Root Rot of Hydroponically Grown Cucumbers Caused by Zoospore-Producing Isolates of *Pythium intermedium*

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ABSTRACT

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Pythium intermedium is reported for the first time as a root pathogen of hydroponically grown cucumbers. Catenulate and deciduous sporangia were capable of direct (formation of germ tubes) and indirect (formation of zoospores) germination.

In 1985, mature cucumber (*Cucumis sativus* L.) plants showing root rot were obtained from a commercial hydroponic facility in England. Isolations from necrotic roots consistently yielded pure cultures of *Pythium intermedium* de Bary (5). This fungus is the only species of *Pythium* that produces catenulate and deciduous hyphal swellings. During the course of our investigations, zoospores were observed in aqueous suspensions of hyphal swellings prepared for pathogenicity tests. According to Van der Plaats-Niterink (5) and Watanabe (6), sporangia and zoospores are not produced by this species, although zoospores were reported in 1943 by Middleton (1). In a more recent study of *Pythium* spp. associated with necrotic feeder roots of apple, Sewell (3) noted that isolates of *P. intermedium* formed zoospores in vesicles with no apparent evacuation tubes.

Pathogenicity of *P. intermedium* on cucumbers and the documentation of zoospore production by this species are presented in this paper.

MATERIALS AND METHODS

Stock cultures of *P. intermedium* obtained from diseased cucumber roots were maintained at 20 C on 10% V-8 juice agar (VJA) medium.

Pathogenicity tests. Pathogenicity tests were conducted in a greenhouse under hydroponic conditions as previously described (4). Three 1-wk-old cucumber seedlings, started in a nursery in peat pellets, were transferred into holes cut into each of four Styrofoam flotation boards. The boards were then placed in 13.5-L plastic tubs containing a continuously aerated nutrient solution. Tubs were located in a temperature-controlled box, and the nutrient solution was adjusted to 20 C before transplanting. One week after transplanting, 20 ml of a suspension of hyphal swellings of *P. intermedium* (containing approximately 1,000 hyphal swellings per milliliter) was added to two tubs. The suspension of hyphal swellings was obtained by syringing the surface of a 4-day-old VJA culture of the fungus with 15 ml of sterile distilled water (SDW). Dislodged hyphal swellings were decanted into a sterile beaker and diluted with SDW; numbers of swellings were estimated with a hemacytometer. Plants in noninfested tubs served as controls. After 2 wk of growth in the tubs, fresh weights and heights of shoots and fresh weights of roots were recorded for each treatment.

Microscopic observation. Suspensions of hyphal swellings, obtained as described above, were incubated at 24 C in sterile plastic petri dishes containing SDW or solidified VJA agar and periodically observed with a light microscope. Specimens for scanning electron microscopic observation were prepared as follows: Hyphal swellings were collected on a Millipore filter (0.45-μm pore diameter) after various incubation times. Small sections of filter were mounted on a specimen holder and plunged into liquid nitrogen. The samples were examined in a scanning electron microscope (Cambridge Instrument Ltd. S.200) fitted with a cryotransfer system (Hexland Ltd. CT 1000) (2). No chemical fixatives or solvents were used. All specimens were sputter-coated with gold.

RESULTS AND DISCUSSION

P. intermedium caused a significant reduction in shoot height and fresh shoot weight (Table 1). No significant differences were recorded in fresh root weights between inoculated and noninoculated treatments. However, roots of inoculated plants showed a slight brownish coloration, whereas those of noninoculated plants did not. The fungus was consistently recovered only from roots of inoculated plants. Our results constitute the first report of the occurrence of this fungus as a root pathogen of hydroponically grown cucumber plants. The extent of damage caused by this fungus in the commercial greenhouse in England was not determined. However, our results confirm a report by Watanabe (6) in Japan regarding the susceptibility of cucumber seedlings to *P. intermedium*. He showed in greenhouse pathogenicity trials that soil isolates of the fungus caused preemergence damping-off and stunting of plants.

