Transmission and Control of *Pythium aphanidermatum* in an Ebb-and-Flow Subirrigation System

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ABSTRACT

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Pythium aphanidermatum was consistently transmitted to healthy potted cucumber (*Cucumis sativus* 'County Fair') plants grown in an ebb-and-flow subirrigation system when the irrigation solution was artificially infested. In contrast, transfer of the fungus was low and sporadic when some plants growing in infested medium were placed in the system among plants growing in noninfested medium. Metalaxyl was more effective than etridiazole in controlling *Pythium* infection when granular formulations were incorporated into the growing medium.

Runoff and drainage of greenhouse irrigation water containing fertilizers and pesticides have been targeted as potential sources of contamination of both surface water and groundwater. Greenhouse irrigation systems in which water is recycled are promising technologies for reducing runoff to minimize the effect on surface water and groundwater quality. However, the possible spread of waterborne, root-infecting pathogens, particularly pythiaceous fungi, in such systems poses a significant threat to crops irrigated with recycled water (1,4,8,12,20,24).

Pythium spp. are a major concern in the greenhouse subirrigation system known as "ebb and flow" or "flood and drain." This system, which recirculates water or fertilizer solution, is widely used in many countries in Europe and is being more widely adopted in North America. Potted plants are placed on specially designed benches or concrete floors, which are periodically flooded with water or fertilizer solution. The water enters the potting medium by capillary action, and the roots are thus moistened. After a specified time, the nutrient solution is drained away and stored in a tank, from which it is recycled to the benches or concrete floors when moisture is again needed.

Although Thinggaard and Middelboe (22) reported the occurrence of several *Pythium* spp., little qualitative or quantitative information has been published (10) on the potential of these fungal pathogens to spread and cause crop losses in the ebb-and-flow subirrigation system. In a preliminary study (18), we found that *P. aphanidermatum* (Edson) Fitzp. and *P. ultimum* Trow can be transported via recirculated nutrient solutions to greenhouse-grown cucumber (*Cucumis sativus* L.) and geranium (*Pelargonium* × *hortorum* L. H. Bailey). In the present study, our objectives were to investigate further the transmission of *P. aphanidermatum* in an ebb-and-flow subirrigation system and to evaluate the effectiveness of soil fungicides in controlling infection incited by *P. aphanidermatum* in such a system.

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MATERIALS AND METHODS

Ebb-and-flow subirrigation system. The ebb-and-flow subirrigation system consisted of wooden trays (2 × 0.30 × 0.1 m) with a drain hole through which water flowed away into 30-L reservoirs. The inside of each tray was lined with transparent polyethylene sheeting to prevent leakage during flooding. At watering, a silicone rubber plug was inserted into the drain hole, and a submersible electric pump (Little Giant Pump Co., Oklahoma City, OK), placed on the bottom of each reservoir and connected to the trays by a rubber tube, pumped nutrient solution into the trays to a depth of 2 cm. At the end of each flooding period, the silicone rubber plugs were removed, and the trays were tilted slightly so that all liquid drained away from the pots.

The reservoirs contained a nutrient solution of 300-ppm nitrogen prepared from a commercial, water-soluble 15% N-16% P_2O_5-17% K_2O fertilizer (Peters Fertilizer Products, Fogelsville, PA). The electrical conductivity of the fertilizer solution was adjusted weekly throughout the experiments by addition of water or fertilizer to maintain the initial level of 2.0 mmho·cm^{-1}.

Plant production. Seeds of cucumber cultivar County Fair were sown in soilless medium (Metro Mix 350; Grace Horticultural Products, W. R. Grace & Co., Cambridge, MA) in plastic trays in a walk-in chamber (20–23 C) under 40-W Grolux and cool-white fluorescent light with average irradiance of 4.5 erg·sec^{-1}·cm^{-2}. Ten-day-old seedlings were transplanted into 9-cm round plastic pots containing Metro Mix 350 and were used in the transmission tests 4 days after transplantation.

Inoculum production. *P. aphanidermatum* (U3, obtained from Patricia Sanders, Department of Plant Pathology, The Pennsylvania State University) was grown on water agar in petri dishes (9 cm in diameter) at 30 C. In an initial pathogenicity study, this isolate caused damping-off and stem base necrosis on County Fair cucumber.

The general method of inoculum production was similar to that used in other studies (5,11,14). Specifically, 50 ml of sterile V8 broth (200 ml of V8 juice, 3 g of $CaCO_3$, 800 ml of distilled water) was dispensed into 125-ml flasks and infested with two mycelial disks (5 mm in diameter) taken from a 5- to 7-day-old culture of the fungal isolate. The flasks were capped with aluminum foil and incubated at 22 C in the dark on an orbital shaker (100 rpm). Two-week-old cultures were removed from the flasks and vacuum-filtered in a Buchner funnel (10 cm) to remove the broth. The harvested culture mats, containing mycelium and oospores, were homogenized with distilled water in a blender at low speed for 15 sec, and the resulting slurry was used as inoculum.

