Survey of *Pythium* and *Phytophthora* spp. in Irrigation Water Used by Colorado Commercial Greenhouses

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SUMMARY. The purpose of this study was to determine if irrigation water is a source of *Pythium* and *Phytophthora* spp. introduction into Colorado greenhouses. Nine greenhouses took part in the study; three each used municipal, well, or surface water as their irrigation supply. Water samples were collected from each greenhouse three times during Summer 1993. Samples were filtered, filter pads were incubated on selective media, and isolated pathogens were used to inoculate susceptible *Cucumis sativus* L. and *Lupinus polyphyllus* Findl. indicator plants. *Pythium rostratum* Butler and *P. dissotocum* Drechsler were isolated from surface water supplies. No *Phytophthora* was found in any water source. No differences were found in stem length or leaf number on inoculated versus control cucumbers or lupines. It was determined that both species of *Pythium* recovered are weak pathogens. Apparently, pathogenic *Pythium* and *Phytophthora* spp. are introduced into greenhouses in three counties in Colorado via means other than water supply.

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Root diseases caused by species of *Pythium* and *Phytophthora* fungi often cause persistent problems throughout the greenhouse industry. A wide variety of plants can be affected regardless of the time of year. Both genera reportedly have been introduced into cropping systems via contaminated or recycled irrigation water (Ali-Shtayeh et al., 1991; Gill, 1970; Pitis and Colhoun, 1983; Sanogo and Moorman, 1993; Shokes and McCarter, 1979; Thompson and Allen, 1974; vonBroembsen, 1984), infested potting media (Favrin et al., 1986), and insects (Goldberg and Stanghellini, 1990). Water used for irrigation of commercial greenhouse crops comes from one of three sources: municipal, well, or an open body of water (i.e., holding pond). The objective of this project was to determine if irrigation water is a source of *Pythium* and *Phytophthora* spp. introduction into greenhouse cropping systems.

Materials and methods

WATER SAMPLING AND FUNGUS DETECTION. Water sampling and fungus detection methods were those outlined by Ali-Shtayeh et al. (1991) and vonBroembsen (1984), with slight modifications. Water from nine greenhouses (three each irrigated by municipal, well, and holding-pond water) with a documented pythiaceus root disease history was sampled once a month from June to Aug. 1993. Twelve liters of irrigation water was collected from each greenhouse. Samples from municipal and well water sources were collected by holding three opened 4-L plastic bottles under the tap after letting the water run for 1 min. Samples from holding ponds were collected by holding three opened 4-L plastic bottles 2 to 3 cm below the water surface until they were filled. All bottles were capped when full, shaded from sunlight, and returned to the laboratory for filtering within 1 h. Water temperatures were recorded during collection.

Water samples collected from a single greenhouse were recombined into a 19-L plastic bucket and thoroughly stirred before filtration through 0.45-mm filter pads. Two hundred milliliters of irrigation water was filtered through one pad. Three filter pads per water sample (one pad per petri dish) were inverted onto

pimaricin-vancomycin-PCNB (PVP) (Tsao and Ocana, 1969), or pimaricin-vancomycin-PCNB-hymexazol (PVPH) (Tsao and Guy, 1977) selective medium and incubated in the dark at 25 °C for 24 h to allow colony development. Resulting fungal colonies were transferred to pure culture for taxonomic identification. Identification of *Pythium* spp. was based on the keys of Waterhouse (1967) and van der Plaats-Niterink (1981). Species reported as pathogenic in the literature were used in pathogenicity studies.

PATHOGENICITY STUDIES. Pathogenicity tests were conducted on cucumber and lupine seedlings in a research greenhouse at the W.D. Holley Plant Environmental Research Center, Colorado State University, between 31 Jan. and 25 Feb. 1994. Fourteen-day-old *C. sativus* var. Poinsett 76 seedlings were transplanted into 10-cm round plastic pots containing 50/50 (by volume) sphagnum peat-shredded rockwool and used immediately for inoculation studies. *Lupinus polyphyllus* var. Galleria Pink seedlings were grown in a 128-cell plug tray, then transplanted to the 10-cm pots. All pots were placed on the bench at 25-cm spacings and hand watered. The average greenhouse temperature was 21 °C under natural light conditions and daylength.

