Use of Safflower Seedlings For the Detection and Isolation of Phytophthora cactorum From Soil and Its Application to Population Studies

Z. Banihashemi and J. E. Mitchell

Associate Professor, Department of Plant Protection, College of Agriculture, Pahlavi University, Shiraz, Iran; and Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706, respectively.

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ABSTRACT

A new method was developed to detect *Phytophthora cactorum,* an incitant of apple collar rot in, and to isolate it from, naturally infested soil. A 15-mm layer of infested soil was placed around safflower seedlings that had been transplanted into steamed soil. The pathogen caused water-soaking of the hypocotyl at or above the soil line within 4-14 days, after which the affected tissue turned light-brown and collapsed, causing the seedling to topple over. Typical sporangia were formed within 4-24 hours when infected parts were washed and floated on tap or distilled water at 20-22 C. The pathogen frequently could be obtained in pure culture when an infected area occurring above the soil line was plated on a selective medium. All isolates obtained by the safflower method were pathogenic to apple seedlings. *Phytophthora cactorum* was isolated by this method from soil samples obtained throughout the year around apple trees with and without disease symptoms. The pathogenicity of several species of soil-borne fungi were tested against safflower, but only an infrequently occurring isolate of a fungus which appeared to be a *Pythium* species caused comparable infection. In a field soil artificially infested with *P. cactorum,* the activity of the pathogen increased during the first 30 days of incubation at temperatures from 4-28 C, but declined later. After 120 days, the infection was least in soil incubated at 28 C, and remained highest in that incubated at 4 C. The viability of sporangia of *P. cactorum* introduced into natural soil decreased within 2 weeks at temperatures from 4-28 C, and few sporangia survived beyond 45 days at 20 C or higher.

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Phytophthora cactorum (Leb. & Cohn) Schroet. is an important soil-borne plant pathogen of many crops, especially woody plants. Tucker in 1933 (29) listed 85 host species belonging to 54 genera and 33 families. Later, Smith (23) was able to infect 43 woody host plants by artificial inoculation. Nienhaus (17) carefully studied the host range of *P. cactorum* and found that of 184 reported hosts, 157 could be infected by the pathogen.

In spite of a wide host range, there is little information on the behavior of *P. cactorum* in soil. This is, in part, due to the lack of a suitable method for detecting the pathogen in naturally infested soils. Despite extensive work on

isolation of *Phytophthora* species from soil by means of selective agar media (8, 9, 11, 15, 18, 21, 24, 28), no reports of successful isolations of *P. cactorum* from naturally infested soil on available antibiotic media have been found. The pathogen has been recovered from autoclaved and nonautoclaved soils artificially infested with zoospores (15, 21). The difficulty of using selective antibiotic media is that fast-growing species of *Pythium* generally present in most soils overgrow *Phytophthora* colonies. Baiting has been widely used to isolate *Phytophthora* spp. from soil. *Phytophthora cactorum* has been isolated from naturally infested soil by baits such as apple fruit (16, 20, 30), pear fruit (1, 20) and blue lupine seedlings (19). The apple fruit technique is not specific for *P. cactorum* and has been used for isolation of other *Phytophthora* species such as *P. cinnamomi* (4, 16), *P. citricola* (16, 20), *P. cryptogea* (16), and *P. syringae* (15, 20, 30). Pear fruit has been reported to be a better bait than apple for isolating *P. cactorum* from soil (1, 30). Van der Scheer (30) studied conditions affecting the recovery of *P. cactorum* from soil by hard apple and pear fruit. Although raising soil temperature increased the isolation efficiency, the amount of fruit decay by soil saprophytes was still high. This technique requires the availability of fresh, hard apple or pear fruit and also subsequent isolation of the pathogen from infected tissues on culture media for species identification.

The present investigation was undertaken to develop a feasible method of detection of *P. cactorum* and its isolation from natural soil, and to study the behavior of the pathogen in soil.

