

Effects of Temperature on Pythium Root Rot of Spinach Grown Under Hydroponic Conditions

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ABSTRACT

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The effect of nutrient solution temperatures on the cyclic occurrence of *Pythium aphanidermatum* and *P. dissotocum* as causal agents of root rot of hydroponically grown spinach was investigated. Both species grew well and produced zoospores in comparable numbers at temperatures between 17 and 27 C. Infecting hyphae of both species penetrated spinach roots, without appressorium formation, within 15 min after inoculation with zoospores at 23 C, the transitional temperature that governed the predominance of either *P. aphanidermatum* or *P. dissotocum*. Both species also caused significant yield reductions at all temperatures tested (17, 21, and 27 C) except *P. aphanidermatum* at 17 C. However, the rapidity of

symptom development varied between the two species. Severe root rot and/or plant death occurred within 3-4 days after inoculation with *P. aphanidermatum* at 21 and 27 C, whereas severe root rot, but no plant death, occurred only after 7 days of incubation following inoculation with *P. dissotocum* at 21 and 27 C. Differences in pathogenicity at specific temperatures give a temporary competitive advantage to the favored species with respect to rapidity of host colonization and subsequent fungus reproduction. Metalaxyl, at 5 µg a.i./ml in the nutrient solution, effectively controlled root rot.

Additional key words: disease control.

In 1981, root rot was the primary limiting factor in commercial hydroponic production of spinach, *Spinacea oleracea* L., in Arizona. Both *Pythium aphanidermatum* (Edson) Fitzp. and *P. dissotocum* Drechsler were responsible for the disease. These two organisms were found to alternate in predominance as the causal agent of root rot during the growing season (1). The reason for the cyclic occurrence of these two species appeared to be related to the temperature of the recirculating nutrient solution. During the summer production season, when solution temperatures ranged from 23 to 27 C, *P. aphanidermatum* was the predominant species isolated from diseased plants; but during the winter production season, when solution temperatures ranged from 17 to 23 C, *P. dissotocum* was the predominant species.

The primary objective of this study was to determine specific effects of temperature on the cyclic occurrence of these two *Pythium* species as causal agents of root rot of hydroponically grown spinach.

MATERIALS AND METHODS

Stock cultures of *P. aphanidermatum* and *P. dissotocum*, originally isolated from diseased spinach plants, were maintained at 24 C on 10% V-8 juice agar (VJA) medium. All temperatures reported in this study, unless otherwise specified, refer to temperatures of nutrient solutions. Ambient temperatures in greenhouse studies ranged daily from 23 to 36 C (mean = 28 C).

Mycelial growth and zoospore production. The effect of various incubator temperatures on mycelial growth of *P. aphanidermatum* and *P. dissotocum* was determined by measuring colony radii after 24 hr of growth on VJA contained in 9-cm-diameter petri dishes. Fungal colonies originated from 5-mm-diameter plugs cut from a 48-hr-old VJA culture of each fungus.

The effects of various temperatures on zoospore production was determined by placing three 7-mm-diameter plugs of a 48-hr-old VJA culture of each fungus in petri dishes containing 20 ml of either sterile distilled water or a complete nutrient solution. Zoospore numbers were visually estimated on a rating scale of 0-5 (0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-50, 4 = 51-200, 5 = >200 zoospores per microscope field at ×50). The maximum rating was recorded during a 72-hr incubation period at various temperatures. Each experiment was replicated twice and repeated three times.