Inocula were produced as described in the literature (Bates and Stanghellini, 1984; Gill, 1970; Sanogo and Moorman, 1993). Isolates recovered during filtration were grown on V-8 juice agar plates in the dark for 7 days at 25 °C. Inoculation of cucumber and lupine were made by committing two petri dish cultures in 100 mL sterile distilled water. Thirty milliliters of the resulting inoculum was poured at the base of each plant on 31 Jan. 1994. All studies were replicated once, with three plants per replicate.

Plant height (shoot length) measurements, from the base of the stem to the base of the highest leaf, were taken twice a week from 31 Jan. to 25 Feb. 1994. Leaf counts were taken at the end of the experiment and data were subjected to analysis of variance. Treatment means were compared using Duncan's multiple range test. Plant roots and crowns were plated out on PVP or PVPH upon plant death or termination of the experiment. Culturing procedures were as follows:

Table 1. Presence (+) or absence (–) of *Pythium* and *Phytophthora* spp. in irrigation water surveyed from nine different Colorado greenhouse operations during Summer 1993.

Water source	Greenhouse	Sampling dates (1993)	Water temp (°C)	<i>Pythium</i>	<i>Phytophthora</i>
Pond	1	17 June	18.7	+	–
		12 July	20.8	+	–
		23 Aug.	20.5	+	–
	2	17 June	17.5	–	–
		12 July	20.9	–	–
		23 Aug.	19.4	–	–
	3	17 June	23.8	+	–
		12 July	24.2	+	–
		23 Aug.	22.7	+	–
Well	4	21 June	25.6	–	–
		14 July	16.9	–	–
		25 Aug.	22.0	–	–
	5	21 June	13.4	–	–
		14 July	14.4	–	–
		25 Aug.	15.0	–	–
	6	24 June	16.7	–	–
		19 July	21.1	–	–
		30 Aug.	22.2	–	–
City	7	21 June	20.9	–	–
		14 July	23.2	–	–
		25 Aug.	25.9	–	–
	8	24 June	15.1	–	–
		19 July	16.5	–	–
		30 Aug.	18.8	–	–
	9	24 June	32.5	–	–
		19 July	19.6	–	–
		30 Aug.	20.6	–	–

plants were removed from pots and roots and crowns were washed in tap water, blotted dry, cut into small pieces, surface disinfested, and placed on the respective media.

Results

Pythium spp. was recovered from two holding-pond water sources. *Phytophthora* spp. was not recovered from

any water source sampled. All well and city water sources and one holding-pond water source sampled were free from *Pythium* spp. (Table 1). *Pythium dissotocum* was identified in all three filtered subsamples taken from Greenhouse 1, with an average temperature at sampling of 20 °C. *Pythium rostratum* was identified in all three filtered subsamples taken from Green-

house 3, with an average temperature at sampling of 23 °C (Tables 1 and 2).

Pathogenicity tests were performed on cucumber and lupine because *P. dissotocum* and *P. rostratum* were reported to infect these plants (Farr et al., 1989). Plant height and leaf counts between treatments were not significantly different when compared to the control in either plant

Table 2. Presence (+) or absence (–) of *Pythium* spp. recovered from *Cucumis sativus* and *Lupinus polyphyllus* inoculated in pathogenicity studies. The *Pythium* was extracted from two greenhouse surface irrigation water supplies.

Plant tested	Rep/plant number	<i>Pythium dissotocum</i>	<i>Pythium rostratum</i>	Control
Cucumber	1/1	+	–	–
	1/2	+	+	–
	1/3	–	+	–
	2/1	–	–	–
	2/2	+	+	–
	2/3	+	+	–
Lupine	1/1	+	+	–
	1/2	+	+	–
	1/3	–	+	–
	2/1	+	+	–
	2/2	+	+	–
	2/3	+	+	–

Table 3. Final mean leaf numbers and stem lengths of *Cucumis sativus* and *Lupinus polyphyllus* plants inoculated with *Pythium* spp. based on analysis of variance and Duncan's multiple range test ($p < 0.05$). Means are averages of six plants per treatment.

Plant tested	Treatment	Mean leaf no.	Mean stem length (cm)
<i>Cucumis sativus</i>	<i>P. dissotocum</i>	4.4 a	14.7 a
	<i>P. rostratum</i>	4.2 a	14.6 a
	Control	5.0 a	15.7 a
<i>Lupinus polyphyllus</i>	<i>P. dissotocum</i>	6.7 a	17.6 a
	<i>P. rostratum</i>	7.3 a	17.0 a
	Control	9.0 a	19.4 a

(Table 3). *Pythium dissotocum* was recovered from four of six inoculated cucumber plants and five of six lupine plants. *Pythium rostratum* was recovered from four of six cucumbers and all six lupines. *Pythium* spp. was not recovered from the uninoculated controls (Table 2).

