Etiology

Identity, Virulence, and Isolation Frequency of Seven *Phytophthora* spp. Causing Root Rot of Raspberry in New York

W. F. Wilcox

Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456.
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ABSTRACT

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A syndrome of declining raspberry stands was typified by: premature chlorosis, wilting, and death of floricanes; poor primocane emergence and survival; and a red-brown necrosis of the root cortex of affected plants. The disease syndrome was observed throughout New York State, primarily on red raspberries but occasionally on purple or black raspberry cultivars, and frequently was correlated with physical or environmental conditions promoting excessive soil moisture in affected locations. *Phytophthora* spp. were isolated from symptomatic plants of seven red raspberry cultivars, two purple raspberry cultivars, and one black raspberry cultivar on 17 of 20 farms from which samples were taken. Isolates were identified as *P. fragariae* (13 farms), *P. megasperma* (seven farms), *P. cryptogea* (two farms), *P. cactorum* (two farms), *P. citricola* (one farm), an unidentified homothallic *Phytophthora* sp. (two farms), and an unidentified heterothallic *Phytophthora* sp. (two farms). When red raspberry plants (cultivars Heritage and Taylor) were grown in artificially infested soil mix and flooded for 48-hr intervals every 2 wk, *P. fragariae* and *P. citricola* were extremely virulent, causing complete root rot and plant death; *P. megasperma*, *P. cryptogea*, and the unidentified homothallic *Phytophthora* sp. were moderately to highly virulent, causing 46–96% root rot and a 20–80% incidence of plant death; and *P. cactorum* and the unidentified heterothallic *Phytophthora* sp. were only mildly virulent. In similar pathogenicity tests, *P. citricola* caused crown rot and death of Mahaleb and Mazzard cherry seedlings, whereas *P. fragariae* and the unidentified homothallic *Phytophthora* sp. caused only negligible root necrosis; the same isolates of *P. fragariae* also failed to produce typical red stele symptoms on inoculated Catskill and Blakemore strawberry plants.

Additional keywords: *Rubus idaeus*, *Rubus occidentalis*, soilborne diseases.

Phytophthora spp. have been associated with dieback and decline phenomena in raspberry plantings since 1937, when Waterston reported sporangia and oospores similar to those of *P. citricola* Sawada to be produced on diseased raspberry root tissue in Scotland (20). In 1958, McKeen (13) reported a race of *P. fragariae* Hickman pathogenic to loganberry roots in British Columbia and suggested that a similar fungus caused root degeneration of raspberry, although he was unable to obtain a pure culture of the pathogen from this host. The following decade, Converse and Schwartze (6,7) implicated a *Phytophthora* sp. as a primary etiologic agent of "raspberry wet soil root rot" in the Pacific Northwest, initially suspecting the fungus as a race of *P. fragariae* but later identifying it as *P. erythroseptica* Pethyb. More recent reports of Phytophthora root rot on raspberry and the associated pathogenic species include those from Australia, *P. cryptogea* Pethyb. and Laff. (18); the Federal Republic of Germany, *P. erythroseptica* (17); France, an unidentified species resembling *P. fragariae* (16); and the British Isles, a highly pathogenic form of *P. megasperma* Drechsler (8). In addition, *P.*

syringae (Kleb.) Kleb., *P. drechsleri* Tucker, *P. cactorum* (Leb. & Cohn) Schroet., *P. cambivora* (Petri) Buisman, *P. megasperma* var. *megasperma* Waterhouse, and two unidentified *Phytophthora* spp. also have been isolated from symptomatic raspberry roots in the British Isles, although these species generally have been deemed nonpathogenic to weakly virulent on raspberry in subsequent pathogenicity tests (8,15).

Decline and dieback of raspberry plantings are common phenomena in New York State, particularly where soil type, topography, or environmental conditions result in regular or extended periods of excessive soil moisture. Although affected plants traditionally have been diagnosed as suffering from winter injury or "wet feet," the symptoms and distribution of such plants often are typical of other deciduous fruit crops affected by Phytophthora root or crown rots. Consequently, this study was initiated to investigate the occurrence and distribution of Phytophthora root rot of raspberry in New York State, and to determine the relative frequency and virulence of the *Phytophthora* species involved. A brief portion of this work has been published previously (24).

MATERIALS AND METHODS

Isolation of *Phytophthora* spp. From 1984 through 1986, 20 farms throughout New York State were visited on which dead or dying raspberry plants with predominantly necrotic root systems were observed. Root systems of four to six symptomatic plants were recovered from each farm, placed into individual plastic bags with enough soil to prevent rapid dessication, and transported to the laboratory in an ice chest. The roots then were washed under running tap water to remove soil, and pieces (about 5 mm^2) of necrotic cortical tissue from the woody roots and crown, or segments of necrotic rootlets 1–2 cm long, were excised with a razor blade, surface-disinfested by a 1–2-sec immersion in 70% ethanol, and blotted dry on a paper towel. Twenty tissue pieces from each plant were plated onto a modified P_5ARPH selective medium (10) containing the following ingredients per liter of distilled water: Corn meal agar (CMA; Difco Laboratories, Detroit, MI), 17 g; pimaricin, 5 mg; sodium ampicillin, 250 mg; rifampicin, 10 mg; PCNB, 25 mg; and hymexazol, 50 mg. Plates were incubated at 19 C in the dark and examined daily for 1 wk. Emerging colonies of *Phytophthora* spp. were subcultured and stored on slants of CMA or unclarified V-8 juice agar (V8A; 14) until they could be identified.

Identification of *Phytophthora* spp. Isolates were identified on the basis of colony morphology, mycelial characteristics, cardinal growth temperatures, and the production, morphology, and dimensions of sporangia, oogonia, and antheridia. For colony morphology and growth temperature studies, a 4-mm-diameter mycelial plug of each isolate was transferred to CMA and/or V8A poured to a standard thickness (20 ml of medium per 90-mm-diameter petri dish), and incubated at 3 C increments from 4–28 and 30–36 C for 4–8 days in the dark. Sporangia were produced by cutting 4-mm-diameter disks from the advancing margin of a colony growing on V8A (5 ml per 65-mm-diameter petri dish), and floating these disks on 10 ml of 1.5% nonsterile soil extract (14) for 9–96 hr at 19 C under fluorescent light. Production and morphology of sex organs were studied after 3–7 wk of incubation at 21 C in the dark on a modified CV8A medium (5) containing the following constituents per liter: supernatant of centrifuged V-8 juice, 100 ml; $CaCO_3$, 0.5 g; β-sitosterol (dissolved in 5 ml of heated ethanol), 30 mg; tryptophan, 20 mg; $CaCl_2 \cdot 2H_2O$, 100 mg; thiamine-HCl, 1 mg; Bacto agar (Difco), 17 g; and distilled water, 900 ml. Those isolates that failed to form sex organs in single culture were subsequently grown on plates of the same medium with either a known A_1 isolate of *P. cryptogea* (University of California, Riverside collection P1088), a known A_2 isolate of *P. drechsleri* (University of California, Riverside P1087), or alone, and incubated as before.

