were investigated: the temperature at which remoistened and flooded soils were incubated, the volume of distilled water added to remoisten air-dried soils, the period of time remoistened soils were incubated before flooding, and the photoperiod during incubation. Except for the parameter being examined, extended baiting procedures were as described above using five cotyledon baits for each soil subsample.

Incubation temperatures of 16, 20, 25, and 28 C were compared using 10 jars per temperature, each with 60 ml of an orchard soil. After air drying, replicate jars of soil were placed at one of the four temperatures for remoistening and baiting. The experiment was conducted four times. Data are presented as the mean proportion of replicate jars that had at least one cotyledon bait colonized by *P. cactorum*.

Varying the volume of water used to remoisten air-dried soil was compared using three 30-ml subsamples, each in a deep petri dish,

of each of three different orchard soils (i.e., nine soil subsamples per volume). After air drying, soil subsamples were remoistened with 10, 12, 14, or 16 ml of distilled water per dish. Ten milliliters of water was the minimum volume that consistently kept 30-ml soil subsamples moist (i.e., prevented drying out) throughout the preflooding incubation period, and 16 ml of water consistently kept soil subsamples puddled (i.e., matric water potential = 0) during incubation. The experiment was repeated and the data, being similar, were pooled. Periods of 1, 2, and 3 days of incubation after remoistening were compared in a similar manner except that all air-dried soils were remoistened with 10 ml of distilled water per dish.

To determine if photoperiod affected detection of *P. cactorum*, the same soils and procedures in the previous two experiments were used. However, after air drying, dishes containing soil subsamples were exposed to total darkness or to a 16-hr photoperiod during the entire extended baiting process. This experiment was conducted three times, and, as before, data were pooled.

In all four experiments, data from the three different soils were combined for analysis. Differences among treatments were compared in contingency tables, and the categorical data, which consisted of success or failure in detecting *P. cactorum*, were analyzed by chi-square tests. Data in the experiments on various remoistening volumes and periods of incubation after remoistening were tested for linear trends by calculating the regression coefficient, b, and its standard error, s_b, from the proportion of successes for each treatment (24). Significance was then determined by computing a normal deviate, $Z = b/s_b$.

Persistence of *P. cactorum* in air-dried soil. Ten jars, each containing 60 ml of a test soil, were subjected to repeated baitings by the extended bioassay to determine the number of drying-wetting cycles through which *P. cactorum* was active. At the end of each baiting, water was decanted and the soil subsamples were allowed to air dry 7 days before the next baiting. This was continued until no *P. cactorum* was detected in any jar for two consecutive cycles.

Extended baiting also was used to determine the length of time *P. cactorum* would persist in air-dried soil. Approximately 2.5 L of naturally infested soil was air dried and stored in an open polyethylene bag in the laboratory, where the temperature ranged from 22 to 30 C. Two weeks later and every 4 wk thereafter, three 30-ml aliquots were placed in deep petri dishes and subjected to extended baiting. At each 4-wk interval that *P. cactorum* was not
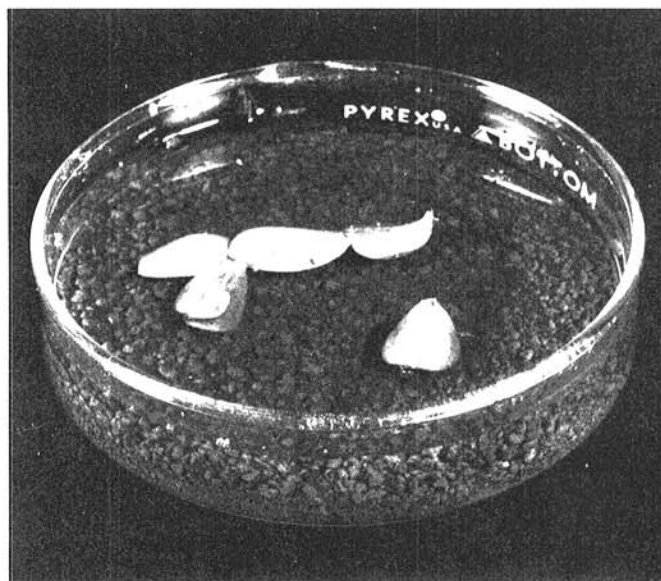

Fig. 1. Apple cotyledons in use as baits to detect *Phytophthora cactorum* in naturally infested soil. Thirty milliliters of a sieved soil sample in a deep petri dish (100×20 mm) was flooded with 60 ml of distilled water, and five cotyledons were floated on the surface.

detected, water was removed from the three dishes, the soils were air dried for 7 days, and the subsamples were baited a second time. This experiment was continued until no *P. cactorum* was detected in two consecutive 4-wk intervals.

