Host Range and Influence of Nutrition, Temperature, and pH on Growth of *Pythium violae* from Carrot

J. K. SCHRANDT, Graduate Student, R. M. DAVIS, Extension Specialist, and J. J. NUNEZ, Staff Research Associate, Department of Plant Pathology, University of California Davis 95616

ABSTRACT

Schrandt, J. K., Davis, R. M., and Nunez, J. J. 1994. Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Dis. 78:335-338.

The host range of *Pythium violae,* the cause of cavity spot of carrots, was determined in artificially infested potting mix in the greenhouse and in naturally infested field soil. Six new symptomless hosts were identified in greenhouse pathogenicity tests: cowpea, broccoli, celery, cucumber, sugar beet, and watermelon. Cowpea and cauliflower were also infected in natural field soil. The susceptibility of three previously reported hosts of *P. violae,* carrot, alfalfa, and wheat, was confirmed. The utilization of 10 carbon sources, 13 nitrogen sources, and three vitamins by *P. violae* was examined in liquid culture under controlled conditions. Although some growth was observed on all carbon and nitrogen sources tested, optimum growth of *P. violae* occurred when fructose, sucrose, or glucose was the sole source of carbon and when arginine, serine, or glutamine was the sole source of nitrogen. *P. violae* also utilized certain polysaccharides and oils, e.g., potato starch and wheat germ oil, and did not require an exogenous source of vitamins. The optimum temperature range for growth was 16–22 C, and the optimum pH range was 5.5–8.0.

Cavity spot of carrot, characterized by sunken, elliptical lesions on the taproots, causes economic losses by reducing carrot quality. Cavity spot has often been attributed to infection by *Pythium* species, especially *Pythium violae* Chesters & C.J. Hickman (3,8,18,20). Although *P. violae* has been isolated from the roots of a few other crops, that is, alfalfa, wheat, and lettuce (4,9,15), little is known about its host range or natural biology; and it has only rarely been recovered from soil (5). *P. violae* also has not been reported to cause economic damage to any crop other than carrots. An assessment of the potential role of other plant species in the survival of *P. violae* is needed, as other hosts could maintain or increase the population of the fungus in the absence of carrots.

There are no reports enumerating *P. violae* populations in the soil. If quantitative information can be obtained on soil populations of *P. violae,* it may be possible to relate populations to levels of disease. Studies on the nutrition, temperature, and pH requirements of *P. violae* may aid in the development of a method for enumerating *P. violae* in the soil. The types of organic and inorganic compounds *P. violae* utilizes in the laboratory may also provide clues to its preferred natural habitat (1,7,12). This report describes the host range of *P. violae* and its utilization of some carbohydrates, polysaccharides, amino acids, and vitamins in synthetic media. Additionally, the effects of pH and temperature on growth were determined.

Accepted for publication 30 December 1993.

© 1994 The American Phytopathological Society

MATERIALS AND METHODS

Inoculum production. Vermiculite (500 ml) amended with V8 juice (200 ml of water, 500 ml of V8 juice, 0.5 g of $CaCO_3$), twice autoclaved at 121 C for 45 min, was inoculated with three cornmeal agar plugs (0.8 cm diameter) from 5-day-old cultures of two isolates of *P. violae* (originally isolated from cavity spot lesions on carrots collected from commercial fields in Kern County, CA). The inoculated V8-vermiculite was incubated at room temperature (about 23 C) in the dark for 3 wk. To standardize the inoculum concentration of *P. violae* in the substrate, 0.1-g aliquots were plated directly on modified PARP (0.4 ml of pimaricin, 0.25 g of ampicillin, 0.01 g of rifampicin, 0.025 g of pentachloronitrobenzene, 17 g of Difco cornmeal agar, 1 L of distilled water) (11). The substrate was evenly distributed over the surface of each of eight PARP plates and incubated in the dark for 36 hr. The number of colony-forming units (cfu) was counted, and the inoculum of *P. violae* was diluted with potting mix (1:1:1, peat:sand:bark) to obtain a final concentration of about 350 cfu/g of potting mix.