Pathogenicity tests. Pathogenicity tests were conducted with minor modifications of previously described methods (14,21). Inocula were prepared by thoroughly moistening 250 cm^3 of fine vermiculite with 175 ml of V-8 juice broth in a 500-ml Erlenmeyer flask, autoclaving twice, and infesting with the desired isolate of *Phytophthora*. When more than one isolate of a given *Phytophthora* sp. was examined, each originated from a different farm. After 3 wk of incubation at 22 C, the substrate was rinsed with tap water to remove excess nutrients and thoroughly mixed with a potting medium containing 1 volume pasteurized sandy loam field soil:2 volumes fine vermiculite, at a rate of 20 cm^3 of inoculum per 1,000 cm^3 of potting medium. Controls received an uninoculated vermiculite-broth mixture at the same rate.

Host material consisted of red raspberry (*Rubus idaeus* L.) plants (Heritage and Taylor cultivars) propagated by meristem tip culture in a commercial greenhouse (Nourse Farms, South Deerfield, MA). After arrival from the nursery, plants were first grown in the above-mentioned pasteurized potting medium for 3 wk to increase the size of their root systems; they then were separated into groups on the basis of shoot length (approximately 10–20 cm), distributed evenly to provide five replicate plants per isolate in a completely randomized design for each cultivar, and transplanted into individual 0.95-L plastic containers filled with infested potting medium. Two weeks after transplanting and at 2-wk intervals thereafter, most plants were flooded for a 48-hr period by plugging the drainage hole at the bottom of their potting containers and adding water until 5–10 mm of free water collected on the soil surface. Additionally, one group of uninoculated plants was left unflooded to determine the effect of periodic waterlogging in the absence of a *Phytophthora* sp. Plants otherwise were watered as necessary between flooding episodes and fertilized weekly with a modified Hoagland's solution (21).

At the end of the 13-wk experimental period, disease severity was assessed for each isolate on the basis of fresh weights of roots and shoots, a visual estimate of the percentage of the root mass rotted, and the proportion of plants dead. The experiment was conducted twice in a greenhouse in which the soil temperature ranged from 18 to 26 C, and natural light was supplemented as necessary to provide a 16-hr photoperiod.

RESULTS

Field symptoms. Plants with Phytophthora root rot usually occurred in clusters and most often were found in soils with relatively poor internal drainage or in sections of the planting where water was most prone to accumulate. In some instances, the disease appeared to have begun in swales or at the bottom ends of rows, then spread uphill over time. Infected floricanes characteristically bore a reduced number of weak lateral shoots and leaves that often turned yellow or scorched along the margins or between the veins (Fig. 1A). Floricanes of severely infected plants wilted and died before harvest. Plant density within disease foci typically was sparse, and the number of emerging primocanes sharply limited, in stark contrast with the unaffected pattern of primocane emergence when floricane collapse was caused by winter injury, cane borers, or canker fungi. Primocanes that emerged within disease foci sometimes wilted and died (Fig. 1B), occasionally after developing necrotic lesions at their base (8); adjacent primocanes that did not collapse often were stunted. The most consistently diagnostic symptom of the disease was a characteristic red-brown necrosis of the cortex of infected roots, which was readily visible after scraping away the overlying epidemis, oftentimes revealing a distinct margin between healthy and diseased tissue (Fig. 1D). Necrosis sometimes extended into the crown region, in which case such a margin was almost always readily apparent (Fig. 1C). The disease was most frequently found on red raspberry plants, including the cultivars Heritage, Hilton, Latham, Newburgh, Reveille, Taylor, and Titan; however, it also was confirmed on the purple raspberry (*R. idaeus* × *R. occidentalis* L.) cultivars Brandywine and Royalty and once on the black raspberry (*R. occidentalis*) cultivar Bristol.

Identity and frequency of *Phytophthora* spp. *Phytophthora* spp. were isolated from symptomatic plants on 17 of the 20 farms from which samples were taken. Isolates were identified as *P. fragariae*, 13 farms; *P. megasperma*, seven farms; *P. cryptogea*, two farms; *P.*

Fig. 1. Symptoms of red raspberry plants affected with Phytophthora root rot. **A,** Floricane of Hilton cultivar infected with *P. fragariae*. Note marginal and interveinal scorching of leaves. Primocane from same plant also appears to be wilting (arrow); **B,** Cluster of young primocanes of Hilton cultivar wilting and collapsing as a result of infection by *P. fragariae*; **C,** Root and crown system of a plant (Titan cultivar) infected with *P. megasperma*. Epidermis has been scraped away to reveal underlying cortex and distinct margin between healthy and red-brown necrotic tissue (arrow). Picture taken just before harvest; **D,** Portion of the primary root system of a mature plant (Hilton cultivar) infected with *P. fragariae*. Epidermis has been scraped away to reveal necrotic (right side of picture) and healthy (left) cortical tissues. Arrow points to distinct margin between healthy and necrotic zones.

cactorum, two farms; *P. citricola*, one farm; an unidentified homothallic *Phytophthora* sp., two farms; and an unidentified heterothallic *Phytophthora* sp., two farms (Fig. 2). Because of inconsistencies in the literature regarding the taxonomy of many *Phytophthora* spp. associated with raspberries, the following descriptions of the New York raspberry isolates are given as a basis for their identification and to facilitate comparison with previous reports. Data for individual isolates are listed in Table 1.