Susceptibility of apple cotyledons to *Phytophthora* species. Seventy-five isolates representing eight species of *Phytophthora* from various fruit tree and woody hosts were assayed. The isolates originated from seven states (California, Kentucky, Maryland, New York, Ohio, Oregon, and Wisconsin) and three other countries (Australia, Germany, and Mexico). The total number of isolates of each species tested and the number used from each host were: 24 isolates of *P. cactorum*, apple 11, strawberry four, ginseng four, rhododendron two, pear one, peach one, and walnut one; 17 isolates of *P. megasperma*, apple nine, cherry four, apricot two, and unknown two; 10 isolates of *P. cambivora* (Petri) Buisman (seven of mating type A1 and three of A2), cherry four, apple three, almond two, apricot one; 10 isolates of *P. citricola* Sawada, avocado four, rhododendron three, azalea one, *Ceanothus* one, mugho pine one; nine isolates of *P. cryptogea* Pethybridge and Lafferty (seven of A1 and two of A2), apple five, almond two, cherry two; three isolates of *P. syringae* (Klebahn) Klebahn, apple two, almond one; one isolate of *P. drechsleri* Tucker (A1) from cherry; and one isolate of *Phytophthora* sp., isolate NY.180 (A1), from apple.

Each isolate was grown on V-8 juice agar (200 ml of V-8 juice, 2 g of $CaCO_3$, 17 g of agar, 800 ml of distilled water) for 3 days at 25 C (except for *P. syringae*, which was grown at 19 C). Five 5-mm agar disks were removed from the colony periphery of each isolate, placed in a sterile small glass petri dish (60 × 15 mm), and flooded

Fig. 2. Sporangia of *Phytophthora cactorum* produced directly on apple cotyledons that were used to bait a naturally infested orchard soil. **A,** Abundant sporangia proliferating around the periphery of a cotyledon. (×4) **B,** Characteristic papillate sporangia (arrows) of *P. cactorum* (×109).

with 15 ml of nonsterile soil extract solution. This solution was prepared by stirring 15 g of soil in 1 L of distilled water for 4 hr and allowing the suspension to settle for at least 4 hr. The supernatant was decanted and centrifuged for 10 min at 44,000 g followed by filtration through Whatman No. 1 filter paper. Two cotyledons were floated in each dish and dishes were placed at 19 C under continuous light. After 24 hr, agar disks were examined for sporangia and then all plates were chilled for 1 hr at 4 C, returned to room temperature, and after an additional 30 min examined for motile zoospores. This procedure was repeated after another 24 hr. At 4 and 7 days after the initial flooding, cotyledons were examined for necrosis and the presence of sporangia.

RESULTS

Extended vs. direct baiting. These two methods were compared over a 2-yr period during which 28 field soil samples and 176 rhizosphere soil samples were subjected to both types of assay. In field samples, *P. cactorum* was detected seven times (25%) by direct baiting and 21 times (75%) following the extended procedure. In rhizosphere samples, collected from 2-yr-old nursery-grown apple trees (S. N. Jeffers and H. S. Aldwinckle *unpublished*), direct baiting was successful only six times (3.4%), whereas extended baiting was successful 153 times (86.9%). Of the 204 total samples, *P. cactorum* was detected in 6% of the samples by direct baiting and in 85% of the samples by extended baiting. In only one instance was direct baiting successful and extended baiting unsuccessful. The extended baiting bioassay was effective using seedling, cotyledon, and leaf piece baits.

Baiting systems. On seedling and seedling piece baits, sporangia of *P. cactorum* were observed growing only from or around visibly necrotic areas; plating of healthy-appearing baits onto PAR selective medium never produced *P. cactorum*. Consequently, only necrotic baits were examined for the presence of *P. cactorum*, and healthy-appearing baits were taken to indicate that the fungus was absent.

Apple seedlings were very satisfactory as baits for *P. cactorum* in naturally infested soils that were subjected to either direct or extended baiting procedures. The hypocotyl region at the water level was colonized most often; a firm, orange-brown lesion developed that frequently extended several centimeters above or below the water level. The area immediately behind root tips was another site of colonization. The distinctive, papillate sporangia of *P. cactorum* were easily recognized when hypocotyls and root systems were immersed in water and examined at 40–60×. No more than 7 days' incubation was required for symptom development. An additional 7 days' incubation did not improve detection with any of the baits tested. Baits were, therefore, examined routinely at 4 and 7 days after flooding for symptoms and signs.

In a preliminary experiment using the extended bioassay to compare seedling piece baits, there was no difference in the number of replicate soil subsamples in which *P. cactorum* was detected by cotyledons, leaf pieces, or hypocotyl segments; each type of bait was successful in five out of five replicates. However, there were differences in the total number of baits colonized in all five replicate subsamples combined: 73% of the cotyledons were successful compared with 56 and 45% of the leaf pieces and hypocotyl segments, respectively. In addition, hypocotyl segments sank to the bottom and very few sporangia were produced. These baits were, therefore, judged unacceptable. Leaf pieces, although relatively successful as baits for *P. cactorum*, had the following drawbacks: few sporangia were produced, pieces frequently were colonized by *Pythium* species (presumably because of the wounded periphery), and those colonized by *P. cactorum* were often difficult to identify macroscopically.