Pathogenicity. Pathogenicity tests were conducted on hydroponically grown spinach plants in an environmentally controlled greenhouse. Four 3-wk-old spinach seedlings, started in a nursery in peat pellets, were transferred into holes cut into each of 16 Styrofoam flotation boards (32 × 27.5 × 2.0 cm). Planted boards were then placed in 13.5-L plastic tubs (37.5 × 33 × 13.5 cm) containing a continuously aerated (300 cc/min per tub) nutrient solution. Tubs were located in a temperature-controlled box, and the temperature of the nutrient solution was regulated by using air as a heat exchange medium (6). The nutrient solution was equilibrated to desired temperatures prior to transplanting. After transplanting, an intact 48-hr-old VJA petri dish (9 cm diameter) culture of *P. aphanidermatum*, *P. dissotocum*, or *P. aphanidermatum* plus *P. dissotocum* was added to each tub. For dual inoculation, one-half of a culture of each fungus was used. Preliminary studies showed that zoospores of both species were produced within 3 hr after placement of cultures in the tubs. Plants in uninfested tubs served as controls. After 3 wk of growth in the tubs, fresh weights of shoots and dry weights of roots were recorded for each treatment. Prior to dry weight determinations, one to three root segments (each 4 cm in length) were collected from each plant in each tub, rinsed in running tap water for 10 min, blotted dry, plated on 2% water agar, and incubated at 24 C. Fungal isolates were identified.

Root penetration. Spinach seedlings were grown hydroponically in the greenhouse, as described above, for 8 days at 23 C. Individual plants were brought to the laboratory and transferred to 445-ml plastic cups, and roots were suspended in 90 ml of half-strength nutrient solution. Zoospores of *P. aphanidermatum* and *P. dissotocum*, produced as described above, were then added to the

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nutrient solution. The number of zoospores per cup, estimated by counts on a hemacytometer, was 4,500 and 4,800/ml for *P. aphanidermatum* and *P. dissotocum*, respectively. At various time intervals (from 0.25 to 15 hr) after infestation and incubation at 23 C, plants were removed from the cups and the roots were severed directly into 2.5% glutaraldehyde in Sorensen's phosphate buffer, pH 7.2. After fixation, roots were rinsed in the buffer, postfixed with 1% OsO₄ for 2 hr, rinsed in distilled water, and dehydrated in a graded acetone series. Roots were dried overnight in a CO₂ critical-point dryer (Polaron Instruments Inc., Watford, England), mounted onto stubs with double-sided cellophane tape and coated with 25–30 nm of 60:40 gold/palladium in a sputter coater (Coating Unit E5100; Polaron Instruments Inc.). Specimens were then examined on a scanning electron microscope at 20 kV. Roots from uninoculated plants served as controls.

Plant-to-plant transmission. Six-week-old spinach plants, infected with either *P. aphanidermatum* or *P. dissotocum*, were used for studies of plant-to-plant transmission under hydroponic conditions. Roots of infected plants were thoroughly rinsed in running tap water. Infected plants were then placed in tubs containing healthy 6-wk-old spinach plants and incubated at 23 C. Disease development was monitored over a 7-day period during which 200- to 500-ml samples of the nutrient solution were

periodically collected from each tub and assayed for the presence of zoospores. The samples were passed through 2- μ m pore diameter Millipore filters which were then inverted on a selective medium (4) in petri dishes. Origins of the developing colonies from either zoospores or other debris were identified after 48 hr of incubation at 25 C.

Studies with metalaxyl. The effects of metalaxyl (Ridomil 2E; Ciba-Geigy Corporation) on zoospore production, root penetration, and control of root rot of spinach by *P. aphanidermatum* and *P. dissotocum* were investigated. Zoospore production in sterile distilled water containing metalaxyl at 1, 2, 5, 10, 50, and 100 μ g a.i./ml was determined as described above. Similarly, the efficacy of metalaxyl on control of root rot was determined by using the respective cultural methods and procedures described above except that the plants were grown hydroponically in the nutrient solution containing 5 μ g a.i. of metalaxyl per milliliter. All experiments were repeated twice.

RESULTS

Mycelial growth and zoospore production. Both species grew equally well at temperatures between 10 and 25 C (Fig. 1). Growth of *P. aphanidermatum*, however, far exceeded that of *P. dissotocum* at temperatures above 25 C although *P. dissotocum* was capable of growth at these high temperatures.