Host range. Seeds of potential hosts were planted in the inoculated potting mix in plastic pots (21 cm diameter × 21 cm deep) and grown in the greenhouse at temperatures of 22–25 C. Six to 10 seeds were planted per pot and subsequently thinned to four seedlings per pot. There were four replicate infested pots for each host and an equal number of noninfested pots. The pots were arranged in a completely randomized design and watered daily. Hosts included alfalfa (*Medicago sativa* L. cv. Moapa), wheat (*Triticum aestivum* L. cv. D6 301), carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Arcang. cv. Dominator), broccoli (*Brassica oleracea* L. cv. Gem hybrid), celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers. cv. 5270R), sugar beet (*Beta vulgaris* L. cv. Beta 4823), cowpea (*Vigna unguiculata* (L.) Walp. cv. California Blackeye no. 5), cucumber (*Cucumis sativus* L. cv. Straight Eight), tomato (*Lycopersicon esculentum* Mill. cv. Glamour), clover (*Trifolium repens* L. cv. Ladino), kidney bean (*Phaseolus vulgaris* L. cv. Linden Red), watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai cv. Crimson Sweet), onion (*Allium cepa* L. cv. Vega), cotton (*Gossypium hirsutum* L. cv. SJ2), potato (*Solanum tuberosum* L. cv. Idaho), corn (*Zea mays* L. cv. Showcase), pumpkin (*Cucurbita pepo* L. cv. Connecticut Field), safflower (*Carthamus tinctorius* L.), cantaloupe (*Cucumis melo* L. cv. Topmark), pea (*Pisum sativum* L. cv. Little Marvel), and bean (*P. vulgaris* L. cv. Blue Lake).

At 4, 6, and 8 wk after sowing, a seedling from each pot was lifted from the soil, washed, and examined under a dissecting microscope for the presence of lesions on the roots. Lesions and random sections of roots 1 cm long, collected at various locations on the root infrastructure, from small feeder rootlets to larger taproots, were washed for 5 min under running tap water. The root sections were then submerged for 1 min in 0.5% NaOCl, rinsed with sterile distilled water, plated on PARP (4 root sections per plate × 2 plates per plant × 4 plants per harvest = 32 root sections/plant/harvest) and incubated for 5 days at 20 C. Putative colonies of *P. violae* were then transferred to 1.5% water agar containing sterile grass blades (*Poa* spp.) and incubated in the dark for development of reproductive structures. After 5–7 days of incubation, the colonies were identified according to their morphological characteristics (17). The experiment was repeated once.

In the spring of 1991, two consecutive field trials were conducted in a commercial carrot field in Kern County with a known history of cavity spot. The following 11 crops were tested for susceptibility to *P. violae* in the field: kidney bean (cv. Red Kidney), corn (cv. Payco 648), tomato (cv. Early Pak), cantaloupe (cv. Top Mark), watermelon (cv. Sugar Baby), alfalfa (cv. Moapa-69), cotton (cv. SJ2), barley (*Hordeum vulgare* L., NK blend, 425 and 428, 50% each), cowpea (cv. California Blackeye no. 5), onion (cv. Vega), and cauliflower (*B. oleracea* cv. White Rock). The seeds were sown by hand in a randomized complete-block design with four replications. Each experimental unit was a single bed 3 m long; beds were spaced 0.96 m apart. The row was seeded every 5.1–7.6 cm, or somewhat less for smaller seed. Three seedlings were sampled from each row approximately 4 wk after sowing and sampled again at 6 and 8 wk. The roots of harvested plants were washed and visually inspected for lesions under a dissecting microscope. Root samples were collected at random and placed on PARP (four root pieces per plate, three plates per plant) using the standard tissue-plating technique previously described. The percentage of roots infected with *P. violae* was recorded after incubation in the dark for 5 days at 20 C.

