

Filtration as a Method for Controlling *Pythium* Root Rot of Hydroponically Grown Cucumbers

N. P. GOLDBERG, Research Specialist, M. E. STANGHELLINI, Professor, and S. L. RASMUSSEN, Senior Research Specialist, Department of Plant Pathology, University of Arizona, Tucson 85721

ABSTRACT

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To test the efficacy of filtration of zoospore-infested water for the control of *Pythium* root rot of cucumber (*Cucumis sativus*) growing in recirculating hydroponic greenhouse culture, cucumber seedlings were transplanted into separate hydroponic tanks. Each tank received water from a zoospore-infested source tank. The infested water was recirculated three times through a 20- μ m filter or through the 20- μ m filter and then a 7- μ m filter. Within 24 hr after the first recirculation cycle, 67% of the plants in the tank receiving infested water passed through only the 20- μ m filter were infected; within 3 days, all plants were infected. None of the plants in the tank receiving water passed through the 20- μ m and 7- μ m filters were infected until 1 day after the third and final recirculation cycle. The fungus was recovered from the surface (0 mm) and middle (8 mm deep) of the 7- μ m filter but not from the inner core (16 mm deep). Thus, the 7- μ m filter effectively removed the fungus from infested water. Although plants in the tank receiving water passed through the 7- μ m filter eventually became infected, shore flies (*Scatella stagnalis*) were suspected as introducing the pathogen into this tank.

One of the most common pathogens causing root disease in hydroponically grown vegetables is *Pythium aphanidermatum* (Edson) Fitzp. (1,7). Once introduced into a recirculating hydroponic system, the pathogen is rapidly and uniformly dispersed via motile or encysted zoospores, and all plants within the system become infected. Devastating crop losses have been reported for tomatoes (4,6,7), spinach (1), and cucumbers (4,6). Metalaxyl was shown experimentally to be effective in controlling *Pythium* species in hydroponics (1) but is not registered for use in this type of cultural system. Ultraviolet irradiation kills zoospores in nutrient solutions but was not effective when a flow rate similar to that used in commercial facilities was studied

(8). Filtration of the nutrient solution to physically remove motile and encysted zoospores (10–12 μ m in diameter) from the system has potential as a method of control. However, experimental evidence

of the effectiveness of this method is lacking. The objective of this research was to investigate the potential use of filtration as a strategy for control of *P. aphanidermatum* in recirculating hydroponic systems.

MATERIALS AND METHODS

Three separate aboveground hydroponic cultural tanks, each 2.4 \times 1.2 \times 0.5 m, were used (Fig. 1). Each tank contained 850 L of a continuously aerated (2,400 cm³/min) nutrient solution. The nutrient solution in each tank contained 972 g of fertilizer (Hydrosol), 200 g of magnesium sulfate, 544 g of potassium nitrate, and 875 g of calcium nitrate. The temperature of the nutrient solution was maintained at 30 C \pm 1 C by submersible water bed heaters, one heater per tank, in order to provide an optimum temperature for the fungus and for disease development (1).

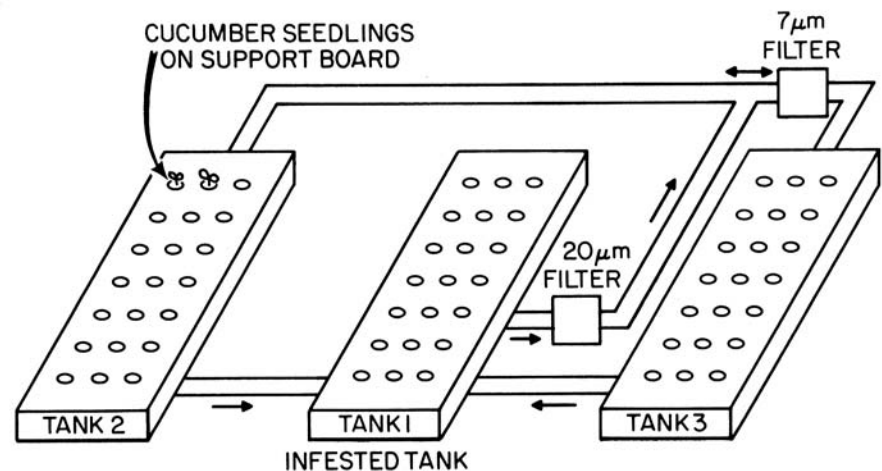


Fig. 1. Recirculating hydroponic system for studying the efficacy of filtration in controlling root rot of cucumber caused by *Pythium aphanidermatum*.