Zoospore production in sterile distilled water varied considerably between the two species at the various temperatures (Fig. 2). However, between 17 and 27 C (the range in temperatures of the nutrient solution used in commercial production of spinach) (1), zoospore production was similar. Zoospore production in sterile nutrient solution was less than in sterile distilled water but, again, similar numbers of zoospores were produced by both species between 17 and 27 C.

Pathogenicity. *P. dissotocum* caused significant yield reductions at all temperatures (Table 1). Stunting and root rot occurred 7 days after inoculation at all temperatures except 17 C where no root rot was observed. However, roots at 17 C were visibly discolored and root elongation appeared to be impaired. Significant, but more dramatic, yield reductions were recorded for *P. aphanidermatum* at incubation temperatures of 21 and 27 C but not at 17 C (Table 1). Severe stunting and/or plant death occurred within 3–4 days after inoculation at 21 and 27 C, respectively. In plants inoculated with both organisms, symptom development and disease severity resembled those caused by the more virulent pathogen at each temperature. Isolations from roots of plants inoculated with individual species consistently yielded only the species used as inoculum. Both species were reisolated from infected root tissue inoculated with both species except at 17 C at which *P. aphanidermatum* was not consistently reisolated.

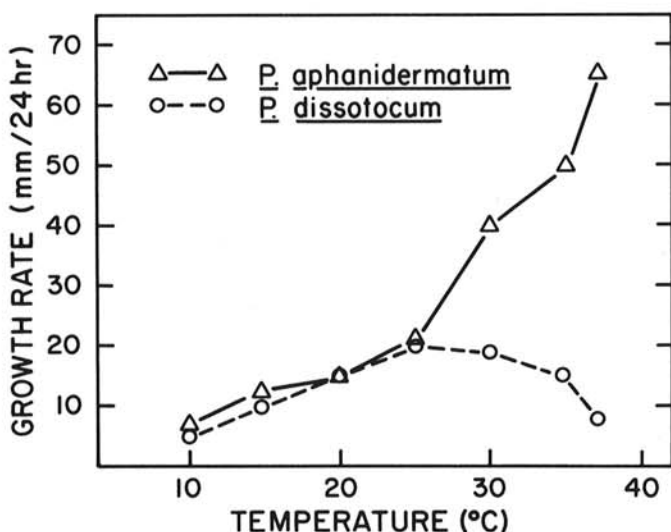


Fig. 1. Vegetative growth of *Pythium aphanidermatum* and *P. dissotocum* on V-8 juice agar at various temperatures. Data presented are the means of three repeated experiments.

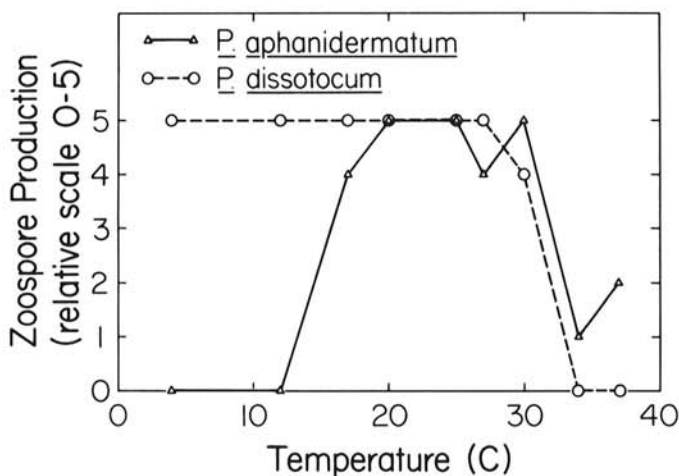


Fig. 2. Zoospore production by *Pythium aphanidermatum* and *P. dissotocum* in sterile distilled water at various temperatures.