MATERIALS AND METHODS.—Soils from around apple trees with and without disease history were collected during the period from October to May, 1973, screened to remove debris, and stored at 4 C in plastic bags. The moisture content of the soil was approximately 10%.

Artificial inoculum of the pathogen was prepared by growing the fungus for 3 weeks in cornmeal-sand medium (300 ml white sand, 15 ml cornmeal, and 100 ml deionized water, autoclaved for 20 minutes). This inoculum was then mixed with either steamed or nonsteamed loam-sand (1:1, v/v) mixture, generally at 1:100 dilution, and incubated at 20-22 C until used.

Sporangia and zoospores were obtained by growing the pathogen on V-8 agar in petri plates until the colony reached a diameter of 5 cm. The uncolonized agar was then removed, and the culture flooded with sterile distilled water and incubated at room temperature for 5-10 days, during which time sporangia developed. Zoosporogenesis was induced by cooling cultures at 4 C for 1 hour after which time zoospores were collected, counted, and diluted as required for inoculation. Intact sporangia were obtained by removing mycelial mats from the surface of flooded plates. These were sonicated for 5 seconds to free sporangia from mycelium, and the suspension was immediately mixed with natural soil (about 6% moisture), known to be initially free of the pathogen. At this time, the initial population of the pathogen was determined on pimaricin medium.

A modified antibiotic agar medium reported earlier (21, 28) and supplemented with 100 μg/ml Tergitol NPX (24) was used for the isolation of *P. cactorum* from diseased safflower or apple tissues and also from soil infested with sporangia. The medium without the antibiotics contained the following ingredients per liter: 1 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g $CaSO_4 \cdot 2\ H_2O$, 0.02 g thiamine HCl, 1 g DL-threonine, 5 g sucrose, 0.1 ml corn oil, 0.1 ml Tergitol NPX, 20 g agar, and 1 liter distilled water. After 1-liter batches were autoclaved for 20 minutes and cooled to 60 C, 200 mg of vancomycin and 10 mg pimaricin were added. When bacterial contamination was a problem, 175,000 units of Polymyxin B were also added.

TABLE 1. Detection of *Phytophthora cactorum* in soil samples collected from around diseased apple trees

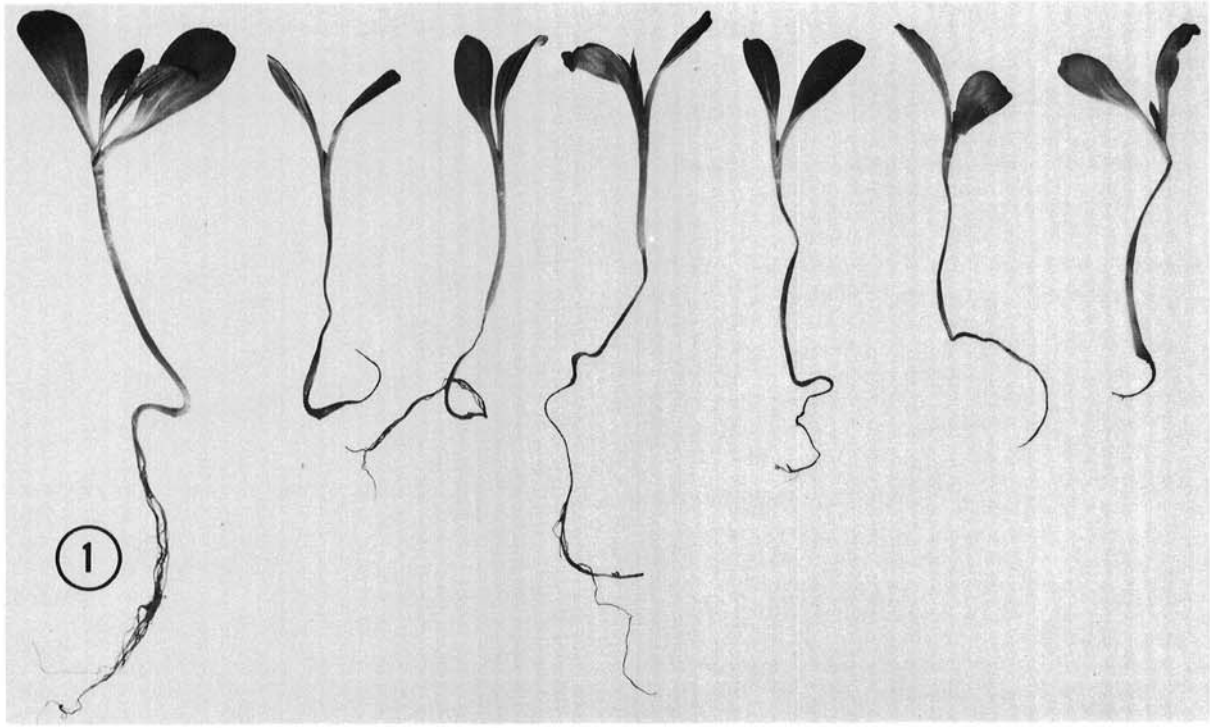
Soil no.[a]	Safflower seedlings diseased[b] (%)	Diseased safflower seedlings from which *P. cactorum* was isolated (%)
1	0	0
2	4	100
3	73	100
4	73	63
5	82	100
6	43	100
7	13	33
8	20	0
9	36	90