P. fragariae (isolates of this type previously were designated *Phytophthora* sp. #1 [24]). Mycelial growth was very poor at all temperatures on CMA, resulting in small, uneven colonies with individual hyphae sparsely branched and often coiled. Growth was relatively slow but much more luxuriant on V8A, producing uniform colonies with profuse aerial mycelium (Fig. 3A). Isolates made slight growth at 4 C, optimum growth at 19–22 C, and little or no growth at 28 C; no isolates grew at 30 C (Table 1). Oogonia were produced readily in single culture after 3–7 wk on CV8A, were usually tapered at the base, and averaged 39.6 μm in diameter. Oospores were plerotic, averaging 35.5 μm in diameter with a mean wall thickness of 5.6 μm. Spheroidal to ovoidal antheridia were predominantly amphigynous, occasionally paragynous, and averaged 17.1 × 13.8 μm (Fig. 3C). Nonpapillate sporangia, borne singly on undifferentiated sporangiophores, were ovoid to obpyriform, sometimes tapered at the base, and proliferated internally by both nesting and extension. Sporangium dimensions averaged 46.9 × 30.2 μm, with a mean length:breadth ratio of 1.53. In comparison, four isolates of *P. fragariae* recovered from strawberry (obtained from J. L. Maas, USDA, Beltsville, MD, and subsequently designated NY 365, NY 366, NY 367, and NY 368) made practically no growth on CMA after 7 days at 22 C but produced colonies on V8A that were similar in morphology and cardinal growth temperatures to the raspberry isolates, although they grew more slowly. These same isolates produced nonpapillate, internally proliferating sporangia that were morphologically very similar to the raspberry isolates, but produced no oospores after 7 wk on CV8A (Table 1).

P. megasperma. All isolates were typical of the large oogonium/low temperature isolates of *P. megasperma* previously described on other deciduous fruit crops (14,23); isolates of this type also have been designated *P. megasperma* var. *megasperma* (19) and have been placed into the recently designated "BHR" subgroup of *P. megasperma* by Hansen et al (9). Colonies produced at 22 C were uniform and appressed on V8A with very little aerial mycelium, whereas those on CMA were uniform to radial, produced no aerial mycelium, and had characteristically broad hyphae with numerous secondary and tertiary branches arising at right angles from the primary hyphae (Fig. 4A and B). Colony growth for all isolates on CMA was slight at 4 C, optimal at 22–25 C, and slight to nil at 30 C; no isolates grew at 33 C. Oogonia were produced in abundance after 3 wk in single culture on CV8A, averaged 49.1 μm in diameter, and contained thick-walled oospores averaging 44.5 μm in diameter. Antheridia were almost entirely paragynous, very rarely amphigynous, and averaged 16.8 × 13.1 μm (Fig. 4D). Sporangia were ovoid to obpyriform, nonpapillate, rounded at the base, proliferated internally, and averaged 57.8 × 36.2 μm, with a mean length:breadth ratio of 1.61 (Fig. 4C).

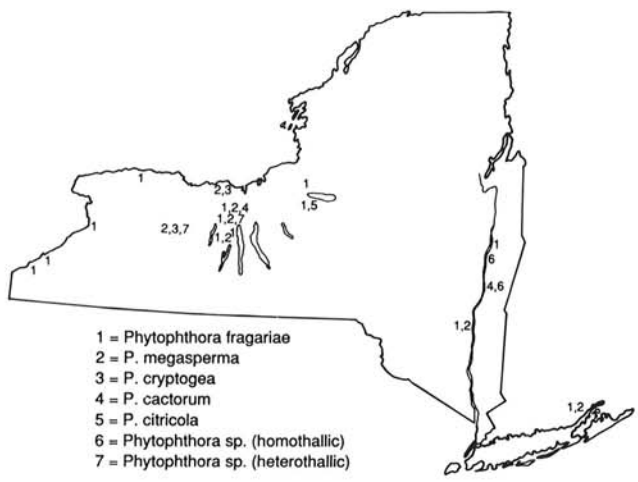

Fig. 2. Frequency and distribution of *Phytophthora* spp. isolated from declining raspberry plants in New York State.

P. cryptogea (isolates of this type previously were designated *Phytophthora* sp. #3 [24]). Isolates provisionally identified as *P. cryptogea* produced radial to rosette colonies with slight aerial growth on CMA, and rosette colonies with pronounced tufts of aerial growth on V8A (Fig. 3H). Colony growth for all isolates on CMA was slight at 4 and 7 C, optimal at 22–25 C, with only a trace of growth at 33 C, and no growth at 36 C. Hyphal branching angles were much more acute than were those of most other *Phytophthora* spp. examined (Fig. 3I). Sporangia were nonpapillate, ovoid to obpryriform, rounded at the base, proliferated internally, and were produced singly on undifferentiated sporangiophores of uniform width (Fig. 3G). Sporangia were relatively small, averaging 41.0 × 29.4 μm, with a mean length:breadth ratio of 1.40. Sex organs were not produced in single culture on CV8A but were produced when each isolate was paired with a known A_2 mating type of *P. cryptogea*.

P. cactorum. Colonies were uniform and lacked aerial mycelium on CMA; on V8A, colonies were uniform and appressed, with extremely short aerial mycelium producing a distinct powdery appearance (Fig. 3D). Isolates made no growth on CMA after 7 days at 4 C and only slight growth at 7 C; growth was optimum at 22–25 C and nearly as strong at 30 C, but no growth occurred at 33 C. Primary hyphae produced a moderate number of secondary hyphae that branched at right angles, tapered to a point, and gave rise to few tertiary hyphae (Fig. 3E). Distinctly papillate, caducous sporangia were produced sympodially, did not proliferate internally, and averaged 41.0 × 33.4 μm (Fig. 3F). Oogonia were produced abundantly in single culture on CV8A, averaging 27.4 μm in diameter. Plerotic oospores averaged 26.0 μm in diameter. Antheridia were entirely paragynous.

P. citricola. Colonies were radial to rosette with no aerial mycelium on CMA, producing a distinct chrysanthemum pattern with abundant, short aerial mycelium on V8A (Fig. 4E). Mycelial growth was nil on CMA after 7 days at 4 and 7 C, optimum at 28–30 C, and nil at 33 C. Primary hyphae were broad, even, and sparingly branched; secondary hyphae were long, even, and arose at right angles from the primary hypha (Fig. 4F). Sporangia were primarily ovoid, had a single semipapillate apex, did not proliferate internally, and averaged 49.7 × 34.7 μm (Fig. 4G). Short-stalked oogonia were produced abundantly in single culture on CV8A and averaged 32.7 μm in diameter, containing plerotic oospores averaging 29.5 μm in diameter. Antheridia were attached paragynously both adjacent and distal to the oogonial stalk (Fig. 4H).