TABLE 1. Yield of spinach cultured hydroponically at several temperatures in the presence and absence of *Pythium aphanidermatum* and *P. dissotocum*

Treatment	Average tissue weights (g) at the indicated temperatures ^x					
	Shoots			Roots		
	17 C	21 C	27 C	17 C	21 C	27 C
Control	33.3 a ^y	88.5 a	50.3 a	0.52 a	1.60 a	1.04 a
<i>P. aphanidermatum</i>	28.3 ab	11.1 d	0.00 ^z	0.50 a	0.29 c	0.00 ^z
<i>P. dissotocum</i>	17.4 c	66.9 b	13.3 b	0.36 ab	1.10 b	0.18 b
<i>P. aphanidermatum</i> and <i>P. dissotocum</i>	15.6 c	28.0 c	0.00 ^z	0.27 b	0.60 c	0.00 ^z

^x Average fresh weight of eight or 10 shoots and dry weight of roots after 3 wk of growth in hydroponic culture.

^y Data presented are from individual experiments with replications combined. Means followed by the same letter within a column are not significantly different ($P=0.05$) according to Duncan's multiple range test.

^z All plants died within 3–4 days after inoculation.

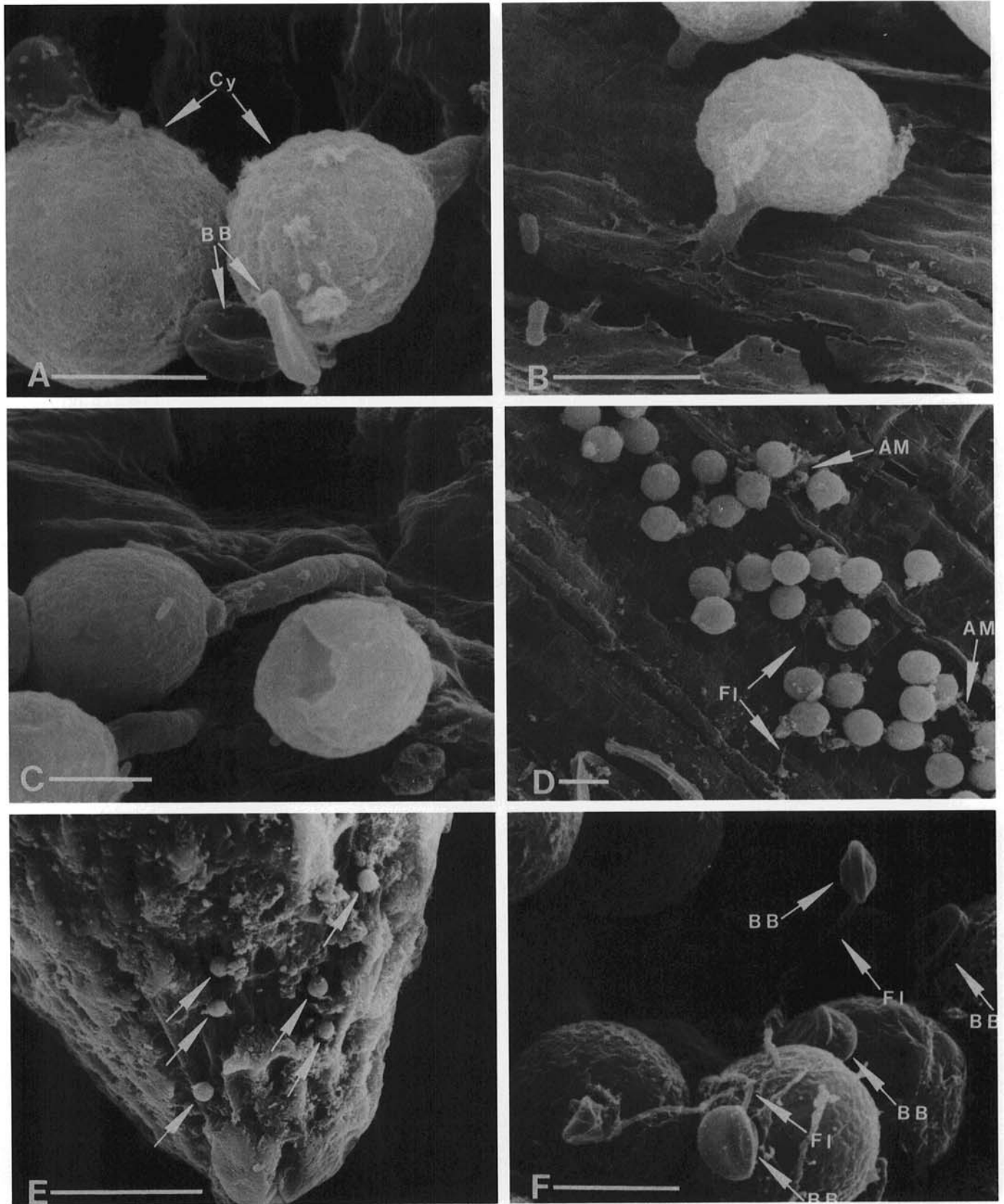


Fig. 3. Scanning electron micrographs showing the penetration process of spinach roots by *Pythium aphanidermatum* (P.a.) and *P. dissotocum* (P.d.). **A**, Direct penetration by P.a., beadlike bodies (BB) still adhering to the cyst wall (Cy), 15 min after inoculation. Bar = 5 μ m. **B**, Direct penetration by P.d., 15 min after inoculation. Bar = 5 μ m. **C**, Direct penetration of roots of plants grown in a solution containing 5 μ g of metalaxyl per milliliter, 30 min after inoculation. Bar = 5 μ m. **D**, Accumulation of P.a. zoospore cysts on unwounded roots, note flagella (Fl) with distal beadlike bodies and adhesive material (AM), 15 min after inoculation. Bar = 10 μ m. **E**, Encysted zoospores of P.a. present on root cap (arrows), 15 min after inoculation. Bar = 50 μ m. **F**, Cysts of P.a. showing flagella (Fl) apparently winding up in the beadlike bodies (BB). Bar = 5 μ m.

Plant-to-plant transmissions. Root rot of healthy spinach plants occurred within 7 days after plants infected with either *P. aphanidermatum* or *P. dissotocum* were placed in the hydroponic tubs containing healthy plants. Zoospores and unidentified residue were identified as the origin of colonies developing on a selective medium subsequent to periodic plating of the nutrient solution during the 7-day incubation period.