Nutritional requirements. The influence of carbon, nitrogen, and vitamin sources on mycelial growth of three isolates of *P. violae* was determined using a liquid basal medium of the following composition: 0.5 g of $MgSO_4 \cdot 7H_20$, 1.0 g of KH_2PO_4, 0.1 g of $CaCO_3$, 2.0 g of glucose, 100 μg of thiamine hydrochloride, 1.0 g of asparagine, 0.01 g of β-sistosterol, 1 ml of trace elements (Pridham and Gottlieb trace salts [16] amended with 0.1 g of $NaMoO_4 \cdot 2H_2O$), and distilled water to make 1 L. The pH of the basal medium was adjusted to 6.5 with 0.1 N NaOH before and after autoclaving. Heat labile compounds, such as the carbohydrates, were sterilized through a millipore filter (pore size 0.22 μm) and added to the autoclaved medium.

Erlenmeyer flasks (250 ml), each containing 75 ml of the defined medium, were inoculated with 8-mm-diameter mycelial plugs from 3-day-old cultures of *P. violae* transferred from intermediary plates of 1.5% Difco water agar. All treatments were replicated three times. Liquid cultures were grown at 24 C on a rotary shaker operating at 100 rpm. After 12 days, the mycelial mats were harvested, washed in distilled water, dried for 24 hr at 24 C, and weighed.

The following carbon sources were tested: glucose, sucrose, fructose, cellobiose, raffinose, xylose, galactose, mannose, rhamnose, and fucose. Each carbon source was added to the glucose-free basal medium at a rate of 2 g/L. Complex carbon sources included potato starch, sodium polypectate, cellulose, wheat germ oil, Tween 20, and safflower oil. The oils were added to the basal medium at a rate of 1 g/L (equivalent to other carbon sources). When oil was added, the medium was blended at high speed to ensure even distribution.

Nitrogen sources included glutamine, ammonium nitrate, proline, sodium nitrate, serine, phenylalanine, ammonium sulfate, asparagine, arginine, methionine, histidine, tryptophan, and lysine. These sources substituted for asparagine in the basal medium and were added at a rate of 0.215 g of nitrogen per liter.

Vitamins included biotin and pyridoxine, which replaced thiamine in the basal agar medium at a rate of 5 μg/L and 100 μg/L, respectively. Mycelial disks (5 mm diameter) were transferred from intermediary plates of the test medium; colony growth was recorded at 48 and 72 hr after the last transfer.

Optimal temperature and pH. Vegetative growth of three isolates of *P. violae* on potato-dextrose agar was determined at temperatures ranging from 8 to 28 C at 2-degree intervals. Radial growth of the colonies in each of four replicate plates was measured at 48 and 72 hr. The experiment was repeated twice.

To determine the effect of pH on growth of *P. violae,* Difco cornmeal agar was adjusted to pH 4.0–10.0 at 0.5 intervals with 0.1 N NaOH and 1 N HCl. The plates were incubated at 20 C, and measurements were recorded after 48 and 72 hr. Treatments were replicated four times, and the experiment was repeated twice.

All data were analyzed with the MSTAT-C statistical program (Version 1.42, Michigan State University, East Lansing) for analysis of variance. Means were separated by the least significant difference test.

RESULTS

Host range. Only carrot, alfalfa, and wheat seedlings grown in inoculated potting mix developed visible root lesions by the eighth week after sowing. Root lesions were not apparent on the other crop species. No symptoms were observed on the aerial parts of any of the plants throughout the experiment. Because differences in the incidence and severity of symptoms were not observed between the two isolates of *P. violae,* the results were combined. *P. violae* was reisolated from nine hosts 6 and 8 wk after sowing (Table 1). The highest percentage of recovery was from carrot and alfalfa. *P. violae* was successfully reisolated from cowpea, broccoli, celery, cucumber, sugar beet, and watermelon grown in inoculated potting mix, but no symptoms were apparent on the roots of these hosts. The fungus did not cause lesions, nor was it reisolated from the other crop plants. No root lesions were observed on plants grown in noninoculated potting mix.