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Two-day-old cucumber (*Cucumis sativus* L. 'Toska 70') seedlings, germinated aseptically on 2% water agar in petri dishes, were transplanted in Oasis Horticultubes (Smithers-Oasis, Kent, OH). The seeded cubes were placed in holes (37 mm in diameter) cut in Styrofoam flotation boards (2.3 × 1.0 × 0.03 m), each board holding 21 seedlings spaced 30 cm apart (1). One board was floated on top of the nutrient solution in each tank, and the seedlings were allowed to grow for 25 days. Then two plants were removed from tank 1 and their roots were placed in a suspension of *P. aphanidermatum* zoospores. Zoospores were produced from two 15-mm-diameter culture plugs from a 4-day-old V8 agar culture. The plugs were placed in a sterile petri dish and flooded with 20 ml of aerated, sterile, deionized water. The dish was incubated overnight at room temperature (25 C) (2). Microscopic observation showed that the zoospores were attracted to and encysted on roots within 1 min and that root penetration occurred shortly after zoospore encystment, confirming findings of previous studies (2,5). After 1 hr, these two plants were returned to tank 1 and served as the source of inoculum for infestation of plants in this tank. In a repeat of this experiment, the source plants were 7 days old at the time of inoculation.

Five days after infestation of tank 1, 1 L of nutrient solution was collected from the center of the tank. The nutrient solution was passed through Millipore filters (0.45 μm), and the filters were

inverted onto water agar. Colonies of *Pythium* growing on these plates originated from propagules approximately 10 μm in diameter, indicating that zoospores or zoospore cysts were the source. No attempt was made to quantify the population of zoospores in tank 1. The infested nutrient solution was recirculated of tank 1 at a flow rate of 114 L/min for 30 min (Fig. 1). Infested nutrient solution in tank 1 was first passed through a 20-μm-cartridge filter (model CFT25 with C5625 filter, Jacuzzi, Little Rock, AR). Then about 57 L/min was diverted into tank 2 and returned to tank 1, and about 57 L/min was diverted through a 7-μm-cartridge filter (model PCY1RFG16 with R1F070 filter, Pall Process Filtration Corporation, East Hills, NY) into tank 3 and also returned to tank 1 by a submersible pump.

A portion of the root system was excised from 12 plants in each tank 3 days after infestation of tank 1 and 1 day after each of the first two recirculation cycles. Root samples were collected from all plants in all tanks after the third and final recirculation. Ten root segments (2 cm long) per plant were blotted dry on paper towels, placed on 2% water agar amended with 200 μg/ml of streptomycin sulfate, and incubated at 37 C for 24–48 hr. Additionally, after the final recirculation cycle, the surface (0 mm), middle (8 mm deep), and inner core (16 mm deep) of the 7-μm filter were sampled for the presence of

the pathogen (Fig. 2). Twelve pieces of filter, each 15 mm², were incubated as described for roots. *Pythium* species isolated from the roots or filter were subcultured on V8 juice medium (2% agar, 10% V8 juice, 0.1% CaCO₃) and identified to species (9). The experiment was repeated.

RESULTS AND DISCUSSION

The results of these experiments are given in Table 1. In the first experiment, *P. aphanidermatum* was isolated from roots of all cucumber plants from tank 1 sampled 3 days after infestation, and all of the plants in tank 1 were dead within 6 days (Fig. 3A). None of the plants in tanks 2 and 3 were infected before the first recirculation cycle. One day after initiation of recirculation, the fungus was isolated from eight (67%) of the 12 plants from tank 2, which had received water passed through a 20-μm filter; all of the plants in tank 2 were infected after the second recirculation cycle and were dead within 6 days. After two consecutive recirculation cycles, the fungus was not isolated from any plants in tank 3, which had received water passed through 20-μm and 7-μm filters (Fig. 3B). One day after the final recirculation cycle, however, the fungus was isolated from one plant in tank 3, and within the next 3 days all plants were infected. In the second experiment, results were similar but onset of infection was later (Table 1), presumably because the seedlings that served as the source