Root penetration. Hyphae from zoospores of both *P. aphanidermatum* and *P. dissotocum* penetrated spinach roots within 15 min after inoculation at 23 C (Fig. 3). Penetration was accomplished by approximately 8 and 26% of the accumulated zoospore cysts of *P. aphanidermatum* and *P. dissotocum*, respectively, after 15 min, and by 64 and 68% after 30 min. Zoospores of both species were predominantly attracted to the region of root elongation but were also observed on root hairs and root caps. Penetration occurred without the formation of appressoria. A very small portion of the cysts (less than 1%) produced penetration hyphae with slightly swollen bases where they contacted the roots. Numerous cysts of both species produced what Colt (5) referred to as an adhesive.

Other events observed prior to or during penetration included retraction and loss of flagella. The stages of flagellum retraction included flagella with a distal beadlike body (Fig. 3D) and flagella which appeared to be rolling-up and forming beadlike bodies (Fig. 3F). One or two beadlike bodies were often observed attached to cyst walls (Fig. 3A). Beadlike bodies were apparently lost either before (Fig. 3B) or after (Fig. 3A), root penetration.

Metalaxyl studies. Zoospore production by both species in sterile distilled water always received a rating of 5. The production of zoospores by *P. aphanidermatum* never exceeded a rating of 1 in the presence of metalaxyl at any of the tested concentrations, and no zoospores were produced at metalaxyl concentrations above 10 µg a.i./ml. *P. dissotocum*, however, produced zoospores with ratings of 1-3 at 1 and 2 µg a.i. metalaxyl per milliliter and often received a rating of 1 at 5 and 10 µg a.i. metalaxyl per milliliter. Although metalaxyl was inhibitory to zoospore production at all concentrations above 10 µg/ml, both species were reisolated after the 72-hr incubation period from the plugs incubated in all concentrations tested.