Lesions were not observed on the roots of any of the crops planted in the field. However, *P. violae* was reisolated from the roots of alfalfa, cauliflower, and cowpea in 6- and 8-wk-old plants. The fungus was not recovered from 4-wk-old seedlings of any of the crop plants. Above-ground symptoms were not seen in any of the plants. In the first field trial, a *Pythium* species identified on grass culture as *P. irregulare* Buisman was isolated on two separate occasions from cotton and tomato seedlings. *Pythium ultimum* Trow was isolated in the second field trial from watermelon.

Nutrition. Because there were no significant differences between the mycelial growth of the different isolates of *P. violae,* results were combined and analyzed together. There were significant differences in mycelial growth in media containing single sources of carbon (Table 2). *P. violae* did not grow in the basal medium in the absence of carbon. Fructose was the most effectively utilized carbohydrate, followed by glucose and

Table 1. Recovery of *Pythium violae* from roots of potential hosts in greenhouse pathogenicity tests 6 and 8 wk after sowing seeds in infested potting mix

	Recovery of *P. violae*[y] (%)			
	Trial 1		Trial 2	
Host	6 wk	8 wk	6 wk	8 wk
Carrot	28.1 a[z]	50.0 a	31.2 a	62.5 a
Alfalfa	22.0 ab	43.7 ab	15.6 ab	37.5 b
Cowpea	12.5 bc	25.0 bc	12.5 b	31.2 bc
Wheat	9.4 c	18.7 c	15.6 ab	31.2 bc
Broccoli	6.2 c	18.7 c	3.1 b	18.7 bc
Celery	3.1 c	18.7 c	6.2 b	12.5 c
Cucumber	6.2 c	12.5 c	6.2 b	18.7 bc
Sugar beet	0	6.2 c	9.4 b	18.7 bc
Watermelon	0	6.2 c	0	0
LSD ($P = 0.05$)	11.86	20.79	16.27	20.60

[y] Successful isolations from eight root sections per plant, replicated four times, on semiselective agar. Data represent combined results from two isolates of *P. violae*. *P. violae* was not recovered from tomato, clover, kidney bean, onion, cotton, potato, corn, pumpkin, safflower, cantaloupe, pea, or bean.

[z] Means in a column followed by a common letter are not significantly different according to LSD. Means converted to percentages after analysis of data.

sucrose. *P. violae* also utilized more complex sources of carbon, such as potato starch, with dry weights comparable to the simple sugars, i.e., about 89.0 mg (*data not presented*). However, the weight of mycelial mats of *P. violae* grown in liquid culture with wheat germ oil was relatively high at 144.2 mg. To determine if the growth of another *Pythium* species was similarly enhanced by wheat germ oil, radial growths of *P. violae* and *P. ultimum* were compared on solid agar medium amended with wheat germ oil and on standard Difco cornmeal agar. After 24 hr, growth was significantly and similarly enhanced for both species with the addition of wheat germ oil to the agar (*data not presented*).

P. violae utilized a wide range of nitrogen sources (Table 3). The fungus did not grow in the basal medium in the absence of nitrogen. Among the nitrogen sources tested, arginine, serine, and glutamine resulted in maximum growth. Tryptophan and ammonium sulfate were poor sources of nitrogen for growth. *P. violae* did not require an exogenous supply of thiamine, biotin, or pyridoxine for growth (*data not presented*).

Temperature and pH. *P. violae* grew optimally at about 20 C (Fig. 1). Growth was similar over the pH range 5.5–8.0 but decreased rapidly below pH 5 (Fig. 2). The fungus grew relatively well at very high pH. Results of repeated experiments were combined.

DISCUSSION

In greenhouse pathogenicity tests, carrot, which exhibited typical cavity spot lesions, was confirmed as one of the primary hosts of *P. violae*. Necrotic lesions were also apparent on roots of wheat and alfalfa grown in *P. violae*-infested soil. *P. violae* was previously recovered and described as pathogenic on roots of wheat and ryegrass in western Australia (4) and was isolated from feeder rootlets of alfalfa in the Central Valley of California (9). Hancock reported that *Pythium* species are most pathogenic on alfalfa when the root system is most rapidly expanding and that *P. violae* was the most common species isolated from alfalfa rootlets in California during the cool months (9).