Transmission of *P. aphanidermatum*. To determine whether *P. aphanidermatum* could become established in the ebb-and-flow system, we designed two treatments in addition to a control. In the first treatment (infested water), the nutrient solution held in the system's reservoirs was infested by the addition of 2.3 g (fresh weight) of fungal culture mat homogenized with 8.5 ml of distilled water (inoculum slurry) per liter of nutrient solution. In the second treatment (infested pot), 4.25 ml of inoculum slurry was buried 3–4 cm deep in the growing medium around the root systems of plants in two pots. Three hours after inoculum was applied, the pots were blotted dry and placed in the center of trays containing noninfested pots. In the control treatment, no inoculum was added to the reservoirs or the pots. The inoculum level used was determined in a preliminary study.

Three tests were conducted during 1990 in a greenhouse. The average daily maximum and minimum temperatures were 32.7 and 16.5 C, respectively, during

test 1 (8–28 June), 29.2 and 16.7 C during test 2 (7–28 July), and 28.6 and 16.8 C during test 3 (6–30 August). There were 44 plants per treatment in test 1 and 40 plants per treatment in tests 2 and 3. The plants were contained in four replicate trays arranged in a randomized complete block design. Plants were watered every other day for the first 10–14 days of each test and daily thereafter. Flooding episodes lasted 10 min. Inoculation was performed and irrigation with fertilizer solution began on the starting date of each test.

Soil fungicide application. Two granular soil fungicides, metalaxyl (Subdue 2G, Ciba-Geigy) at 2% a.i. and etridiazole (Truban 5G, Mallinckrodt Inc., St. Louis, MO) at 5% a.i., were incorporated separately into soilless medium (Metro Mix 350) at the label rates of 1.2 and 185.5 mg a.i./L, respectively, 4 hr before seedlings were transplanted. In these tests, *P. aphanidermatum* was introduced into the ebb-and-flow system by infestation of the irrigation solution only.

The design of the experiment was a split plot with a randomized complete block arrangement of the whole-plots factor. Two levels of the pathogen (infested and noninfested) were assigned to the whole plots, and the three fungicide treatments (none, metalaxyl, or etridiazole) were assigned to the subplots. For each of the six combinations of pathogen level and fungicide treatment, 13 potted plants were placed on each of three replicate trays.

Two tests were conducted during 1990 in a greenhouse. The average daily maximum and minimum air temperatures were 26 and 17 C, respectively, during test 1 (26 September–20 October) and 25.6 and 16 C during test 2 (5–30 November). As in the transmission tests, plants were watered every other day for the first 10–14 days of each test and daily thereafter, flooding lasted 10 min, and inoculation was performed and irrigation with fertilizer solution began on the starting date of each test.

Assessment of disease incidence and fungal viability. Throughout each test, plants were observed for the appearance of stunting, damping-off, and stem base necrosis. At the end of each test, the number of plants with each symptom (except stunting) was recorded. Plant height was measured when stunting was either the most obvious symptom or the only symptom.

Roots were collected from each plant in each treatment and thoroughly washed in running tap water. Four or five root segments were taken from each plant and plated on a *Pythium*-selective medium (TP: 200 ml of cornmeal agar, 0.04 g of pimaricin, 0.01 g of penicillin, and 0.01 g of polymyxin) (3).

To monitor the viability of *P. aphanidermatum* in the subirrigation system and to determine whether and when the fungus became established in the nutrient solution, we assayed the recycled liquid weekly by passing 240 ml of the solution through 0.8-μm Millipore filters, which were then inverted on the TP medium. An additional filter assay was performed at the end of each test.

Root pieces and filters were incubated at 30 C in the dark for 2 days. *Pythium* was identified according to Van der Plaats-Niterink's key (23).

Statistical analysis. Plant height was subjected to an analysis of variance. Where necessary, data relevant to percentages were transformed by an arcsine-square root transformation before analysis of variance. Treatment means were compared using Fisher's protected least significant difference or Student's *t* test procedures (19).

RESULTS

Transmission of *Pythium aphanidermatum*. In test 1, *P. aphanidermatum* was transmitted to more plants in the infested water treatment than in the infested pot treatment (Table 1). No stem base necrosis or damping-off was observed in the infested pot treatment, whereas all of the plants in the infested water treatment showed stem base necrosis and 12% damped off. Plant height was reduced about 60% in the infested water treatment compared to the control, and the fungus was recovered from all plants and reservoirs. Plants in the infested pot treatment did not differ significantly in height from control plants; however, the fungus was isolated from 18% of the plants in the infested pot treatment but not from the nutrient solution used to irrigate those plants.