Cotyledons functioned well as baits and did not have the drawbacks of hypocotyls and leaf pieces. Firm, brown colonized cotyledons were easily discernible from uncolonized ones, which remained green and symptomless. Abundant sporangia of *P. cactorum* were produced around the entire periphery of affected cotyledons and were easily recognized when cotyledons were immersed in fresh water and examined microscopically at 40–60×
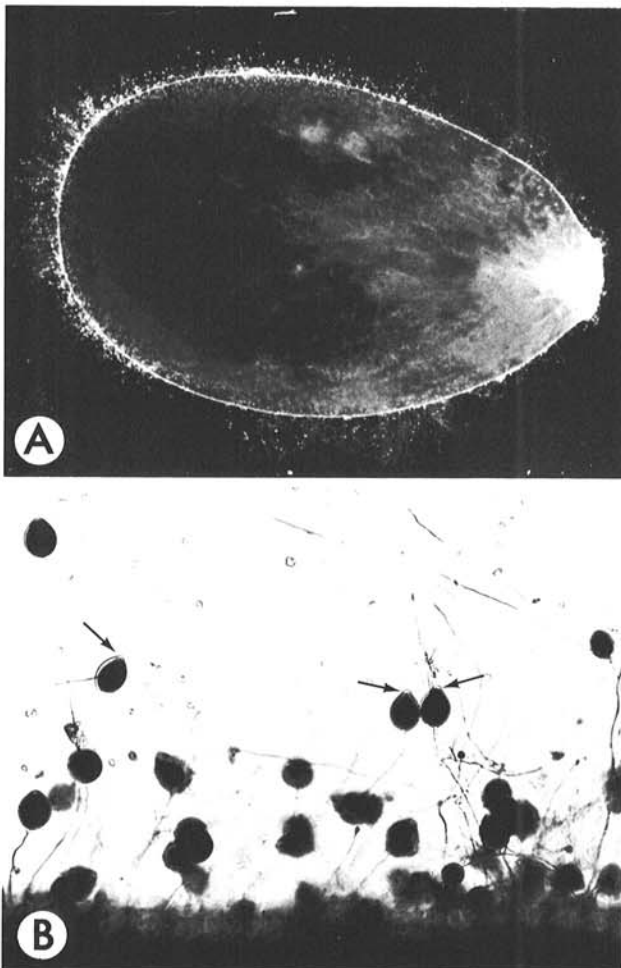
(Fig. 2). Colonization and interference by *Pythium* species was rarely a problem, presumably due to the intact perimeter of cotyledons. If *Pythium* species did colonize these baits and *P. cactorum* was also present, sporangia of *P. cactorum* were readily distinguished from those of *Pythium* species based on size and shape.

The effectiveness of cotyledons compared to leaf pieces was examined further in eight separate trials using the extended procedure (Table 1). The two types of baits were either in separate vessels (two trials) or were in the same vessels (six trials). Both were equally effective at detecting *P. cactorum* in replicate soil subsamples ($P = 0.66$, Table 1). At no time did one bait type and not the other detect the fungus in a test soil. However, based on the total number of baits colonized in all trials, a significantly greater proportion of cotyledons compared to leaf pieces was colonized by *P. cactorum* ($P < 0.005$, Table 1).

The effectiveness of cotyledons compared to seedlings also was examined, again using the extended procedure (Table 2). In seven of 10 trials, cotyledons and seedlings were incubated in the same vessels, and in the other three trials, different baits were incubated in separate vessels. Cotyledons and seedlings were equally successful at detecting *P. cactorum* in replicate subsamples ($P = 0.84$, Table 2). *P. cactorum* was never detected in a given soil by one and not the other bait. However, cotyledons were more efficient than seedlings at detecting *P. cactorum*. There was consistently a greater proportion of successful cotyledons than successful seedlings per trial, and the total proportion of cotyledons compared with that of seedlings colonized by *P. cactorum* was highly significant ($P < 0.005$, Table 2).

Pear and apple fruits were unsuccessful baits for *P. cactorum* in our experiments. Apples were used in one trial with 10 soil subsamples baited directly and then baited by the extended procedure. None was successful. In three trials with pear fruits as baits, 15 replicate soil subsamples were baited directly, and 25 were baited by the extended procedure. The fungus was detected in a single replicate using each baiting method. When all these soil subsamples were air dried and baited again following the extended protocol with either leaf pieces or cotyledons as baits, *P. cactorum* was detected in each soil subsample.

Parameters affecting the extended baiting bioassay. Incubation temperature had a highly significant effect on detection of *P.*

cactorum in naturally infested soil (Fig. 3). A chi-square test statistic, with three degrees of freedom, was 63.54, calculated from the total number of soil subsamples in which *P. cactorum* was detected in all four trials. A chi-square value greater than this occurs with a probability of $P < 0.005$. At 20 C, the fungus consistently was detected in more soil subsamples ($7/10$, $8/10$, $10/10$, and $7/10$) than at the three other incubation temperatures, and variability among trials was least. Detection at 25 C was sporadic, ranging from $0/10$ to $10/10$ successful replicates. In only a few subsamples per trial was *P. cactorum* detected when incubation was at 16 C ($0/10$, $5/10$, $1/10$, and $3/10$), and the fungus was never detected when soil subsamples were incubated at 28 C. Similar data resulted when the total number of baits colonized in all replicates at each temperature was counted.