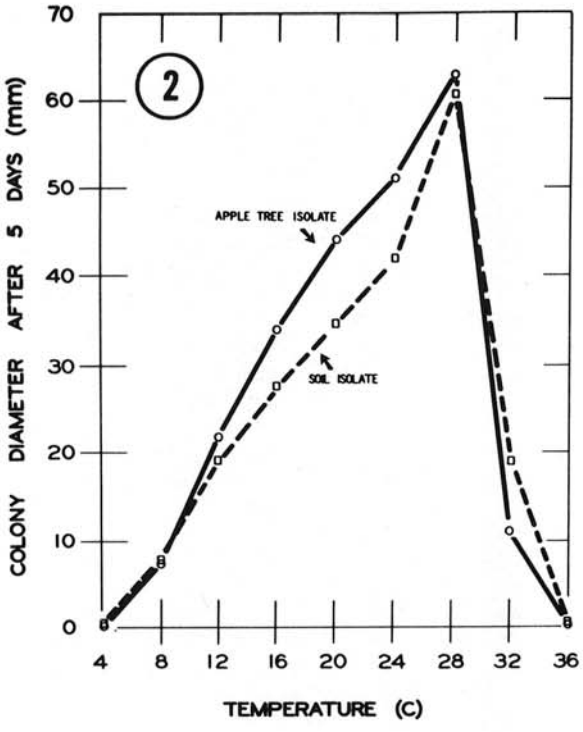
[a]Soils 1 and 2 from around trees with purpling leaves. Soils 3 through 9 from sites where dead or dying trees had been removed. All soils were collected on 30 November 1973.
[b]Safflower seeds were planted in naturally infested soils.

Cultures containing mainly oospores with few sporangia were obtained by growing a single zoospore isolate in V-8 broth containing 30 mg beta-sitosterol per liter in medicine bottles at 20 and 22 C in the dark for 2-4 months. The mycelial mats with oospores were sonified for 30 seconds to disperse oospores and then frozen for 24 hours at −20 C to kill mycelia and sporangia (2). The concentration of oospores was determined by use of a haemocytometer, and adjusted to the desired level. The suspension was atomized on to the surface of a thin layer of soil which was then mixed for 30 minutes in a twin-shell blender and diluted with untreated, pathogen-free soil to obtain the desired concentration of oospores in each sample.

Six herbaceous plants were selected for use as baits from among the various hosts reported for this pathogen. These were pea (*Pisum sativum* L., 'Perfection') (23), melon (*Cucumis melo* L., 'Pride of Wisconsin') (29), snapdragon (*Antirrhinum majus* L., tall rust-resistant, Northrup King & Co.) (10), tomato (*Lycopersicon esculentum* Mill, 'Marglobe') (5), cowpea [*Vigna sinensis* (Torner) Savi, 'California Black Eye'] (31) and safflower (*Carthamus tinctorius* L., 'Nebraska-10') (25).