In test 2, plants in the three treatments did not differ significantly in height (Table 1). In the root assays, *P. aphanidermatum* was isolated from 80% of the plants in the infested water treatment, compared to only 3% in the infested pot treatment. Nonetheless, only 35% of plants in the infested water treatment showed an observable symptom (stem base necrosis), and all plants in the infested pot treatment were symptomless. No damping-off was observed in any of the treatments. The fungus was recovered from all reservoirs in the infested water treatment but not in the control or infested pot treatments.

In test 3, stunting was the most obvious symptom (Table 1), and plants in the infested water treatment were significantly shorter than those in the control and infested pot treatments. No stem base necrosis or damping-off was observed in any treatment. The fungus was isolated from 78% of the plants and from all reservoirs in the infested water treatment but was not recovered from any plants or reservoirs in the other treatments.

Effect of soil fungicide application on infection. In both soil fungicide tests, the pathogen significantly affected plant height (Table 2). Plants in infested treatments were significantly shorter than those in noninfested treatments. A significant pathogen × fungicide interaction ($P = 0.0001$ in test 1, 0.0012 in test 2) was detected, indicating that the effect of the fungicides was different between the levels of infestation. In test 1, this interaction effect was accounted for by the significant difference in height of plants in the infested-no fungicide and noninfested-no fungicide treatments on the one hand and in the infested-etridiazole and noninfested-etridiazole treatments on the other hand. In test 2, however, the pathogen × fungicide interaction was attributable only to the difference in height of plants in the infested-etridiazole and noninfested-etridiazole treatments.

In test 1, 62% of plants in the infested-no fungicide treatment showed stem base necrosis, compared to 39% in the infested-etridiazole treatment. Plant mortality was 11% and 3% in the infested-no fungicide and infested-etridiazole treatments, respectively. No stem base necrosis or damping-off was recorded in the infested-metalaxyl treatment. The fungus was never recovered from roots of plants in the noninfested treatments. However, fungus isolation differed significantly among the infested treatments. Ninety and 85% of plants in the infested-no fungicide and infested-etridiazole treatments, respectively, had the fungus in their roots, as opposed to only 5% in the infested-metalaxyl treatment.

In test 2, visible symptoms expressed on plants were similar to those in test 1. In the infested-etridiazole treatment, the proportion of stem base necrosis was 46%, compared to 28% in the infested-no fungicide treatment. Thirteen percent of plants in the infested-etridiazole treatment died; no mortality was recorded in the infested-no fungicide and infested-metalaxyl treatments. The fungus was not recovered from roots of any plants in the noninfested treatments or from roots of plants in the infested-metalaxyl treatment but was isolated from 80% of plants in the infested-etridiazole treatment and 39% of plants in the infested-no fungicide treatment. In both tests, the pathogen was recovered from all reservoirs containing infested irrigation water and was never isolated from reservoirs containing noninfested water.

DISCUSSION

Potential avenues of introduction of *Pythium* spp. in greenhouse production systems include infested irrigation water (2,6,17), infested potting media (5,13), and insects (9). These fungal pathogens have been reported in hydroponic systems, which were originally thought to be the answer to eliminating root diseases caused by soilborne pathogens (25). Because the basic principle of the ebb-and-

flow system is similar to that of recirculating hydroponics, we hypothesized that *Pythium* spp. could spread and cause major damage on crops grown in the ebb-and-flow system. We chose cucumber because its high level of susceptibility makes it an excellent model plant for examining the worst possible case of *Pythium* transmission in potted plants grown in the ebb-and-flow system.

Under the conditions of this study, *P. aphanidermatum* was consistently transmitted to more plants when inoculum was present in the irrigation solution than when it was added to the growing medium of a limited number of plants. The decrease in disease incidence noted in tests 2 and 3 compared to test 1 could have been the result of the cooler environment during these tests. In most cases, the fungus was recovered from the root systems even if there were no visible symptoms on the aboveground or belowground parts of the plants. Stanghellini and Kronland (20) reported this type of subclinical infection in hydroponically grown lettuce infected by *P. dissotocum,* and Favrin et al (5) reported it in greenhouse-grown cucumbers infected by various species of *Pythium.*

Although transmission was not extensive when infested pots were intermixed with healthy potted plants, it is important to note that some transfer did occur. However, because the pathogen was not recovered from the recycled water in the infested pot treatment, the question arises as to how some plants became infected. It is possible that the pathogen was present in the water but at a very low level, and if so the amount of water we sampled (240 ml) may have been too small for detecting the fungus. We believe that the large amount of water used for flooding the trays increased the likelihood of some propagules coming into contact with potted plants. In the ebb-and-flow system we used, drainage was complete, few or no roots grew out of the pot drain holes by the end of the tests, and no insect problems were encountered. Roots that did emerge from the pot drain holes did not extend more than 1 cm from the edges of the pots. Consequently, we cannot attribute the pot-to-pot transmission of the pathogen to root-to-root contact, undrained water, or insects.