The volume of water added to remoisten air-dried soil subsamples affected detection of *P. cactorum* (Table 3). Although there was not a significant difference among proportions of soil subsamples in which the fungus was detected ($P = 0.22$), there was a highly significant difference in the total proportion of baits colonized ($P < 0.005$). When linear trends in the proportions were calculated, both sets of data produced significant regression coefficients (Table 3). The optimum volume of distilled water added to a 30-ml volume of soil that was air dried was 10 ml. This produced both the greatest number of replicate soil subsamples in which *P. cactorum* was detected and the greatest number of colonized baits.

Varying the period of incubation after remoistening also had a highly significant ($P < 0.005$) effect on detection of *P. cactorum*, based on either the proportion of soil subsamples in which the fungus was detected or the total proportion of baits colonized (Table 4). Likewise, highly significant ($P < 0.001$) regression coefficients confirmed the linear trends in these two sets of data (Table 4). An incubation period of 3 days was more effective than an incubation period of either 2 days or 1 day.

Detection of *P. cactorum* by extended baiting was not affected by photoperiod. Incubation of soil subsamples in total darkness resulted in the fungus being detected in $17/18$ replicates in which

TABLE 3. The effect of different volumes of water added to remoisten air-dried soil on the detection of *Phytophthora cactorum* in naturally infested soils using an extended baiting procedure and apple cotyledon baits[a]

Volume of water (ml)	Proportion detecting *P. cactorum*	
	Replicates[b]	Baits[c]
10	15/18	56/90
12	12/18	44/90
14	12/18	40/90
16	9/18	26/90
Analysis[d]		
$X^2(P)$	4.5 (0.223)	20.52 (<0.005)
$b(s_b)$	−0.05 (0.025)	−0.05 (0.012)
$Z(P_z)$	−2.01 (0.044)	−4.44 (<0.001)

[a] Cotyledons were from apple seedlings from open-pollinated seed (seed parent cultivar Grimes Golden). Extended baiting: 30-ml soil subsamples were air dried for 3 days at room temperature, remoistened with specified volume of distilled water, and incubated for 3 days at 20 C. Subsamples were flooded with 60 ml of distilled water, and five baits were added per subsample. Soils then were incubated for 7 days at 20 C.
[b] Proportion of soil subsamples that had at least one bait colonized by *P. cactorum*. Three subsamples of each of three different orchard soils were used for each volume of water; the experiment was repeated and data were pooled.
[c] Total proportion of baits colonized by *P. cactorum* in all soil subsamples (five baits per subsample × 18 replicates).
[d] X^2 = Chi-square statistic with 3 df; P = probability of a greater chi-square value occurring; b = regression coefficient; s_b = standard error of b; Z = normal deviate = b/s_b; P_z = significance level associated with Z (24).
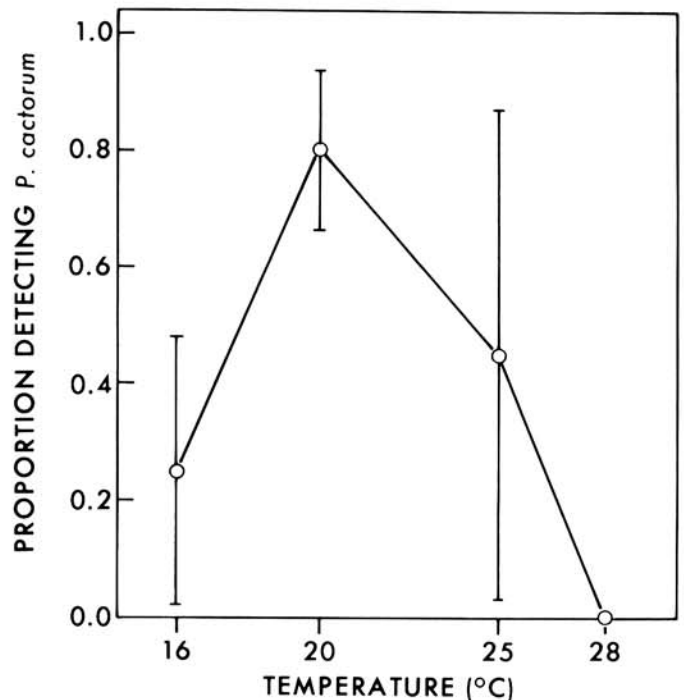

Fig. 3. Effect of incubation temperature on the detection of *Phytophthora cactorum* in naturally infested soil using an extended baiting procedure (see text) with apple cotyledons. Each point is the mean proportion of replicate soil subsamples that had at least one cotyledon bait out of five colonized by *P. cactorum*. Means (open circles) and standard deviations (bars) are of four trials with 10 replicates for each temperature per trial.

82/90 baits were colonized, whereas incubation with a 16-hr photoperiod resulted in the fungus being detected in 18/18 replicate subsamples in which 87/90 baits were colonized. However, sporangia appeared to be more abundant on cotyledons incubated in the light than on those incubated in the dark.