Homothallic *Phytophthora* sp. (isolates of this type previously were designated *Phytophthora* sp. #2 [24]). Isolates grew very slowly on CMA, producing uniform to radial colonies; on V8A, growth rate also was slow but nearly twice that on CMA, producing uniform, appressed colonies lacking aerial mycelium (Fig. 4I). Hyphae were undulating and frequently branched on CMA, with branches arising at relatively acute angles (Fig. 4J). Growth was nil after 7 days on V8A at 4 and 7 C, optimum at 25 C, slight at 33 C, and nil at 36 C. Distinctly papillate sporangia frequently were ovoid, averaging 40.6 × 29.6 μm (Fig. 4K), but occasionally were distorted, developing long necks and dual apices; sporangia did not proliferate internally. Oogonia characteristically had tapered bases to which antheridia always were attached paragynously (Fig. 4I); mean diameters of oogonia and oospores were 35.2 μm and 32.2 μm, respectively.

Heterothallic *Phytophthora* sp. (isolates of this type previously were designated *Phytophthora* sp. #4 [24]). Colonies were uniform to radial with slight aerial growth on CMA and were uniform with abundant aerial growth on V8A (Fig. 3K). Hyphae produced on CMA were sparingly branched, with secondary hyphae frequently growing in a zigzag pattern giving rise to short tertiary projections

(Fig. 3L). Isolates grew slowly at 4 C, made optimum and rapid growth at 25–28 C, grew strongly at 33 C, and made no growth at 36 C. Sporangia were broadly ovoid, averaging 54.8 × 45.8 μm (length:breadth ratio = 1.20), rounded at the base, and proliferated internally by nesting and extension (Fig. 3J). Sex organs were not produced in single culture, but were produced when paired with an A_2 mating type of *P. cryptogea*.

Pathogenicity tests. Although all *Phytophthora* spp. isolated from dead or declining raspberry plants were pathogenic in greenhouse tests, they differed markedly in virulence towards red

TABLE 1. Dimensions of reproductive structures and colony growth rates of *Phytophthora* species isolated from raspberry in New York State

	Sporangium dimensions (μm)[a]			Diameter (μm)[b]		Antheridium dimensions (μm)[b]		Colony growth rate/(mm/day) Temperature (C)						
Isolate	Length	Breadth	l/b	Oogonium	Oospore	Length	Breadth	7	16	22	25	28	30	33
NY328	36.9	24.0	1.54	38.8	34.2	16.7	13.5	0.1[c]	1.1	1.4	0.9	0	0	0
NY329	57.6	37.7	1.53	39.1	35.7	16.4	13.0	0.1	0.9	1.7	2.1	0.2	0	0
NY331	50.8	32.2	1.58	41.3	37.6	19.1	15.0	0.2	2.7	4.3	3.7	0.2	0	0
NY332	42.6	28.6	1.49	40.7	37.0	18.0	13.4	0.1	2.3	4.0	3.4	0.7	0	0
NY334	52.4	33.3	1.58	42.4	38.0	16.4	13.2	0.2	2.0	3.3	2.3	1.0	0	0
NY335	46.6	30.2	1.54	40.1	36.2	17.2	13.6	0.1	1.6	3.4	1.7	0.5	0	0
NY339	51.3	32.4	1.58	39.6	36.0	18.2	16.1	0.1	1.3	2.9	3.0	1.0	0	0
NY340	48.0	31.5	1.52	38.7	33.9	17.5	14.5	0.2	2.6	3.7	3.2	1.0	0	0
NY385	47.6	28.4	1.68	37.4	33.2	15.8	13.1	0.2	1.6	3.0	2.4	0.7	0	0
NY386	46.1	35.6	1.29	40.7	36.6	14.7	12.0	0.1	1.3	2.3	1.0	0.2	0	0
NY387	40.5	27.6	1.47	41.2	36.9	17.7	13.5	0.1	0.6	1.0	0.9	0.1	0	0
NY388	42.6	26.6	1.60	38.6	33.9	16.8	13.8	0.2	1.4	3.6	3.9	0.6	0	0
NY389	47.2	24.4	1.66	36.0	32.6	18.8	14.6	0.1	1.7	1.3	1.3	0	0	0
$\bar{X}$	46.9	30.2	1.53	39.6	35.5	17.1	13.8	0.14	1.6	2.8	2.3	0.48	0	0
(s)	(5.48)	(4.11)	(0.09)	(1.74)	(1.76)	(1.23)	(1.04)	(0.05)	(0.63)	(1.11)	(1.1)	(0.38)		
P. fragariae (strawberry)														
NY365	48.9	30.8	1.59	...[f]	...	...	...	0.1[c]	1.3	1.9	1.6	0	0	0
NY366	57.5	34.3	1.68	...	...	...	...	0.1	0.9	0.3	0.3	0.1	0	0
NY367	57.2	36.2	1.58	...	...	...	...	0.1	1.1	0.9	0.6	0.1	0	0
NY368	48.0	29.8	1.61	...	...	...	...	0	1.0	1.1	1.0	0.1	0	0
$\bar{X}$	52.9	32.8	1.62	...	...	...	...	0.08	1.1	1.1	0.87	0.08	0	0
P. megasperma														
NY312	56.1	40.6	1.38	48.4	43.1	15.6	12.3	0.6[d]	2.6	3.4	2.7	2.0	1.7	0
NY318	51.9	33.1	1.60	54.3	48.4	16.7	13.2	0.4	3.0	4.6	4.4	3.7	2.0	0
NY319	62.2	37.0	1.71	49.3	45.7	18.0	13.8	0.6	3.3	4.7	4.7	3.9	2.4	0
NY321	62.7	36.7	1.73	51.6	46.6	17.6	13.6	1.0	3.3	4.0	3.6	2.9	0.3	0
NY322	65.3	37.8	1.73	46.9	42.9	17.9	13.8	0.7	3.1	4.6	4.6	3.4	1.4	0
NY330	53.4	38.4	1.39	46.8	41.8	14.7	11.7	0.9	3.6	4.4	3.9	3.3	1.4	0
NY392	52.2	31.0	1.68	48.0	42.1	15.2	11.8	1.0	3.6	4.7	4.7	3.4	1.0	0
NY393	58.9	35.3	1.67	47.3	45.2	18.9	14.2	1.0	3.4	4.1	4.0	3.3	2.2	0
$\bar{X}$	57.8	36.2	1.61	49.1	44.5	16.8	13.1	0.78	3.2	4.3	4.1	3.2	1.6	0
(s)	(5.20)	(3.04)	(0.15)	(2.63)	(2.36)	(1.52)	(0.98)	(0.23)	(0.33)	(0.45)	(0.69)	(0.58)	(0.68)	
P. cryptogea														
NY315	42.2	28.4	1.50	...[f]	...	...	...	0.7[d]	2.7	3.9	3.7	3.4	2.7	0.1
NY316	38.9	30.6	1.27	...	...	...	...	0.7	2.9	4.0	4.0	3.6	2.6	0.1
NY317	40.4	28.9	1.40	...	...	...	...	0.7	2.9	4.0	4.0	3.4	2.6	0
NY320	42.5	29.7	1.43	...	...	...	...	0.7	2.7	3.7	3.7	3.3	2.1	0
$\bar{X}$	41.0	29.4	1.40					0.7	2.8	3.9	3.9	3.4	2.5	0.1
P. cactorum														
NY323	41.7	30.1	1.39	29.9	27.9	15.0	10.8	0.1[e]	3.4	5.1	5.0	5.1	4.7	0
NY327	37.6	27.2	1.38	27.1	25.2	11.7	9.2	0.1	3.1	4.6	4.7	3.6	3.9	tr
$\bar{X}$	39.7	28.7	1.39	28.5	26.6	13.4	10.0	0.1	3.3	4.9	4.9	4.4	4.3	0
P. citricola														
NY333	48.9	35.6	1.68	32.7	29.5	13.2	10.4	0[e]	4.8	7.3	7.3	7.5	7.6	0
Phytophthora sp. (homothallic)														
NY324	37.3	30.1	1.24	34.4	31.2	16.5	11.9	0[e]	1.2	2.8	2.8	2.4	1.9	0.4
NY325	42.0	29.5	1.42	33.6	30.7	16.8	12.3	0	1.3	3.6	3.7	3.3	2.6	1.0
NY326	41.7	29.7	1.40	37.6	34.8	18.0	13.5	0	1.0	2.4	2.4	1.7	1.6	0.1
$\bar{X}$	40.3	29.8	1.35	35.2	32.2	17.1	12.6	0	1.2	2.9	3.0	2.5	2.0	0.5
Phytophthora sp. (heterothallic)														
NY313	56.2	47.6	1.18	...[f]	...	...	...	1.3[e]	4.5	6.3	6.5	6.0	6.0	4.3
NY394	53.3	44.0	1.21	...	...	...	...	0.8	4.7	6.5	6.5	6.0	6.0	5.3
$\bar{X}$	54.8	45.8	1.20	...	...	...	...	1.1	4.6	6.4	6.5	6.0	6.0	4.8