Some have hypothesized that soluble salts tend to accumulate in the potting media when the ebb-and-flow subirrigation system is used. Because high levels of nutrients can predispose plants to Pythium root rot (7,15,16), such salt accumulation may increase disease incidence. In this study, although the salt content of the potting medium was high (S. Sanogo, *unpublished*), no distinct relationship was found between disease development and salt concentration. However, in 1989, poinsettia plants grown in an ebb-and-flow system were affected by *Pythium* spp. in at least one commercial greenhouse in Pennsylvania where the soluble salt content of the growing media was also high (S. Sanogo, *unpublished*).

Symptom development (damping-off, stem base necrosis, and height reduction) was significant in plants grown in infested, fungicide-free medium and in infested, etridiazole-amended medium. In contrast, symptom development was suppressed in plants grown in infested, metalaxyl-amended medium. In addition, the pathogen was readily recovered from roots of plants grown in infested, fungicide-free or etridiazole-amended medium but was not isolated or was isolated to a lesser extent from roots of plants grown in infested, metalaxyl-amended medium. Although the effectiveness of etridiazole differed in the two tests for unknown reasons, the general conclusion can be drawn that metalaxyl was more effective than etridiazole in controlling *Pythium* infection.

The difference between the two fungicides may be explained by the fact that metalaxyl is highly systemic and etridiazole is not. The effectiveness of soil fungicides may vary as a result of adsorption, inactivation, or decomposition by components of the planting media or other interactions with the growing medium (21). Further research is needed to quantify the efficacy and fate of fungicides in the ebb-and-flow subirrigation system.

The results of this study agree with those reported by Hoitink et al (10) and indicate that it is possible for *Pythium* to be transmitted in an ebb-and-flow subirrigation system, particularly if the water becomes heavily infested, which could occur if the water is reused for many crops over several months. Movement of *Pythium* from infested pots to other pots within the ebb-and-flow system does not appear to pose any greater threat to production than in operations where recirculating systems are not used.

Table 1. Effect of *Pythium aphanidermatum* on cucumber grown in an ebb-and-flow subirrigation system

Test Treatment	Plant height[y] (cm)	Plants with stem base necrosis[z] (%)	Plants that damped off[z] (%)	Plants from which the fungus was isolated[z] (%)
Test 1				
Control	67 a	0	0	0
Infested pot	65 a	0	0	18 a
Infested water	27 b	100	12	100 b
Test 2				
Control	96 a	0	0	0
Infested pot	107 a	0	0	3 a
Infested water	91 a	35	0	80 b
Test 3				
Control	67 a	0	0	0
Infested pot	70 a	0	0	0
Infested water	41 b	0	0	78

[y] Average height of surviving plants. Numbers in a column for each test followed by the same letter do not differ significantly ($P = 0.05$) according to Fisher's protected least significant difference procedure.

[z] Total number of plants was 44 per treatment in test 1 and 40 per treatment in tests 2 and 3. Numbers in a column for each test followed by the same letter do not differ significantly ($P = 0.05$) according to Student's t test.

Table 2. Effect of *Pythium aphanidermatum* and soil fungicides on cucumber grown in an ebb-and-flow subirrigation system

	Plant height[y] (cm)		Plants with stem base necrosis[z] (%)		Plants that damped off[z] (%)		Plants from which the fungus was isolated[z] (%)	
Treatment	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Infested								
No fungicide	25	33	62 a	28 a	11 a	0	90 a	39 a
Metalaxyl	49	36	0	0	0	0	5 b	0
Etridiazole	34	27	39 b	46 b	3 b	13	85 a	80 b
Noninfested								
No fungicide	53	40	0	0	0	0	0	0
Metalaxyl	54	35	0	0	0	0	0	0
Etridiazole	50	41	0	0	0	0	0	0

[y] Least significant differences ($P = 0.05$) for comparing means at the same level of infestation were 15.9 and 14.7 in tests 1 and 2, respectively.

[z] Total number of plants was 39 per treatment. Numbers in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Fisher's protected least significant difference procedure (used to compare treatment means after analysis of variance with significant F test) and according to Student's t test (used to compare sets with only two treatment means).

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