Persistence of *P. cactorum* in air-dried soil. Six consecutive cycles of extended baiting were necessary to completely deplete 10 replicate soil subsamples of detectable *P. cactorum*. The fungus colonized all the cotyledon baits in 10/10 replicates during the first two cycles. By the third cycle, *P. cactorum* colonized only 78% of the baits and was detected in 8/10 replicates; in the fourth cycle, *P. cactorum* was not detected. In cycles five and six, *P. cactorum* was detected again, but in only 3/10 and 1/10 replicate dishes, respectively. It was not detected in two subsequent baiting cycles.

Detectable propagules of *P. cactorum* persisted in air-dried soil for 54 wk. The fungus was readily detected for 18 wk with no loss of activity in the bioassay (detection in all three replicates). After 30 wk, activity had declined (detection in 2/3 replicates), and after 46 wk, the fungus was barely detectable (only one cotyledon in all three replicates was colonized during the second extended baiting). After 50 wk, *P. cactorum* was not detected in either baiting but was again detected at 54 wk, by a single cotyledon during the second baiting. The fungus was not detected in subsamples baited 58, 62, 66, or 99 wk after the soil had been air dried. After 101 wk, all of the remaining air-dried soil was divided into three sets of five 30-ml soil subsamples. Sets were moistened for either 5, 7, or 14 days before flooding, but *P. cactorum* was not detected.

Susceptibility of apple cotyledons to *Phytophthora* species. All isolates of a given species except *P. citricola* behaved similarly toward apple cotyledons regardless of host origin (Table 5); a species either colonized or did not colonize cotyledons. Isolates of *P. cactorum* and *P. cambivora* invaded cotyledons aggressively and sporulated readily. Colonization by *P. cryptogea* was noticeably less aggressive, and sporulation was less vigorous. All isolates of *P. citricola* colonized the cotyledons, but three of four avocado isolates were only weakly aggressive, colonizing one of two cotyledons; the other seven isolates, from various hosts, were very aggressive. *Phytophthora megasperma*, *P. drechsleri*, and *Phytophthora* sp. (NY.180) were unable to cause necrosis and sporulate on apple cotyledons even if incubated for an additional 7 days (14 days total), although all produced ample zoospores. *Phytophthora syringae*, which produced relatively few zoospores under our test conditions, also did not cause necrosis nor sporulate on apple cotyledons after 14 days of incubation.

A preliminary experiment with fewer isolates of *P. cactorum*, *P. cambivora*, *P. cryptogea*, *P. megasperma*, and *Phytophthora* sp. (NY.180) showed results similar to those reported here. In a separate experiment, three isolates of *P. megasperma*, one isolate of *P. cryptogea*, and *Phytophthora* sp. (NY.180) did not cause visible necrosis and did not sporulate on leaf disk baits. Nevertheless, after disinfestation for 30 sec in 0.5% NaOCl and plating on PAR or PARH selective medium (16), all leaf disks produced colonies of *Phytophthora*. In a third experiment, isolates of *P. megasperma* and *P. cryptogea* did not colonize apple seedlings used to bait artificially infested soil, but *P. cactorum* and *P. cambivora* did colonize these baits.

DISCUSSION

Selecting a bait that was readily colonized by *P. cactorum* and identifying incubation conditions, both before and after flooding, that favor development and activity of this fungus have increased the sensitivity and effectiveness of our baiting bioassay dramatically. The conventional apple and pear fruit baits used most frequently in the past for detecting *P. cactorum* in orchard soils (18,19,23,30) were unsatisfactory for New York soils in our tests. Reported success with these baits may be due to several factors, including condition and cultivar of fruit used, inoculum density, types of propagules of *P. cactorum* in test soils, and edaphic conditions. In addition, use of fruit baits was time consuming; isolation from lesions on fruits to selective medium was necessary to confirm the presence of *P. cactorum*; and other organisms caused lesions on the baits.

Apple seedlings proved to be successful baits for *P. cactorum*, contrary to previous reports (18,19). The 5- to 6-wk-old seedlings used in these previous studies may have been too old; our experience is that 2- to 4-wk-old seedlings are most susceptible (S. N. Jeffers and H. S. Aldwinckle, *unpublished*). Apple cotyledons and seedling leaf pieces also were satisfactory baits; susceptibility of these was not affected noticeably by plant age. However, cotyledons were not only more sensitive and efficient than the other two but were easier to handle and suffered less from contamination by *Pythium* species.