Discussion and conclusion

Irrigation water is a source of pathogen introduction into greenhouses (Ali-Shtayeh et al., 1991; Gill, 1970; Pitis and Colhoun, 1983; Sanogo and Moorman, 1993; Shokes and McCarter, 1979; Thompson and Allen, 1974; vonBroembsen, 1984). Based on our findings, it is unlikely that *Phytophthora* spp. would be introduced into Colorado greenhouses via irrigation water, since there was no evidence of this fungus in any of the water sampled. *Pythium* spp., however, potentially could be introduced through irrigation water from a holding-pond source. Two different *Pythium* spp.—*P. dissotocum* and *P. rostratum*—were detected. *Pythium dissotocum* can cause significant problems in hydroponic culture of spinach (Bates and Stanghellini, 1984). *Pythium rostratum*, according to van der Plaats-Niterink (1981), is considered nonpathogenic or very weakly pathogenic to plants.

In this study, both *Pythium* species were able to colonize roots and crowns of most inoculated plants. The effects of both fungi on the height and leaf count of cucumber and lupine, however, were not different when compared to the uninoculated control, which leads us to believe that *P. dissotocum* and *P. rostratum* are unlikely to cause significant problems in Colorado greenhouse potted plant culture. Even though the potential for *Pythium* spp. introduction via holding-pond irrigation sources may

exist, pathogenic *Pythium* and *Phytophthora* spp. are more likely to be introduced into greenhouses in three counties in Colorado via sources other than irrigation water.

Literature cited

- Ali-Shtayeh, M.S., J.D. MacDonald, and J. Kabashima. 1991. A method for using commercial ELISA tests to detect zoospores of *Phytophthora* and *Pythium* species in irrigation water. *Plant Dis.* 75:305–311.
- Bates, M.L. and M.E. Stanghellini. 1984. Root rot of hydroponically grown spinach caused by *Pythium aphanidermatum* and *P. dissotocum*. *Plant Dis.* 68:989–991.
- Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. *Fungi on plants and plant products in the United States*. Amer. Phytopath. Soc. Press, St. Paul, Minn.
- Favrin, R.J., J.E. Rahe, and B. Mauza. 1986. *Pythium* spp. associated with crown rot of cucumbers in British Columbia greenhouses. *Plant Dis.* 72:683–687.
- Gill, D.L. 1970. Pathogenic *Pythium* from irrigation ponds. *Plant Dis. Rpt.* 54:1077–1079.
- Goldberg, N.P. and M.E. Stanghellini. 1990. Ingestion–egestion and aerial transmission of *Pythium aphanidermatum* by shore flies (Ephydriidae: *Scatella stagnalis*). *Phytopathology* 80:1244–1246.
- Pitis, J.E. and J. Colhoun. 1983. Isolation and identification of pythiaceae fungi from irrigation water and their pathogenicity on *Antirrhinum*, tomato and *Chamaecyparis lawsoniana*. *Phytopath. Zeitschrift.* 110:301–318.
- Sanogo, S. and G.W. Moorman. 1993. Transmission and control of *Pythium aphanidermatum* in ebb-and-flow subirrigation system. *Plant Dis.* 77:287–290.
- Shokes, F.M. and S.M. McCarter. 1979. Occurrence, dissemination, and survival of plant pathogens in surface irrigation ponds in southern Georgia. *Phytopathology* 69:510–516.
- Thompson, S.V. and R.M. Allen. 1974. Occurrence of *Phytophthora* species and other potential plant pathogens in recycled irrigation water. *Plant Dis. Rpt.* 58:945–949.
- Tsao, P.H. and S.O. Guy. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. *Phytopathology* 67:796–801.
- Tsao, P.H. and G. Ocana. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature* 223:636–638.
- van der Plaats-Niterink, A.J. 1981. Monograph of the genus *Pythium*. *Studies in Mycol.* 21.
- vonBroembsen, S. 1984. Distribution of *Phytophthora cinnamomi* in rivers of the south-western Cape Province. *Phytophylactica* 16:227–229.
- Waterhouse, G. 1967. Key to *Pythium* Pringsheim. CMI Mycological Paper 109.