For the in vitro baiting studies, about 30 g of soil was placed in each petri plate, saturated with sterile distilled water, and the soil surface covered with nylon mesh. Several boiled hemp or rape seeds and also small pieces of McIntosh apple or sour apple fruit [*Malus prunifolia* (Willd.) Borkh.] were scattered on the nylon mesh in contact with soil. After 2-4 days of incubation at room





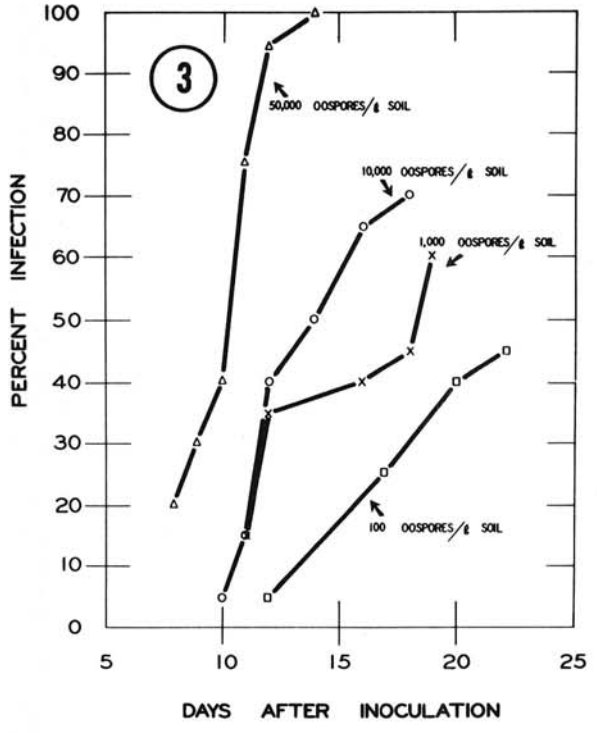


Fig. 1-3. **1)** Symptoms of infection of safflower seedlings by *Phytophthora cactorum*. Control plant on left. **2)** The growth of two isolates of *P. cactorum* during 5 days of incubation at temperatures ranging from 4-36 C. **3)** The relation between oospore concentration in soil and infection of safflower seedlings.

TABLE 2. Pathogenicity of some soil-borne fungi to safflower seedlings[a]

Fungi tested	Percent plants dead at inoculum dilution of	
	1:10	1:100
Phytophthora cactorum (Lebert & Cohn) Schroet.	100[b]	93
Phytophthora megasperma Drechs.	13	13
Phytophthora citricola Sawada	0	0
Phytophthora capsici Leonian	0	0
Aphanomyces euteiches Drechs.	0	7
Pythium sp. no. 1	63	40
Pythium sp. no. 2	20	0
Pythium ultimum Trow.	13	13
Pythium salpingophorum Drechs.	13	7
Pythium aphanidermatum (Edson) Fitzp.	7	20
Pythium vexans deBary	7	0
Pythium sylvaticum Campbell & Hendrix	0	7
None (Control soil)	5	5

[a]Fourteen-day-old cornmeal-sand inoculum mixed with steamed soil and 100 ml of infested soil was placed around 5-day-old safflower plants that had been transplanted into steamed soil. Five replicate cups with five plants each were used for each treatment.

[b]Percent safflower plants infected after incubation for 4 weeks at 24 C in a greenhouse.

temperature, the presence of the pathogen in the baits was ascertained by floating them on sterile distilled water in petri plates to induce sporangium development, or by culturing them on pimaricin medium.