[a] Mean of 40 observations/isolate.
[b] Mean of 25 observations/isolate.
[c] Values for all isolates of this species expressed as average daily increase of colony radius after 7 days on V8-juice agar.
[d] Values for all isolates of this species expressed as average daily increase of colony radius after 7 days on corn meal agar.
[e] Values for all isolates of this species expressed as average daily increase of colony radius after 4 days on corn meal agar.
[f] Oogonia and antheridia not formed in single culture.

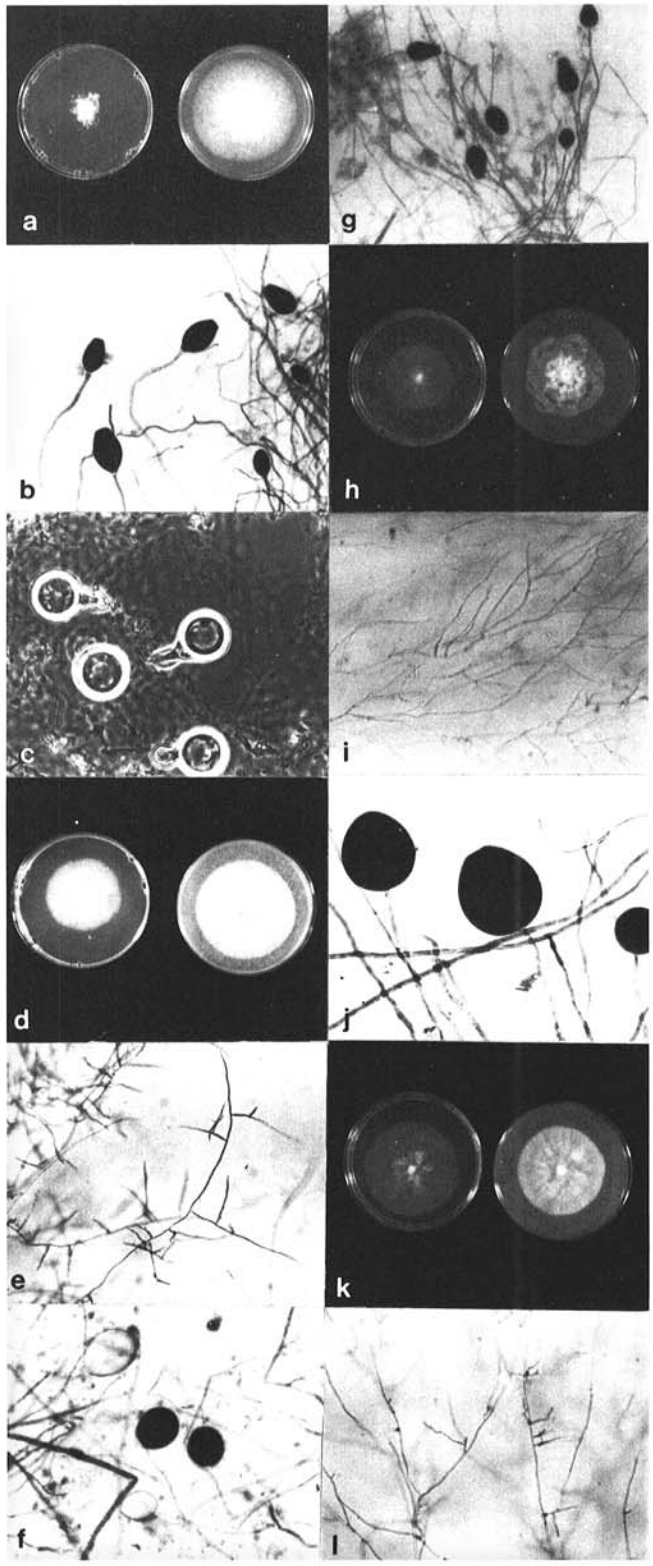

Fig. 3. Morphological features of four *Phytophthora* spp. causing root rot of raspberry. **A–C,** *P. fragariae*: **A,** Colonies formed on corn meal agar (CMA) (left) and V-8 juice agar (V8A) (right) after 8 days of growth at 22 C; **B,** Typical ovoid to obpyriform sporangia (×110); **C,** Oogonia showing funnel-shaped bases and characteristic amphigynous attachment of antheridia (×190). **D–F,** *P. cactorum*: **D,** Colonies formed on CMA (left) and V8A (right) after 5 days of growth at 22 C; **E,** Hyphae formed on CMA at 19 C (×40); **F,** *Papillate sporangia* (×150). **G–I,** *P. cryptogea*: **G,** Obpyriform to ovoid sporangia (×115); **H,** Colonies formed on CMA (left) and V8A (right) after 5 days of growth at 22 C; **I,** Hyphae formed on CMA at 19 C (×40). **J–L,** Heterothallic *Phytophthora* sp.: **J,** Characteristic broadly ovoid sporangia (×205); **K,** Colonies formed on CMA (left) and V8A (right) after 4 days of growth at 22 C; **L,** Hyphae formed on CMA at 19 C (×40).