At 5 µg a.i. of metalaxyl per milliliter of the nutrient solution, the root rots caused in spinach by both *P. aphanidermatum* and *P. dissotocum* were completely controlled. Mean shoot weight was 1.5, 50.3, 58.1, and 59.5 g for the inoculated control, uninoculated control, inoculated metalaxyl-treated plants, and uninoculated metalaxyl-treated plants, respectively. Although control of root rot was achieved, penetration of metalaxyl-treated plants did occur and *P. dissotocum*, but not *P. aphanidermatum*, was reisolated from treated plants.

DISCUSSION

The objective of this study was to determine the role of temperature on the cyclic occurrence of *P. aphanidermatum* and *P. dissotocum* as causal agents of root rot of hydroponically grown spinach. Temperatures, within the range found to occur naturally in the commercial hydroponic system (ie, 17-27 C) were not limiting to mycelial growth or zoospore production of either species. Additionally, scanning electron microscopy showed that both species were able to penetrate spinach roots within 15 min after inoculation at 23 C, the apparent transitional temperature (1) above or below which *P. aphanidermatum* and *P. dissotocum* predominated, respectively.

Our results indicated that the cyclic occurrence of these two species was probably due to differential effects of temperature on the pathogenicity of the two species. Differences in pathogenicity at specific temperatures would impart a temporary competitive advantage and result in an increase in the population and predominance of the more aggressive species. Our studies showed that *P. dissotocum* was more aggressive than *P. aphanidermatum* at 17 C. Thus, *P. dissotocum*, by virtue of its greater pathogenicity at low temperatures, predominated as the causal agent of root rot during the winter production months. This predominance was

maintained until temperature increases became more favorable to *P. aphanidermatum* which then became the dominant species associated with root rot of spinach.

Regarding the nature of the infective propagules of *P. aphanidermatum* and *P. dissotocum*, our results showed that zoospores produced from the roots of diseased plants served efficiently as inoculum in pathogen transmission to healthy plants. Although the role of zoospores as infective propagules in soil has been questioned (10,14), recirculating hydroponic systems provide an ideal environment for the production, unobstructed movement, and rapid dissemination of zoospores. Zoospore attraction, accumulation, encystment, germination, and host penetration occurred within 15 min after inoculation of spinach roots with a zoospore suspension of both *P. aphanidermatum* and *P. dissotocum*. The latter time interval is considerably shorter than those reported previously which ranged from 35 min to 2 hr (5,7,13). In addition, penetration apparently occurred by enzymatic rather than mechanical means since no appressoria were observed. Formation of appressoria, however, is apparently a variable phenomenon (7).

Our observations on the retraction of and formation of beadlike bodies on flagella during zoospore encystment of *Pythium* species have been reported previously (7,11). However, it is not known if the microtubules of the flagella are shed with the beadlike bodies. These beadlike bodies often appeared collapsed, suggesting that they may be empty. If so, the flagellar microtubules may have been reabsorbed by the cyst, which would be a nutritional advantage to the organism. The flagella of primary zoospores in the Saprolegniales are reabsorbed while those of the secondary spore are shed (15). Hemmes and Hohl (9), however, found no evidence of reabsorption of flagellae in *Phytophthora*.

Confirming a previous study (1), metalaxyl, at a concentration of 5 µg a.i./ml in the nutrient solution, was effective in the control of root rot caused in spinach by *P. aphanidermatum* and *P. dissotocum*. Although disease control was achieved, both species were observed to penetrate roots of treated plants and *P. dissotocum* could be reisolated from these roots. These results, coupled with results of our in vitro studies which showed that the chemical is fungistatic, indicate the possibility of the development of tolerant strains of the fungus. Caution should be exercised in the use of metalaxyl, particularly in recirculating hydroponic systems in which a tolerant strain of a pathogen would be rapidly disseminated. The occurrence of metalaxyl-tolerant strains of *Pythium* species and other oomycetes has been reported (2,3,8,12).

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