In later routine tests with safflower, seeds were surface-sterilized in 0.5% sodium hypochlorite for 15 minutes, washed under running tap water for 5-10 minutes, and germinated in vermiculite at 28 C for 5 days. When soils were to be assayed for *P. cactorum,* seedlings were transplanted into 3 cm of a steamed sand-loam mixture in 500-ml paper cups (five per cup). Two days later, 200 ml of the soil to be assayed was added to the cup followed by water to bring the soil just to the point of saturation. This provided a 1.5-cm layer of test soil in contact with the hypocotyl of the seedlings.

In certain experiments, 2-day-old seedlings were transplanted directly into test soil in 10-cm clay pots. Four to five replicate 500-ml paper cups, each with five seedlings, were used in each treatment. These were incubated in the greenhouse at 24-28 C for 3-4 weeks and watered daily as required to keep the soil near saturation unless other treatment was specified. Some experiments were also carried out in growth chambers at various temperatures with 12,580-15,840 lux illumination by cool-white fluorescent and incandescent bulbs.

Plants were examined daily and the number showing disease symptoms were recorded. Affected plants were then removed and the infected parts (usually the hypocotyl) were washed and floated on either distilled or tap water in petri plates and incubated at 20-22 C for 12-48 hours to permit sporangial formation. They were examined at ×50 and ×100 magnifications with an inverted microscope. *P. cactorum* could be easily identified by its characteristic papillate sporangia. This process was continued to the end of each experiment.

RESULTS.—*Isolation of Phytophthora cactorum from soil.*—Soils obtained from around diseased apple trees were tested both by the baiting method and the soil dilution technique in attempts to isolate *P. cactorum.* Baiting with rape seed, boiled hemp seed, or apple fruit tissue was not successful. *Phytophthora cactorum* was not observed in subcultures from 100 such isolation attempts. When soil samples were inserted into apple fruits and the latter incubated in moist jars, rotting developed in some fruits, but *P. cactorum* could not be isolated. Soil dilution plates using several culture media recommended for the isolation of *Phytophthora* species were used, but no *P. cactorum* was detected.

The possibility of detecting and isolating *P. cactorum* by growing susceptible plants in infested soil was tested using six different plant species reported to be hosts of the fungus. Of the six species tested, only safflower was found to be highly susceptible to the pathogen. Some melons showed slight crown decay, but the other plants tested (tomato, cowpea, snapdragon, green pea) did not show any disease symptoms after 1-3 months of incubation. Disease symptoms on safflower appeared first as a small water-soaked lesion on the hypocotyl which enlarged rapidly (Fig. 1) causing the tissue to turn a light-tan and collapse. When seedling tissue infected with *P. cactorum* was floated on water, characteristic sporangia usually appeared within 4-24 hours. When girdled areas were plated on the antibiotic medium, the pathogen usually grew out readily, frequently in pure culture.

When safflower was seeded or transplanted into the soils collected from various apple orchards, from 0-82% of the plants became diseased (Table 1). *Phytophthora cactorum* was isolated from a high proportion of plants showing disease symptoms.

Soil samples were collected on 11 March (soil still frozen) and 2 May at various distances from a few apple trees in an old orchard. *Phytophthora cactorum* was present in all samples with infection being greatest with samples collected in May adjacent to the trees, but with the fungus also being detected in a soil sample obtained 8 m away from an apple tree.

TABLE 3. Dilution end-point of *Phytophthora cactorum* in three naturally infested soils

Soil no.	Proportion of infested soil in mixture with steamed soil[a]					
	1:0	1:2	1:4	1:8	1:16	1:32
3	20[b]	33	13	0	0	0
4	50	0	33	0	0	0
5	100	70	70	40	33	0

[a]Infested soil (100 ml) was placed around 5-day-old safflower seedlings transplanted into steamed soil. Five replicates of five plants each were used for each treatment.

[b]Percentage of safflower seedlings infected with *P. cactorum* after incubation for 4 weeks at 28 C in the greenhouse.