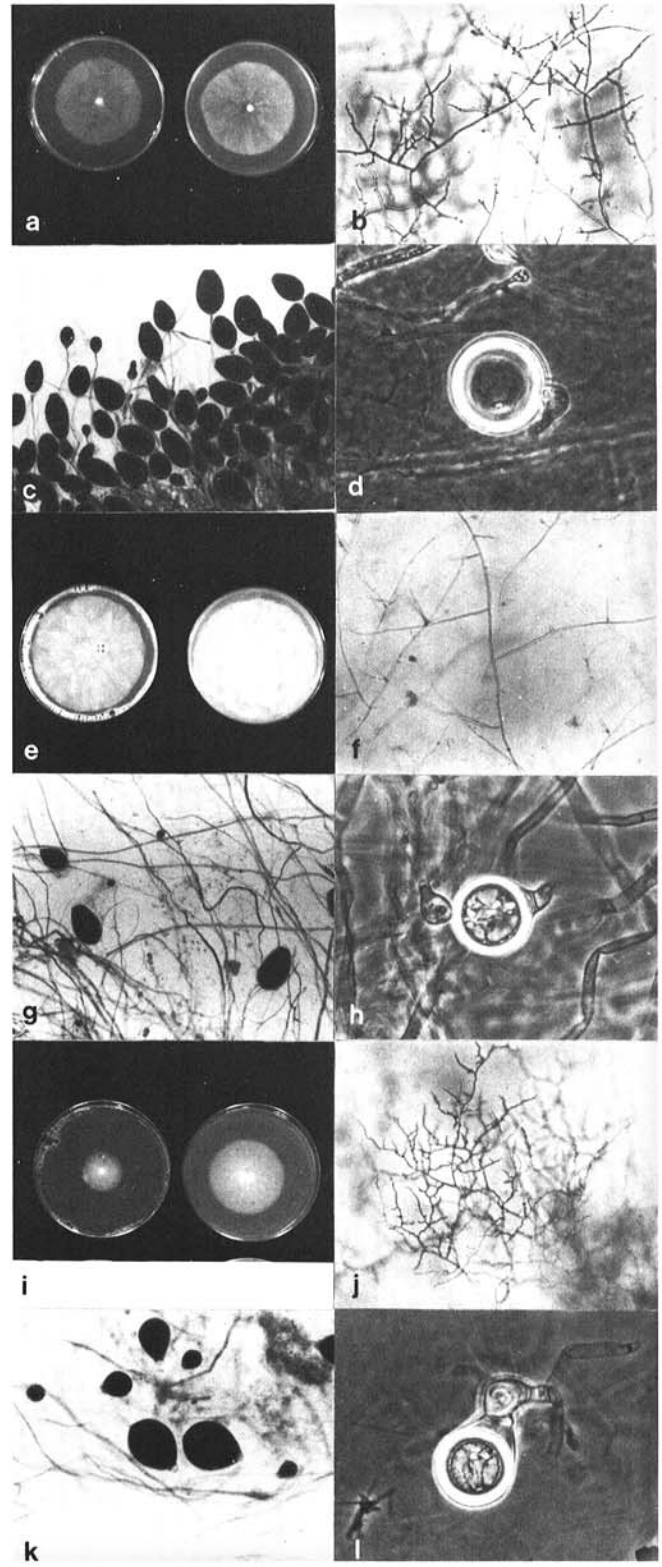

Fig. 4. Morphological features of three *Phytophthora* spp. causing root rot of raspberry. **A–D,** *P. megasperma*: **A,** Colonies formed on CMA (left) and V8A (right) after 5 days of growth at 22 C; **B,** Characteristic branching pattern of hyphae formed on CMA at 19 C (×40); **C,** Typical sporangia (×92); **D,** Oogonium and paragynously attached antheridium (×280). **E–H,** *P. citricola*: **E,** Colonies formed on CMA (left) and V8A (right) after 5 days of growth at 22 C; **F,** Even, sparingly branched hyphae formed on CMA at 19 C (×90); **G,** Semipapillate sporangia (×95); **H,** Oospore within short-stalked oogonium, paragynous antheridium attached distal to oogonial stalk (×375); **I–L,** Homothallic *Phytophthora* sp.: **I,** Colonies formed on CMA (left) and V8A (right) after 8 days of growth at 22 C; **J,** Undulating hyphae formed on CMA at 19 C (×40); **K,** Distinctly papillate sporangia (×150); **L,** Sexual organs, showing characteristic paragynous attachment of antheridium to tapered base of the oogonium (×375).

raspberry. The unidentified heterothallic *Phytophthora* sp. and *P. cactorum* caused minor degrees of root rot and slight decreases in root and shoot weights relative to uninoculated, flooded control plants, whereas *P. megasperma*, *P. cryptogea*, and the unidentified homothallic *Phytophthora* sp. caused moderate to severe levels of root rot, pronounced decreases in root and shoot weights relative to the control plants, and a 20–80% incidence of plant death, depending on the isolate and raspberry cultivar involved (Tables 2 and 3). The three tested isolates of *P. fragariae* and the lone isolate of *P. citricola* were all highly virulent, causing 96–100% root rot and an 80–100% incidence of plant mortality on both Taylor and Heritage red raspberry cultivars (Tables 2 and 3). A repetition of this experiment produced qualitatively similar results (data not shown).

In two additional pathogenicity tests with similar techniques, the same three isolates of *P. fragariae* and the two isolates of the unidentified homothallic *Phytophthora* sp. caused negligible root rot on Mahaleb (*Prunus mahaleb* L.) and Mazzard (*Prunus avium* L. 'Silverbark Mazzard') cherry seedlings, whereas the isolate of *P. citricola* caused complete root rot, crown rot, and death of each of the five replicate seedlings of both cherry species. These same raspberry isolates of *P. fragariae* failed to produce typical red stele symptoms when Blakemore and Catskill strawberry cultivars were planted into artificially infested soil mix and flooded for 48-hr periods in a growth chamber at 15 C.