New symptomless hosts identified in the greenhouse trials included cowpea, broccoli, celery, cucumber, and sugar beet. Cowpea and cauliflower were also found to be naturally infected in the field. Although *P. violae* colonized the roots of these crops, no distinct root rot or

Table 2. Growth of *Pythium violae* in liquid culture containing different carbohydrates as the sole carbon source

Carbon source (2 g/L)	Dry weight (mg)[y]
D-Fructose	121.4 a[z]
D-Glucose	84.7 b
D-Sucrose	81.7 bc
D-Cellobiose	79.0 bcd
D-Raffinose	63.5 bcd
D-Galactose	63.0 bcd
L-Xylose	57.4 cd
D-Mannose	52.8 d
L-Rhamnose	0
D-Fucose	0
LSD ($P = 0.05$)	26.73

[y] Dry weight of mycelial mats grown in liquid culture for 12 days. Values are averages of four replications in each of three experiments with three isolates of *P. violae*.

[z] Means in a column followed by a common letter are not significantly different according to LSD.

Table 3. Growth of *Pythium violae* in liquid culture containing different sources of nitrogen

Nitrogen source (0.215 g/L)	Dry weight (mg)[y]
Arginine	133.1 a[z]
Serine	110.9 ab
Glutamine	107.2 abc
Phenylalanine	89.3 bcd
Asparagine	83.3 cd
Sodium nitrate	64.7 de
Lysine	53.9 ef
Histidine	51.7 ef
Methionine	50.2 ef
Proline	47.7 ef
Ammonium nitrate	46.3 ef
Ammonium sulfate	36.3 f
Tryptophan	36.1 f
LSD ($P = 0.05$)	27.12

[y] Dry weight of mycelial mats grown in liquid culture for 12 days. Values are averages of four replications in three experiments with three isolates of *P. violae*.

[z] Means in a column followed by a common letter are not significantly different according to LSD.

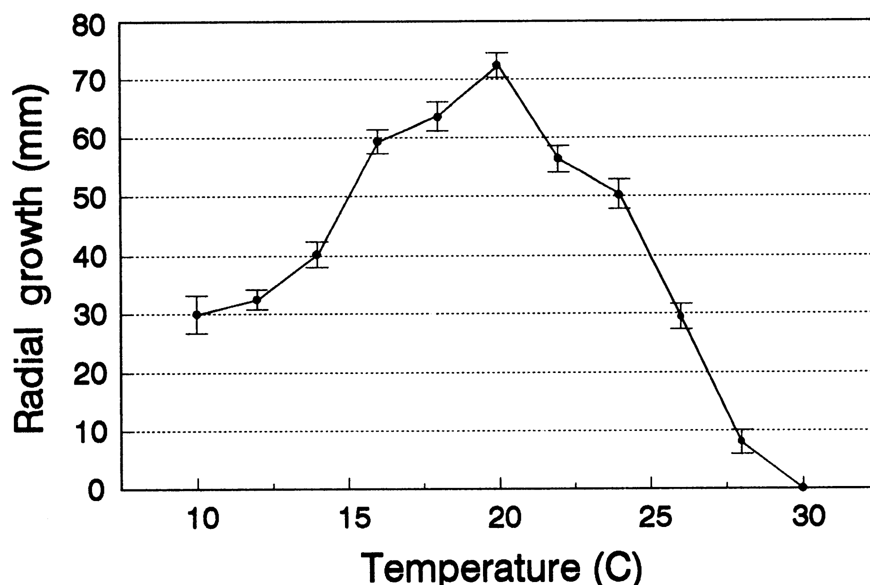

Fig. 1. Effect of temperature on mycelial growth of three isolates of *Pythium violae* on potato-dextrose agar after 72 hr. Each point is the average of four replications in each of three experiments.