TABLE 4. Infectivity of *Phytophthora cactorum* at various dilutions in untreated soil during 4 months of incubation at different temperatures as determined by the safflower seedling test

Proportion of infested soil in mixture with steamed soil[a]	Period and temperature of incubation						
	0 day	30 days			120 days		
		4 C	20 C	28 C	4 C	20 C	28 C
1:0	60[b]	93	100	93	80	80	100
1:2	60	80	100	93	73	60	60
1:4	60	93	93	86	100	60	80
1:8	65	60	100	86	40	70	33
1:16	73	100	93	80	60	13	7
1:32	53	100	86	66	70	60	7
1:64	33	73	80	7	13	13	0
1:128	0	60	73	15	27	0	0
1:256	0	60	80	0	40	0	7

[a]Infested soil prepared by mixing 3-week-old sand-cornmeal inoculum of *P. cactorum* (1:100) with nonsteamed loam:sand (1:1) mixture and incubated in plastic bags at 10% moisture at temperatures from 4 to 28 C for 120 days. After incubation, infested soil was mixed with steamed soil to provide the proportions indicated.

[b]Percentage infection with *P. cactorum* after 5-day-old safflower seedlings were transplanted into the soil and incubated for 4 weeks in a greenhouse at 24 C. Four replicate cups with five plants in each cup were used for each treatment.

TABLE 5. The survival of sporangia of *Phytophthora cactorum* incubated in natural soil at temperatures from 4 to 28 C

Incubation temperature (C)	Survival (%) after incubation for:	
	15 days	47 days
4	38[a]	38
16	81	7
20	46	0
24	72	0.7
28	50	0.5

[a]Percentage survival based on counts made at 0 day by dilution plate method on selective antibiotic medium.

Infections as a function of environmental factors.—Infection of safflower by *P. cactorum* in naturally infested soils was 4, 12, 28, and 50% at 16, 20, 24, and 28 C, respectively, under greenhouse conditions. The maximum infection occurred at 28 C, the optimum temperature for mycelial growth in culture (Fig. 2). Most of the seedlings showed typical symptoms of infection within 14 days after being transplanted into infested soils.

In another experiment, safflower seedlings that had been transplanted into steamed soil were inoculated by adding a layer of natural soil initially free from *P. cactorum* that had been infested with oospores from a 70-day-old culture of the pathogen (1,000 oospores/g soil). After these had been incubated at 24 and 28 C in growth chambers for 4 weeks, 85% of the plants at 28 C were infected with *P. cactorum* as compared with 35% of those incubated at 24 C.

The effect of watering frequency on the assay was determined. Two-day-old safflower seedlings were planted into naturally infested soil in clay pots in a greenhouse at 24 C. Pots were all watered uniformly for 5 days after which the effect of three irrigation schedules was compared: (i) daily watering so that the soil was kept continuously saturated; (ii) watering every 3 days; and (iii) watering every 5 days. At each scheduled watering, soil in pots was saturated and allowed to drain. Pots watered every 5th day were sufficiently dry before the next irrigation that plants showed incipient wilting. The pathogen could be isolated from naturally infested soils irrespective of irrigation schedule used. Plants watered daily had 44% infection and were most vigorous. The number of plants appearing diseased was somewhat greater (50%) in the group least frequently watered, but it was apparent that the watering regime after the 5th day had little effect on the amount of disease that developed.

The effect on infection of the extent of contact of seedlings with infested soil was determined in a series of cups in which layers of infested soil 6-, 4.5-, 3-, 1.5-, and 0-cm deep overlayed non-infested soil layers of 0-, 1.5-, 3-, 4.5-, and 6-cm deep, respectively. Safflower seedlings were transplanted in such a way as to not disturb the position of the layers and with the radicle in the bottom 15-mm of soil. The cups were then incubated for 4 weeks at 28 C in the greenhouse. The minimum depth of infested soil used (1.5 cm) resulted in 55% infection which was comparable to the 65% which occurred with the entire plant in contact with infested soil.