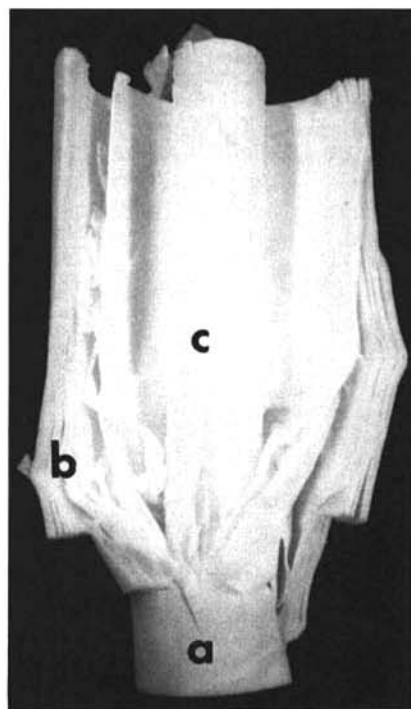


Fig. 2. Sampling depths on the 7-μm cartridge filter (approximately 26 × 6 cm) used to filter water infested with zoospores of *Pythium aphanidermatum*: a = 0 mm (surface), b = 8 mm (middle), c = 16 mm (inner core).

Table 1. Efficacy of filtration of infested water to control root rot of cucumber caused by *Pythium aphanidermatum* in a recirculating hydroponic system^a

Experiment	Percent of plants infected by <i>P. aphanidermatum</i>					
	Days after infestation of tank 1					
	3	7	9	11	14	16
First						
Tank 1	100	100	100	100	100	100
Tank 2	0	67	100	100	100	100
Tank 3	0	0	0	5	100	100
Second						
Tank 1	100	100	100	100	100	100
Tank 2	0	0	33	58	100	100
Tank 3	0	0	0	0	5	100

^aSix, 8, and 10 days after infestation of tank 1, infested water was recirculated through a 20-μm filter to tank 2 and through the 20-μm filter and then a 7-μm filter to tank 3.

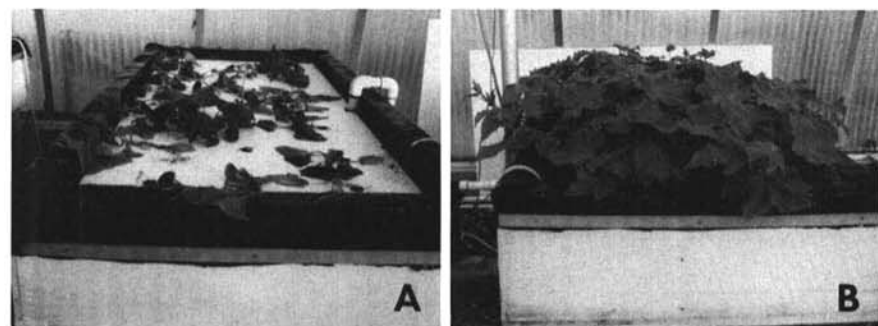


Fig. 3. *Pythium* root rot of cucumbers 5 days after infested water was recirculated from tank 1 to (A) tank 2 through a 20-μm filter and (B) tank 3 through the 20-μm filter and then a 7-μm filter.

of inoculum in the second experiment were younger with smaller root systems than those used in the first experiment and therefore produced less inoculum.

P. aphanidermatum was recovered from the surface and the middle, but not from the inner core, of the 7- μ m filter. Thus, the filter was apparently effective in removing the zoospores from the nutrient solution. Because the plants in tank 3 eventually became infected, however, other means of pathogen introduction and spread into the system were investigated. Shore flies (*Scatella stagnalis* Fallen) are effective aerial vectors of *P. aphanidermatum* (3), and we determined that this insect was the probable reason for fungal contamination of tank 3. In an attempt to eliminate the flies in the second experiment, a clear plastic tarp was draped over the entire tank. Holes in the tarp allowed the seedlings to emerge, and the base of each seedling was painted with grease. Despite these precautions, the fungus entered the system. Although not documented as the source of contamination in the second experiment, the flies were presumed to

be the source because *P. aphanidermatum* was not recovered from the inside of the 7- μ m filter and because the insects could not be totally excluded.

Greenhouse hydroponic systems that utilize a reservoir for recirculating nutrient solution to and from growing chambers are common in commercial vegetable production. Pathogens entering the system at any location are disseminated rapidly to all plants. Fortunately, reports of serious disease problems in the industry are not common. However, devastating crop losses caused by *P. aphanidermatum* have occasionally been reported (1,4,6,7), and because effective control measures are lacking, cultivation of the susceptible crop has sometimes been abandoned (1). Results of this study indicate that a 7- μ m filter effectively removes zoospores of *P. aphanidermatum* from nutrient solution.

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