Previously, cotyledons of other plants have been used successfully as baits; those of eucalyptus and maple have been

TABLE 4. The effect of incubation period of remoistened soil on the detection of *Phytophthora cactorum* in naturally infested soils using an extended baiting procedure and apple cotyledon baits[a]

Incubation period (days)	Proportion detecting *P. cactorum*	
	Replicates[b]	Baits[c]
3	18/18	87/90
2	13/18	46/90
1	3/18	4/90
Analysis[d]		
X^2 (P)	27.7 (<0.005)	153.1 (<0.005)
b (s_b)	0.42 (0.006)	0.46 (0.037)
Z (P_z)	64.3 (<0.001)	12.4 (<0.001)

[a] Cotyledons were from apple seedlings grown from open-pollinated seed (seed parent cultivar Grimes Golden). Extended baiting: 30-ml soil subsamples were air dried for 3 days at room temperature, remoistened with 10 ml of distilled water, and incubated for the specified number of days at 20 C. Subsamples were flooded with 60 ml of distilled water, and five baits were added per subsample. Soils then were incubated for 7 days at 20 C.
[b] Proportion of soil subsamples that had at least one bait colonized by *P. cactorum*. Three subsamples of each of three different orchard soils were used for each incubation period; the experiment was repeated and data were pooled.
[c] Total proportion of baits colonized by *P. cactorum* in all soil subsamples (five baits per subsample × 18 replicates).
[d] X^2 = Chi-square statistic with 2 df; P = probability of a greater chi-square value occurring; b = regression coefficient; s_b = standard error of b; Z = normal deviate = b/s_b; P_z = significance level associated with Z (24).

TABLE 5. Colonization of apple cotyledons by zoospores of eight species of *Phytophthora*[a]

Species	Hosts[b] (no.)	Isolates tested (no.)	Percent successful[c]
P. cactorum	7	24	100
P. cambivora	4	10	100
P. cryptogea	3	9	100[d]
P. citricola	5	10	100[e]
P. megasperma	4	17	0
P. syringae	2	3	0
P. drechsleri	1	1	0
Phytopthora sp. (NY. 180)	1	1	0

[a] For each isolate, two cotyledons (produced on seedlings grown from open-pollinated apple seed, seed parent cultivar Grimes Golden) were floated on a zoospore suspension in a small petri dish (60 × 15 mm) for 7 days at 19 C under constant light.
[b] Number of different host plants from which isolates originated. Hosts included apple, almond, apricot, avocado, azalea, *Ceanothus*, cherry, ginseng, mugho pine, peach, pear, rhododendron, strawberry, and walnut; see text for specific species-host combinations.
[c] Successful colonization of cotyledons was characterized by typical necrosis and subsequent formation of sporangia.
[d] All isolates of *P. cryptogea* were less aggressive in colonizing cotyledons compared with *P. cactorum* and *P. cambivora*.
[e] Three of four isolates of *P. citricola* from avocado were only moderately successful, colonizing one of two cotyledons; seven other isolates of *P. citricola* were as aggressive as *P. cactorum* and *P. cambivora*.

found suitable for detecting *P. cinnamomi* (29) and *P. citricola* (7), respectively. In our experiments, apple cotyledons were susceptible plant organs readily colonized by *P. cactorum*. We recommend using cotyledons of plants grown only from seed of a relatively susceptible parent cultivar (1), such as Grimes Golden or McIntosh as used in this investigation. The genetic diversity of open-pollinated seed and the differential virulence of isolates of *P. cactorum* toward apple (1,15) have not noticeably affected our bioassay. Cotyledons were colonized aggressively by 24 isolates of *P. cactorum* from seven different hosts.

Based on the susceptibility of apple cotyledons to zoospores of eight *Phytophthora* species, cotyledon baits could be expected to detect species other than *P. cactorum*. Of all the species of *Phytophthora* known to attack apple roots (reviewed in [15]) or to be present in apple soil (18,19,23), *P. cactorum*, *P. cambivora*, *P. citricola*, and perhaps *P. cryptogea* would be detectable but not *P. megasperma*, *P. syringae*, *P. drechsleri*, and *Phytophthora* sp. (NY.180). In subsequent field studies, apple cotyledons consistently detected only *P. cactorum*, *P. citricola*, or *P. cambivora* in apple rhizosphere, apple orchard, or nonagricultural soil samples (S. N. Jeffers and H. S. Aldwinckle, *unpublished*). Other baits susceptible to species that do not colonize cotyledons need to be identified; in particular, a bait for *P. megasperma*, an important crown rot pathogen on apple in New York (15), is needed.

Air drying and remoistening soil subsamples before flooding and adding baits (the extended baiting procedure) have produced the greatest improvement in detection of *P. cactorum*. In the 204 field and rhizosphere soil samples assayed by both conventional, direct baiting and by extended baiting, the direct procedure failed to detect *P. cactorum* in 93% of the samples identified as positive by the extended method. Had we relied solely on the direct baiting technique, our conclusions about the occurrence and distribution of this fungus would have been misleading. Recently, others have reached similar conclusions (12,27). By not using an extended procedure, many reports on the absence or relatively low populations of this fungus, and perhaps related species, may be in question.

Bioassay techniques that involve air drying soil or adjusting soil moisture have been beneficial for detecting other *Phytophthora* species. Stack and Millar (27) previously reported the beneficial effects of an extended bioassay for detecting *P. megasperma* f. sp. *medicaginis*; it was this research that motivated our investigations. We have recovered *P. megasperma* from sour cherry orchard soils using our extended baiting technique with cherry leaf disk baits (S. N. Jeffers, *unpublished*), and Drilias et al (7) have adopted the procedure to detect *P. citricola* and *P. cactorum* from soil around maple trees using maple cotyledons. In addition, others have found that incubating field soil samples at a predetermined soil matric potential for a length of time before flooding and adding baits increased the sensitivity of their bioassays (5,12). Several reports have identified the beneficial effect of remoistening air-dried soil on the detection of both *Phytophthora* species and *Pythium* species (11, others reviewed in [27]).