Infection by other soil-borne microorganisms.—In an occasional soil sample, safflowers became diseased, but *P. cactorum* could not be found when seedlings were floated on water or cultured on pimaricin medium. In most of these cases, a culture of a sterile fungus resembling *Pythium* was obtained. In a few cases, *P. sylvaticum* Campbell & Hendrix and *P. salpingophorum* Drechsler (identified by R. G. Pratt) were found.

The cultures isolated from safflowers and a few other fungi listed in Table 2 were tested for pathogenicity. The inoculum used was a 14-day-old cornmeal-sand culture diluted 1:10 and 1:100. One hundred grams of infested soil were placed in each pot around safflower seedlings that

had been transplanted into steamed soil and incubated in greenhouses at 24 C for 4 weeks. The results (Table 2) indicated that only *P. cactorum* and an isolate of the *Pythium*-like organism were significantly pathogenic.

In a further test, the susceptibility of safflower to *P. cactorum, P. capsici, P. citricola, P. megasperma,* and *Pythium vexans, P. sylvaticum, P. aphanidermatum,* and also *Aphanomyces euteiches,* was determined at 24 C in growth chambers. For this test, fungi were grown on PDA for 2-8 days. The content of one plate was blended in 200 ml of sterile distilled water for 10 seconds at low speed and 10 ml of suspension was used to inoculate five plants in each pot. After plants were incubated for 3 weeks, only *P. cactorum* was pathogenic. Attempts to recover the other fungi used as inoculum from crown and roots of safflowers were not successful.

Other baiting methods.—The possibility of reducing the time required to assay soils for *P. cactorum* using safflower tissue in an in vitro baiting method comparable to the alfalfa seedling technique used for the isolation of *P. megasperma* (14) were not successful. Both naturally and artificially infested soils were used. In a few cases, successful isolations were made and *P. cactorum* isolates obtained were pathogenic to apple seedlings, but results were not consistent by this method. *Pythium* species were frequently present in affected baits.

Infectivity and pathogenicity of Phytophthora cactorum isolates.—Zoospore suspensions of isolates of *P. cactorum* obtained by the safflower seedling test from different soils and from safflower baits and infected apple tissues were used to inoculate 14-day-old apple seedlings, cultivar McIntosh. After incubating for 3 weeks at 24 C in a growth chamber, all of the isolates were found to be highly pathogenic to apple seedlings, causing a severe root rot usually accompanied by purpling of the leaves. When healthy appearing roots of most symptomless plants were floated on water and incubated for 24-48 hours, they were found to be infected by *P. cactorum.*

The susceptibility of three commercially grown safflower cultivars, UC-1, US-10, and Gila were compared with Nebraska-10 in naturally and artificially infested soils at 28 C in a greenhouse. In heavily infested soils, all cultivars were equally affected with all plants tested being infected. In a naturally infested soil having a lower inoculum level, however, the infection of Nebraska-10, UC-1, Gila, and US-10 was 80, 47, 40, and 15%, respectively.

The infection of safflower seedlings by *P. cactorum* at different inoculum levels was studied in naturally infested soils and those to which oospores had been added. Orchard soils obtained from around diseased apple trees on 30 November 1973 were diluted with steamed soil, and the activity of *P. cactorum* present determined (27). Not surprisingly, the infection occurring in various soil samples differed substantially (Table 3). More infection occurred in soils collected from around severely diseased trees than from those less affected.

The effect of inoculum level was determined using untreated field soil, initially free of *P. cactorum,* that had been infested with oospores of the pathogen to give concentrations ranging from 10-50,000 oospores/g soil. Infested soil was placed around seedlings transplanted into steamed soil and incubated at 28 C in growth chambers for 4 weeks. Infection occurred when 100 or more oospores/g soil were used (Fig. 3). At the highest inoculum level (50,000 oospores/g), 100% of the safflower seedlings became infected within 15 days. At lower inoculum levels (100 oospores/g), fewer plants became infected, and most of the plants which developed symptoms only did so after 2 weeks of incubation.