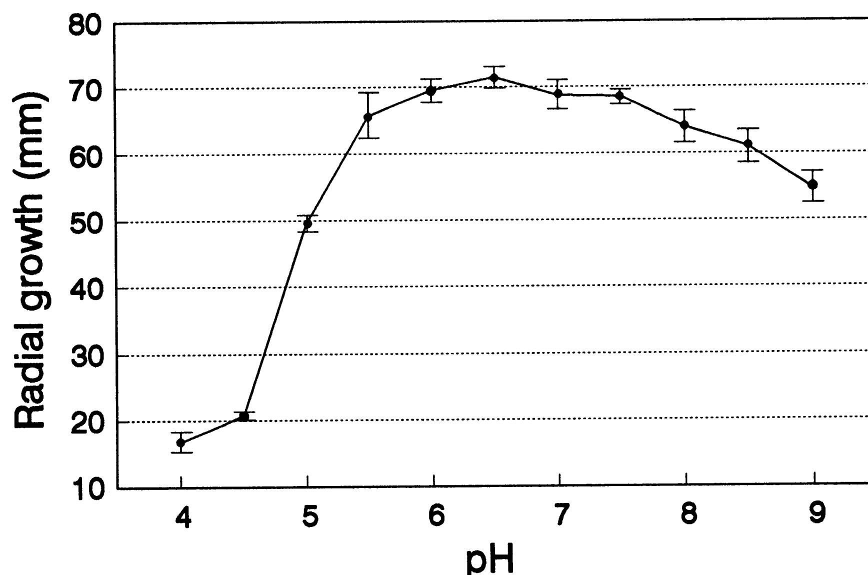

Fig. 2. Effect of pH on mycelial growth of *Pythium violae* on cornmeal agar after 72 hr at 20 C. Each point is the average of four replications with three isolates of *P. violae*.

above-ground symptoms were associated with infection. Many of these susceptible hosts occur in rotations with carrots in California and may maintain or increase populations of *P. violae* in commercial fields.

With regard to nutritional requirements, *P. violae* is not unlike other plant-parasitic *Pythium* species (10). Optimal growth occurred when fructose, glucose, or sucrose was the sole carbon source, which is similar to the results of nutrition studies for *P. ultimum* (13). In addition, *P. violae* was able to utilize certain polysaccharides, e.g., starch and cellulose. Lipids, including triglycerides, also served as sources of carbon; both *P. violae* and *P. ultimum* grew well on wheat germ oil. Even in those instances in which lipids or their components are not the sole carbon source, their presence in the medium may significantly affect the production of sexual spores of Oomycetes (2,10). Doster and Bostock demonstrated that oospore production by *Phytophthora syringae* was greatly increased when wheat germ oil was added to the basal medium (6).

P. violae was also able to utilize all of the naturally occurring forms of nitrogen included in this study. Some amino acids were better sources of nitrogen than inorganic nitrogen. *P. violae* did not require an exogenous supply of vitamins, which is consistent with the growth of most *Pythium* species (10). Although a few species require an exogenous supply of pyrimidine for growth (14), plant-pathogenic species of *Pythium* apparently do not (7).

Results of temperature studies indicate that *P. violae* has a much lower temperature optimum than many other *Pythium* species. Although van der Plaats-Niterink (17) listed 25 C as an optimum temperature for *P. violae,* results from this study are more in agreement with Wagenvoort et al, who found that *P. violae* grew faster at 16 C than at 25 C (19). Vivoda et al (18) found that, at 20 C, *P. violae* was more virulent and caused more lesions per carrot than *P. ultimum.* Previous failures to isolate *P. violae* from cavity spot lesions may be explained by its low temperature optimum and relatively slow daily growth rate. In the natural environment, adaptation to cooler temperatures may partially provide a competitive advantage for *P. violae.*

Growth of *P. violae* was sharply reduced at a pH of about 5.0, but it grew well in highly alkaline conditions. In a survey in California carrot fields, there was no relationship between pH (5.5–7.7) and occurrence of cavity spot (18). This is in contrast to a field study in England, where the incidence of cavity spot was negatively correlated with increasing soil pH (20).