It is conceivable that in the extended procedure drying stimulates or conditions otherwise dormant oospores to germinate and, therefore, enhances the detection of *P. cactorum*. This could be either a direct effect on the oospore itself or an effect on the associated soil microflora that may be suppressing germination. Oospores of *P. megasperma* f. sp. *medicaginis* had increased germination after being subjected to a similar extended baiting procedure (27), and germination of oospores of *Pythium ultimum* and *P. aphanidermatum* was enhanced after an air-drying and premoistening treatment (2,17).

When air-dried soils are remoistened, conditioned oospores presumably germinate and produce sporangia (also suggested in [5,12]). The effect of soil matric potential on sporangium formation in *Phytophthora* is well documented, with sporangia of most species, including *P. cactorum*, forming in moist as opposed to saturated soils (8,22,26). Flooding moistened soils would then promote zoospore release (8,31) and movement towards baits on the water surface. The possibility that extended baiting techniques may enhance detection of other *Phytophthora* species that survive in soil as oospores warrants consideration.

Detection of *P. cactorum* with the extended bioassay occurred over a range of conditions, but there was a definite optimum for each parameter investigated except photoperiod. Detection occurred from 16 to 25 C with an optimum at 20 C and none at 28 C. Previously, optimum oospore germination by *P. cactorum* occurred at 20 C with little or none above 28 C (4,22), and sporangia formed and germinated indirectly best in the range 20–25 C with poor indirect germination above 28 C (9,22).

Detection occurred when 30 ml of soil was air dried and remoistened with 10, 12, 14, or 16 ml of water, but was optimal with 10 ml, the minimum volume that consistently prevented drying out during the preflooding incubation period. This was true for all three of the orchard soils tested. Oospores of *P. cactorum* are known to germinate over a range of soil moistures (26), but sporangia are produced under relatively restricted moisture conditions (8,9,22,26). The range of soil matric potentials produced when air-dried soil subsamples were remoistened with 10 ml of water and incubated for 3 days was apparently most favorable and that produced with 16 ml of water was apparently too wet and relatively unfavorable for sporangium production. Actual soil matric potential values were not measured.

Detection occurred in 100% of replicate subsamples from three different soils when subsamples were remoistened and incubated for 3 days before flooding but in only 72 and 17% of subsamples remoistened and incubated for 2 and 1 days, respectively. Incubation of moistened soils for durations greater than 3 days has been used (5,7,12); however, Gisi (9) reported that sporangium germination of *P. cactorum* was terminated after 2 days. Photoperiod did not affect our detection of *P. cactorum* in three different soils, although there are numerous reports regarding the effects of light on this pathogen (4,9,10,22).

This fungus was capable of persisting for at least 54 wk in air-dried soil and through six consecutive air-drying-remoistening cycles. Discovery of this was made possible with the extended bioassay procedure. Survival of *P. cactorum* in air-dried soil has been reported previously (21,22), but not for such an extended duration. However, other pythiaceous fungi have survived longer under similar conditions. Oospores of *Pythium ultimum* survived for 60 wk (17) and other *Pythium* species were still active after 12 yr in air-dried soil (11). Attempts to eliminate *P. cactorum* and other homothallic species by air-drying tissues and soil containing oospores probably will be unsuccessful.

Our extended baiting method is an efficient bioassay for routinely testing soil samples for the presence of *P. cactorum*. The procedure we have adopted is as follows: 1) air dry three to five 30-ml subsamples of a sieved soil in deep petri dishes (100 × 20 mm) for 3 days at room temperature; 2) remoisten each subsample with 10 ml of distilled water, cover the dishes, and incubate for 3 days at 20 C with a 16-hr photoperiod; 3) flood each dish with 60 ml of distilled water, add four to six apple cotyledons, and continue incubating at 20 C for 7 days; and 4) examine necrotic cotyledons at 4 and 7 days for the characteristic sporangia of *P. cactorum*. Advantages of the method: *P. cactorum* was consistently detected in soils where direct baiting methods failed; cotyledons are readily available, easily handled, and inexpensive; infected cotyledons are discernible macroscopically; sporangia of *P. cactorum* form directly on necrotic baits, making identification possible without subsequent isolation and subculturing; and contamination by *Pythium* species, which has been a problem with some bioassays (5,6), rarely caused interference. With this technique, we can now investigate the ecology of *P. cactorum* in natural soils and the epidemiology of Phytophthora crown rot in apple orchards.

LITERATURE CITED

1. Aldwinckle, H. S., Polach, F. J., Molin, W. T., and Pearson, R. C. 1975. Pathogenicity of *Phytophthora cactorum* isolates from New York apple trees and other sources. Phytopathology 65:989-994.
2. Ayers, W. A., and Lumsden, R. D. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology 65:1094-1100.