DISCUSSION

This appears to be the first report confirming Phytophthora root rot on raspberries in any North American location outside the Pacific Northwest. However, the consistent isolation of *Phytophthora* spp. from dead and dying plants, the widespread occurrence of this disease throughout the fruit-producing districts of New York, and the isolation of *P. fragariae* from symptomatic red raspberry plants on a commercial fruit farm near Wooster, OH (Wilcox and Ellis, *unpublished*), suggest that Phytophthora root rot is a common, albeit previously unrecognized, cause of declining red raspberry stands in the midwestern and northeastern regions of the United States; symptoms typical of Phytophthora root rot also occur on raspberries in the province of Ontario, Canada (1). Although *P. fragariae* was isolated from one black raspberry stand during the course of this study, field symptoms suggested that virus diseases and Verticillium wilt were much more common causes of decline in the black raspberry plantings visited.

The *Phytophthora* sp. most commonly isolated from dead or declining raspberry plants, and which consistently proved to be highly virulent in pathogenicity tests, was identified as *P. fragariae* because it most closely resembled original descriptions and recognized specimens of this taxon in the following cultural and morphological characters: very slow vegetative growth on agar media; uniform and profusely aerial colonies on V8A; inability to grow at temperatures above 25–28 C; homothallism; predominant amphigyny with occasional paragyny; and ovoid to obpyriform, nonpapillate, internally proliferating sporangia produced on single undifferentiated sporangiophores. Isolates so identified were not assigned to *P. erythroseptica* due to the occasional but consistent presence of paragynous antheridia, and cultural characteristics (cardinal temperatures for growth, colony type, and growth rate) markedly different from those reported for this species (19) and observed in the author's laboratory in initial comparative studies with a known isolate (NY 189) of *P. erythroseptica* from potato. Independent investigations conducted in the British Isles by Duncan et al (8) parallel to those reported herein yielded 16 highly virulent isolates from red raspberry that were morphologically indistinguishable from both the raspberry isolate originally identified as *P. erythroseptica* by Converse and Schwartze (7) and three raspberry isolates identified as *P. erythroseptica* var. *erythroseptica* from the Federal Republic of Germany (FRG) (17). However, all such raspberry isolates differed significantly from a known potato isolate of *P. erythroseptica* in cardinal temperatures for growth, mycelial growth rate, colony morphology, existence of paragyny, and sporangial size (8). Although the authors noted that the isolates in question bore "a close resemblance" to *P. fragariae* on the basis of growth rate, colony appearance, and characteristics of oospores and sporangia, they apparently chose not to identify them as such because of failure to induce typical red stele symptoms on inoculated strawberry plants; rather, the authors chose to identify these isolates as an explicit type of *P. megasperma*, a taxon from which they conceded that their isolates

TABLE 2. Pathogenicity and relative virulence of seven *Phytophthora* spp. to Taylor red raspberry plants grown for 13 wk in artificially infested soil mix in a greenhouse

Inoculum		Fresh wt. (g)		% Root[a,b] rot	Plants dead
Phytophthora	Isolate	Roots	Shoots		
Uninoculated, not flooded		65.1 A	22.6 A	1 F	0/5
Uninoculated, flooded		42.9 B	17.6 BC	1 F	0/5
P. cactorum	NY327	42.5 B	20.7 AB	3 F	0/5
Phytophthora sp. (heterothallic)	NY313	33.6 BC	15.8 C	22 EF	0/5
P. cactorum	NY323	26.0 C	14.8 C	11 EF	0/5
P. cryptogea	NY320	14.9 D	8.8 D	80 BC	3/5
P. megasperma	NY321	12.7 DE	9.1 D	68 CD	2/5
P. megasperma	NY318	11.6 DEF	9.3 D	70 CD	3/5
P. cryptogea	NY315	10.4 DEFG	8.5 D	88 AB	3/5
Phytophthora sp. (homothallic)	NY326	3.9 EFG	3.2 E	80 BC	3/5
P. citricola	NY333	2.7 EFG	2.7 E	100 A	5/5
Phytophthora sp. (homothallic)	NY325	1.3 FG	1.7 E	96 A	4/5
P. fragariae	NY331	1.2 FG	0.7 E	100 A	5.5
P. fragariae	NY329	1.1 FG	1.0 E	100 A	5/5
P. fragariae	NY334	0.6 G	0.8 E	100 A	5/5

[a] Mean of five replicate plants per treatment. Values in each column followed by a common letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian K-ratio LSD rule.

[b] Percentage of root mass rotted, based on a visual estimation. Mean separation based upon a $\log_e$ transformation of the data. The appropriate *Pytophthora* sp. was reisolated from diseased roots of at least one plant exposed to each isolate.

TABLE 3. Pathogenicity and relative virulence of seven *Phytophthora* spp. to Heritage red raspberry plants grown for 13 wk in artificially infested soil mix in a greenhouse

Inoculum		Fresh weight (g)[a]		% Root[a,b] rot	Plants dead
Phytophthora sp.	Isolate	Roots	Shoots		
Uninoculated, not flooded		72.2 A	39.7 A	2 D	0/5
Uninoculated, flooded		50.9 B	29.7 B	2 D	0/5
Phytophthora sp. (heterothallic)	NY313	37.6 C	26.2 BC	14 CD	0/5
P. cactorum	NY327	30.0 CDE	30.1 B	5 D	0/5
P. cactorum	NY323	25.9 DE	30.8 B	24 C	0/5
P. megasperma	NY318	21.6 EF	19.6 C	46 B	1/5
P. cryptogea	NY320	20.0 EF	19.4 C	52 B	1/5
P. cryptogea	NY315	18.2 EFG	17.1 C	59 B	1/5
P. megasperma	NY321	18.1 EFG	17.4 C	60 B	2/5
Phytophthora sp. (homothallic)	NY326	9.4 FGH	8.8 D	88 A	2/5
Phytophthora sp. (homothallic)	NY325	6.2 GH	5.8 DE	91 A	3/5
P. citricola	NY333	4.8 H	5.5 DE	90 A	4/5
P. fragariae	NY329	2.5 H	2.1 E	96 A	4/5
P. fragariae	NY331	1.2 H	1.0 E	100 A	5/5
P. fragariae	NY334	1.0 H	1.5 E	100 A	5/5

[a] Mean of five replicate plants per treatment. Values in each column followed by a common letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian K-ratio LSD rule.