Survival of Phytophthora cactorum in natural soil at different temperatures.—Cornmeal-sand inoculum of *P. cactorum* that had been incubated 3 weeks was mixed (1:100) with a nonsteamed sand-loam mixture (1:1, v/v), initially free of the pathogen and incubated at temperatures of 4, 20, and 28 C. Assays were made at 0, 30, and 120 days. At each assay, 5-day-old safflower seedlings were transplanted into infested soil which had been diluted at various proportions with steamed soil (27) and incubated for 4 weeks at 24 C in the greenhouse. In soils that had been incubated 30 days at all three temperatures, the amount of infection (Table 4) increased over that which occurred when inoculum and soil were first mixed (0 days). There was a slightly greater increase of infectivity of inoculum in soil incubated at 20 C than at other temperatures. After 120 days of incubation, however, the infectivity of inoculum in all soils had decreased as indicated by infection occurring at higher soil dilution. This decrease was especially evident in samples stored at higher temperatures.

The ability of sporangia of *P. cactorum* to survive in soil under different conditions was investigated. Sporangia obtained from 15-day-old cultures were mixed with natural soil free from *P. cactorum* and incubated at temperatures of 4, 16, 20, 24, and 28 C. Assays were made after 0, 15, and 47 days by dilution plating on antibiotic medium. Plates were incubated in the dark at 28 C for 5 days. Higher soil dilution made it possible to exclude most of soil fungi present. A decline in viable propagules was noticed after 15 days of incubation at all temperatures (Table 5) and the number of sporangia remaining viable after 47 days of incubation at temperatures above 4 C was drastically reduced.

DISCUSSION.—Various agar media reported to be useful for isolation of *Phytophthora* species from soil have not been successfully used for the isolation of *P. cactorum* from naturally infested soils. This may be related to the nature of surviving propagules in soil. Oospores and chlamydospores are the two important resistant structures in *Phytophthora* (12) and successful isolation of *Phytophthora* from naturally infested soils on dilution plates has been achieved only with chlamydospore-producing species (8, 28). The fact that mycelia and sporangia of *P. cactorum* are short-lived and that oospores have been reported to be the main survival propagules in soil (22) was confirmed in the present investigation. The fact that *P. cactorum* oospores require light for germination (2, 3) has not been appreciated by workers attempting the isolation of this fungus from soil on agar media. Thus, present and previous attempts to isolate oospores from soil may have failed because the germination requirements had not been fulfilled. In the present work it was not possible to find an in vitro baiting technique similar to the lupine test of Chee and Newhook (6), or the alfalfa test of Marks and Mitchell (14).

At the present time, use of intact safflower seedlings as a selective host seems to be the most practical means for isolation of *P. cactorum* from natural soils. The safflower

seedling test is an easy means for this purpose. Safflower seedlings are uniformly susceptible, genetically uniform, and quickly grown. Seeds can be stored and used throughout the year, and the pathogen can be detected under a broad range of environmental conditions. The pathogen can be identified without further isolation, and pure cultures of the fungus can be obtained readily from diseased tissue.

The results of the present studies and those reported by others (7, 13, 26) indicate that the girdling of the seedlings at the soil line seems to be characteristic of *P. cactorum*. Artificial inoculation of safflower seedlings with several *Phytophthora* and *Pythium* species failed to cause typical collapse of the hypocotyl in safflower caused by *P. cactorum*.

The safflower seedling test provides a means to study the population dynamics of *P. cactorum* in natural soils. In the present investigation, the pathogen was found to be present in soil obtained throughout the year from around apple trees with and without disease history. By the use of the safflower seedling test, we investigated the persistence of the pathogen in natural soil incubated at various temperatures. An increase in infectivity that occurred during the first 30 days of incubation may have been due to maturation of oospores by aging (2, 22). A decline in infectivity noted at all temperatures (16-28 C), may have been due to germination of oospores and subsequent lysis or loss of viability of sporangia produced. The fact that sporangia did not survive beyond 47 days at 20 C or higher (Table 5) confirms to a previous report (22) that oospores are the major survival propagules of *P. cactorum* in soil.

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