Since it has not been possible to quantify populations of *P. violae* from naturally infested soils, by either soil dilution or soil-baiting methods, information on the ecology of this pathogen has remained limited and incomplete. At present, the relationship between population densities of *P. violae* in the soil and the occurrence of cavity spot is unknown. *P. violae* may exist in low numbers in the soil, or certain environmental conditions might have to be met for oospores to germinate, both of which, among other reasons, make the population difficult to quantify. With regards to developing a selective medium for the slow-growing *P. violae* that could exclude other *Pythium* spp., no special nutritional factors were identified in this study. *P. violae* utilized the same carbon and nitrogen sources as *P. ultimum* and probably other *Pythium* species as well (13). However, the growth of some *Pythium* spp. occurring in the same soil may be restricted by incubation at relatively low temperatures (15–17 C), which allow near optimum growth of *P. violae.*

LITERATURE CITED

1. Agnihotri, V. P., and Vaartaja, O. 1967. The influence of nitrogenous compounds on growth of *Pythium* species. Can. J. Microbiol. 13:1509-1519.
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. Davis, R. M., Liddell, C. M., Guerard, J. P., Nunez, J., and Vivoda, E. 1988. *Pythium* diseases of carrots in California. (Abstr.) Phytopathology 78:1578.
4. Dewan, M. M., and Savasithamparam, K. 1988. *Pythium* spp. in roots of wheat and rye grass in western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici.* Soil Biol. Biochem. 20:801-808.
5. Dick, M. W., and Ali-Shtayeh, M. S. 1986. Distribution and frequency of *Pythium* species in parkland and farmland soils. Trans. Br. Mycol. Soc. 86:49-62.
6. Doster, M. A., and Bostock, R. M. 1988. The effect of temperature and type of medium on oospore production by *Phytophthora syringae.* Mycologia 80:77-81.
7. Foley, M. F., and Deacon, J. W. 1986. Physiological differences between mycoparasitic and plant-pathogenic *Pythium* spp. Trans. Br. Mycol. Soc. 86:225-231.
8. Groom, M. R., and Perry, D. A. 1985. Induction of "cavity spot-like" lesions on roots of *Daucus carota* by *Pythium violae.* Trans. Br. Mycol. Soc. 84:755-757.
9. Hancock, J. G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75:1112-1120.
10. Hendrix, J. W. 1974. Physiology and biochemistry of growth and reproduction in *Pythium.* Proc. Am. Phytopathol. Soc. 1:207-210.
11. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
12. Moore, L. D. 1964. Effect of carbon source and calcium on growth of *Pythium ultimum.* (Abstr.) Phytopathology 54:901.
13. Ribeiro, W. R. C. 1991. Mycoparasitism by *Pythium periplocum* and related species of *Pythium* with spiny oogonia. Ph.D. diss. University of California, Davis.
14. Ridings, W. H., Gallegly, M. E., and Lilly, V. G. 1969. Thiamine requirements helpful in distinguishing isolates of *Pythium* from those of *Phytophthora.* Phytopathology 59:737-742.
15. Stanghellini, M. E., and Kronland, W. C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by *Pythium dissotocum.* Plant Dis. 70:1053-1056.
16. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Co., Minneapolis, MN.
17. van der Plaats-Niterink, A. J. 1968. Monograph of the genus *Pythium.* Studies in Mycology No. 21. Centraal Bureau Voor Schimmelcultures, Baarn, Netherlands.
18. Vivoda, E., Davis, R. M., Nunez, J. J., and Guerard, J. P. 1991. Factors affecting the development of cavity spot of carrot. Plant Dis. 75:519-522.
19. Wagenvoort, W. A., Blok, I., Mombarg, H. F. M., and Veldhuizen, T. 1989. Cavity spot of carrots in relation to a *Pythium* sp. Gartenbauwissenschaft 54:70-73.
20. White, J. G. 1988. Studies on the biology and control of cavity spot of carrots. Ann. Appl. Biol. 113:259-268.