[b] Percentage of root mass rotted, based on a visual estimation. Mean separation based upon a $\log_e$ transformation of the data. The appropriate *Phytophthora* sp. was reisolated from diseased roots of at least one plant exposed to each isolate.

were morphologically distinct (8). Based on the descriptions and figures published by Duncan et al (8), it seems possible that these isolates from the Pacific Northwest, the FRG, and the British Isles represent the same organism as those from New York and Ohio identified as *P. fragariae* in the present report. It is also possible that the current report from France (16) of a *Phytophthora* sp. that is highly virulent on raspberry, morphologically similar to *P. fragariae*, but nonpathogenic on strawberry may comprise yet another reference to this organism, as may the 1958 report of a new race of *P. fragariae* isolated from loganberry (*Rubus* (*Eubatus*) sp. × *R. idaeus*) in British Columbia (13). Certainly, this group of apparently similar isolates from various parts of the world should be collectively examined to confirm their common identity.

Although I am mindful of the practical utility of reserving *P. fragariae* as a taxon for isolates that cause red stele of strawberry, I am similarly mindful of the confusion engendered by assigning isolates to taxa whose systematic criteria they do not satisfy. For the present, it therefore would seem most desirable to identify raspberry isolates as *P. fragariae* when they satisfy current morphological criteria for placement within this taxon, while remaining cognizant of indicated differences in pathogenicity between strawberry and raspberry isolates of the species. In the future, a large sample of raspberry and strawberry isolates should be thoroughly tested for pathogenic capabilities to confirm these preliminary indications. Such isolates also should be examined in detail for cultural, morphological, and physiological differences that may be sufficiently consistent and pronounced to warrant taxonomic separation solely on these characters.

Although isolated from only one farm, *P. citricola* also was highly virulent in pathogenicity tests; *P. megasperma*, the species second in frequency of isolation, was moderately to highly virulent in these tests and also has caused consistently high levels of root rot and plant mortality on Festival, Latham, NY 114 (Titan × Heritage), Reveille, and Titan red raspberry; on Brandywine and Royalty purple raspberry; and on Bristol and Jewel black raspberry cultivars in similar experiments conducted subsequent to those reported herein (Wilcox, *unpublished*). Both *P. citricola* and *P. megasperma* var. *megasperma* have been isolated from red raspberry in the United Kingdom, but such isolates have not been pathogenic to red raspberry in greenhouse tests (8,15). It seems likely, however, that the contrast in results between the current study and previous British work is due largely to differences in experimental techniques employed in the respective studies. British workers generally have inoculated with a suspension of zoospores without completely saturating the raspberry potting soil, a technique recognized by Duncan et al (8) as providing conditions possibly less conducive to disease development than those experienced in wet, poorly drained field soils. In contrast, plants in the current study were flooded for a relatively prolonged period (48 hr) at regular intervals after inoculation. Significant root rot caused by *P. megasperma* has been reported to occur on Mahaleb cherry seedlings only when seedlings were regularly flooded for 48-hr periods (22), and, in the current study, *P. megasperma* was recovered primarily from raspberries growing in soils whose drainage would be considered marginal for this crop. Thus, although it would appear that relatively wet soil conditions are required before *P. megasperma* becomes a serious pathogen of red raspberry, it is a widely distributed species with a high potential for virulence once proper conditions occur. Similarly, soil flooding treatments can also greatly amplify the virulence of *P. citricola* on Paradox walnut (*Juglans hindsii* (Jeps.) Jeps. × *J. regia* L.) (11), and appear to be an absolute requirement for the development of crown rot of Mahaleb cherry under greenhouse conditions (Wilcox, *unpublished*). However, not all *Phytophthora* spp. recovered from raspberries are influenced to this extent by soil water conditions. Consistent with British results (8), *P. cactorum* was a weak pathogen in the current study, even though the soil was regularly flooded after inoculation. Of the two isolates of *P. cactorum* recovered during this study, one came from a plant from which the highly virulent unidentified homothallic *Phytophthora* sp. also was recovered, whereas the other came from a declining stand from which *P. fragariae* was frequently isolated. These results collectively suggest that *P. cactorum* is but a minor or, perhaps, secondary (8) pathogen on red raspberry.

Heterothallic isolates that produced nonpapillate sporangia were of two distinct morphological and pathological types. Isolates provisionally identified as *P. cryptogea* were moderately to highly virulent and were similar in morphological and cultural characteristics and in protein electrophoretic banding patterns (4) to other isolates identified as *P. cryptogea* that have been recovered from deciduous fruit trees in several states adjoining the Great Lakes; however, this group of isolates differs on the basis of colony morphology and growth rate (21) and protein electrophoretic banding patterns (4) from isolates identified as *P. cryptogea* that have been recovered from deciduous fruit trees in California. The unidentified heterothallic *Phytophthora* sp. appears to be morphologically similar to an unidentified *Phytophthora* sp. described on cherry (21) and walnut (12) trees in California, but was only weakly virulent on raspberry in the current pathogenicity tests. Current criteria for classifying heterothallic, nonpapillate isolates of *Phytophthora* are plainly inadequate for those that do not exhibit unique features placing them within one of the more well-defined taxa (e.g., *P. cinnamomi*) of Waterhouse's Group VI (19). Lack of adequate taxonomic guidelines for such isolates has led to the possibility that different workers may identify significantly different organisms by a common name (e.g., *P. cryptogea*) or, conversely, that different identifications may be applied to a common organism. Until an improved taxonomic system is developed for this group, it is hoped that authors who identify such isolates as *P. cryptogea*, *P. drechsleri*, or "*Phytophthora* sp." will include sufficient descriptive and illustrative detail that comparisons among reports will be facilitated.

Recognition of Phytophthora root rot as a significant cause of declining red raspberry stands in eastern North America should enable growers and advisers to develop an integrated control program using selection and modification of planting sites for maximum water drainage; cultivar selection; and chemical control procedures, as appropriate for individual situations. Whereas the relative susceptibility to *P. erythroseptica* (probably synonymous with *P. fragariae*, as discussed above) has been documented among many raspberry cultivars in the Pacific Northwest (2,3), many other important cultivars popular in eastern North America have not been included in these studies but should be examined. Furthermore, the evidence presented in this study indicates that several additional *Phytophthora* spp. are serious pathogens of red raspberry, suggesting that cultivar and germ plasm reactions to these organisms also should be examined in the future.

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