Laboratory studies showed that hyphal swellings, hereafter called sporangia, of *P. intermedium* were capable of bimodal germination. In the

Table 1. Effects of *Pythium intermedium* on shoot heights and fresh shoot weights of cucumber plants cultured hydroponically[a]

Treatment	Plant height (cm)	Fresh shoot weight (g)
Control	39.0	40.3
Inoculated	26.1*[b]	24.6*

[a] Data collected 14 days after inoculation.
[b] There were three plants per treatment, and each treatment was replicated once. Data were analyzed by analysis of variance. Numbers in the same column followed by an asterisk are significantly different ($P = 0.01$) from the control.

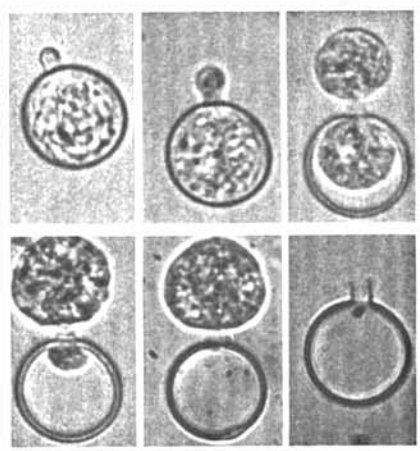

Fig. 1. Sequential stages in the indirect germination of sporangia of *Pythium intermedium*. Sporangium diameter = 15 μm.

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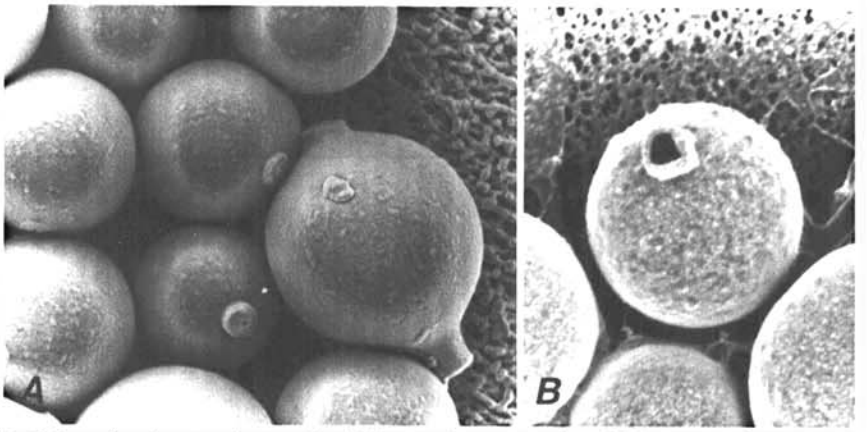

Fig. 2. Scanning electron micrographs of **(A)** catenulate sporangia and **(B)** an evacuation tube on a sporangium of *Pythium intermedium*. Sporangium diameter = 15 μm.

presence of a nutrient source, 98% of the sporangia produced a germ tube. In SDW in the absence of a nutrient source, 10–20% of the sporangia produced zoospores. The remaining population did not germinate. Figure 1 shows sequential stages of evacuation tube, vesicle formation, zoospore cleavage, and zoospore release. Evacuation tubes, measured after zoospore release, ranged from 3 to 5 μm in length. Formation of evacuation tubes occurred approximately 1.5 hr after incubation in SDW, and zoospore release occurred between 30 and 60 min later. The number of zoospores per vesicle ranged from eight to 10, depending on the size of the sporangium. Sporangia ranged from 13 to 23 μm in diameter. Figure 2 shows scanning electron micrographs of the basal plugs of catenulate sporangia and an evacuation tube on a sporangium. The low percentage of sporangia that germinated indirectly could not be increased despite numerous attempts. We noted, however, that zoospore production was related to culture age. Sporangia from cultures older than 10 days germinated with germ tubes only. These results may explain the apparent discrepancy regarding the zoospore-producing capabilities of this fungus.

The production of deciduous sporangia, coupled with the capacity to produce zoospores, makes *P. intermedium* especially suited for rapid dispersal in recirculating hydroponics.

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