3. Banihashemi, Z., and Mitchell, J. E. 1975. Use of safflower seedlings for the detection and isolation of *Phytophthora cactorum* from soil and its application to population studies. Phytopathology 65:1424-1430.

4. Banihashemi, Z., and Mitchell, J. E. 1976. Factors affecting oospore germination in *Phytophthora cactorum*, the incitant of apple collar rot. Phytopathology 66:443-448.

5. Canaday, C. H., and Schmitthenner, A. F. 1982. Isolating *Phytophthora megasperma* f. sp. *glycinea* from soil with a baiting method that minimizes *Pythium* contamination. Soil Biol. Biochem. 14:67-68.

6. Dance, M. H., Newhook, F. J., and Cole, J. S. 1975. Bioassay of *Phytophthora* spp. in soil. Plant Dis. Rep. 59:523-527.

7. Drilias, M. J., Kuntz, J. E., and Worf, G. L. 1984. Sugar maple cotyledons used to detect *Phytophthora citricola* and *P. cactorum* in soils. (Abstr.) Phytopathology 74:845.

8. Duniway, J. M. 1979. Water relations of water molds. Annu. Rev. Phytopathol. 17:431-460.

9. Gisi, U. 1975. Untersuchungen über die Bodenphase von *Phytophthora cactorum* (Leb. et Cohn) Schroet. mit fluoreszen-zoptischer Direktbeobachtung. Z. Pflanzenkr. Pflanzenschutz 82:355-377.

10. Harnish, W. N. 1965. Effect of light on production of oospores and sporangia in species of *Phytophthora*. Mycologia 57:85-90.

11. Hoppe, P. E. 1966. *Pythium* species still viable after 12 years in air-dried muck soil. Phytopathology 56:1411.

12. Ioannou, N., and Grogan, R. G. 1984. Water requirements for sporangium formation by *Phytophthora parasitica* in relation to bioassay in soil. Plant Dis. 68:1043-1048.

13. Jeffers, S. N., and Aldwinckle, H. S. 1983. Use of apple seedlings to detect *Phytophthora cactorum* in apple orchard soils. (Abstr.) Phytopathology 73:368.

14. Jeffers, S. N., and Aldwinckle, H. S. 1984. Baiting *Phytophthora cactorum* from naturally infested soil. (Abstr.) Phytopathology 74:867.

15. Jeffers, S. N., Aldwinckle, H. S., Burr, T. J., and Arneson, P. A. 1982. *Phytophthora* and *Pythium* species associated with crown rot in New York apple orchards. Phytopathology 72:533-538.

16. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.

17. Lumsden, R. D., and Ayers, W. A. 1975. Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum*. Phytopathology 65:1101-1107.

18. McIntosh, D. L. 1964. *Phytophthora* spp. in soils of the Okanagan and Similkameen Valleys of British Columbia. Can. J. Bot. 42:1411-1415.

19. McIntosh, D. L. 1966. The occurrence of *Phytophthora* spp. in irrigation systems in British Columbia. Can. J. Bot. 44:1591-1596.

20. McIntosh, D. L. 1975. An improved agar medium for isolating Phytophthora cactorum from soil. Can. J. Bot. 53:1444-1445.

21. Mes, M. G. 1934. A wilt of snapdragon (*Antirrhinum majus*) in S. Africa. South Afr. J. Sci. 31:281-287.

22. Meyer, D., and Schönbeck, F. 1975. Untersuchungen über die Entwicklung von *Phytophthora cactorum* (Leb. et Cohn) Schroet. im Boden. Z. Pflanzenkr. Pflanzenschutz 82:337-354.

23. 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.

24. Snedecor, G. W., and Cochran, W. G. 1967. Statistical Methods, 6th ed. The Iowa State University Press, Ames. 593 pp.

25. Sneh, B. 1972. An agar medium for the isolation and macroscopic recognition of *Phytophthora* spp. from soil on dilution plates. Can. J. Microbiol. 18:1389-1392.

26. Sneh, B., and McIntosh, D. L. 1974. Studies on the behavior and survival of *Phytophthora cactorum* in soil. Can. J. Bot. 52:795-802.

27. Stack, J. P., and Millar, R. L. 1985. Relative survival potential of propagules of *Phytophthora megasperma* f. sp. *medicaginis*. Phytopathology 75:1398-1404.

28. Suzui, T., and Hoshino, Y. 1979. Collar rot of apple caused by *Phytophthora cambivora* (Petri) Buism. Ann. Phytopathol. Soc. Jpn. 45:344-352.

29. Tsao, P. H. 1983. Factors affecting isolation and quantitation of *Phytophthora* from soil. Pages 219-236 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN. 392 pp.

30. Van der Scheer, H. A. T. 1971. Isolation of *Phytophthora cactorum* from soil in orchards and strawberry fields and differences in pathogenicity to apple. Neth. J. Plant Pathol. 77:65-72.

31. Zentmyer, G. A., and Erwin, D. C. 1970. Development and reproduction of *Phytophthora*. Phytopathology 60:1120-1127.