Additional *Pythium* Species Associated with the Bean Root Rot Complex in Wisconsin's Central Sands

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ABSTRACT

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Pythium aristosporum, P. catenulatum, P. dissotocum, and an unidentified *Pythium* species producing heterothallic sporangia were isolated from roots and hypocotyls of commercial snap beans growing in Wisconsin's Central Sands. Pathogenicity tests showed that all four species caused pruning and discoloration of bean roots. This is the first known report of the association of three additional *Pythium* species with the bean root rot complex in Wisconsin.

In the Central Sands of Wisconsin (an area about 100 miles north of Madison), *Pythium ultimum* Trow and *P. irregulare* Buis. appear to be the major *Pythium* species associated with bean root rot in commercial snap bean fields (1,6,9,10). Little is known about the importance of other *Pythium* species that may be involved in the bean root rot complex as it currently exists in Wisconsin. We assessed four additional *Pythium* spp. repeatedly isolated from commercial snap bean fields distributed throughout this important bean production area of Wisconsin's Central Sands to determine their pathogenicity and possible role in the bean root rot complex.

MATERIALS AND METHODS

Pythium isolates were obtained from snap bean roots and hypocotyls collected 30–40 days after planting in commercial fields throughout the Central Sands of Wisconsin. Hypocotyls and roots were washed in fast-running tap water for 3 min, blotted dry, and cut into 1-mm sections, which were placed onto petri dishes containing a medium selective for *Pythium*. This medium (PVP) consisted of 2.5% Difco agar, 200 ppm of vancomycin (Vancocin HCl), 5 ppm of pimaricin (Pimafucin), and 130 mg of pentachloronitrobenzene (PCNB) (5,7, 10,12). Cultures were incubated 24–48 hr in the dark at 24 ± 3 C before hyphal tips were transferred to fresh petri dishes containing PVP medium. Transfers were allowed to grow for 48 hr before subsequent transfer and maintenance on cornmeal agar (CMA) tube slants. Isolates were tentatively identified using the grass blade technique and the key of Middleton (4,8).

Isolates were tested for pathogenicity in controlled environments with 16-hr light and 8-hr dark photoperiods at 20 ± 3, 24 ± 3, and 28 ± 3 C. Bean seedlings of cultivar Early Gallatin (commercially slurry-treated with captan/chlorpyrifos (Lorsban 50SL) were grown at 24 ± 3 C in vermiculite for 7 days. Individual 7-day-old seedlings, selected for uniformity, were then transplanted in wax cups containing 228 g of silica sand (natural grain), one plant per cup and five replicate plants for each treatment, and randomized on the growth-chamber cart. Roots of seedlings were washed to remove free vermiculite particles before transplanting. Transplants were watered with half-strength Hoagland's solution immediately before inoculation. Plants were inoculated by using a glass spoon to gently remove the sand adjacent to each hypocotyl, then adding a 5-mm-diameter mycelial disk from the edge of an actively growing 4-day-old fungal mat. Sand was pressed gently against the disk to hold it in place. Controls consisted of uninoculated agar disks and isolates of *P. ultimum* and *P. irregulare* known to be pathogenic on beans (3).

Mycelial disks for inoculation were produced using a modified media first reported by Johnson et al (2). Mycelial disks from isolates maintained on CMA tube slants were transferred to petri plates containing the following sterile medium: 20 g of sucrose, 2 g of $NaNO_3$, 0.1 g of K_2HPO_4, 0.5 g of $MgSO_4{:}7H_2O$, 0.5 g of KCl, 0.015 g of $FeSO_4{:}7H_2O$, 15 g of Difco agar, 200 ppm vancomycin, and 5 ppm pimaricin in 1 L of sterile distilled water. Antibiotics were added after the medium had cooled but before dispersal into plates.

Disease severity ratings on roots and hypocotyls were determined 28–32 days after inoculation, on a scale of 0–4 based on symptom comparison with the isolate of *P. ultimum* known to be pathogenic on beans. On this scale, 0 = healthy, no symptoms; 1 = slight, few, and small tan lesions on taproot and lower hypocotyl; 2 = moderate, obvious root pruning and tan root and hypocotyl lesions, a few showing above the sand surface; 3 = severe, extensive root pruning, many coalescing tan lesions on root and lower hypocotyl causing softening of infected tissues, plant stunting, and light brown hypocotyl streaks; and 4 = dead or dying. Disease severity means were calculated and statistically compared using Duncan's multiple range test (11).

RESULTS AND DISCUSSION

Species isolated and found pathogenic on snap beans in this study were as follows: *P. aristosporum* Vanterpool, *P. catenulatum* Matthews, *P. dissotocum* Drechs., and an unidentified *Pythium* species with heterothallic sporangia.

All isolates were pathogenic on snap beans compared with controls (Table 1). Control plants treated with agar disks without mycelium generally had white firm roots, but an occasional rootlet showed tan discoloration near the terminal end. *Pythium* was never isolated from these areas. In dramatic contrast, controls inoculated with *P. ultimum* had soft roots with severe root pruning and reddish brown discoloration that in some cases extended up the hypocotyl. *P. ultimum* and *P. irregulare* were clearly more pathogenic than the other *Pythium* spp. at 20 C, but this difference was not as apparent at 24 and 28 C. The latter

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Table 1. Effect of temperature on disease severity of snap bean seedlings inoculated with *Pythium* species isolated from commercially grown snap beans in Wisconsin[a]

Pythium species	Disease severity[b]		
	20 C	24 C	28 C
Control (agar disk plug only)	0.4 a[c]	0.7 a	0.4 a
P. ultimum	3.2 e	2.6 de	2.2 c
P. irregulare	3.0 e	1.8 c	1.4 b
P. aristosporum	2.2 d	2.4 d	2.6 cd
P. dissotocum	1.8 cd	1.8 c	1.6 b
P. catenulatum	1.0 b	1.0 ab	1.6 b
Pythium spp., heterothallic sporangia	1.4 b	1.4 bc	1.5 b
P. aristosporum + *P. dissotocum* + *P. catenulatum* + *Pythium* spp. with heterothallic sporangia	2.9 e	3.0 e	3.0 d

[a] Seven-day-old seedlings of cultivar Early Gallatin grown in a controlled environment of 16 hr of light and 8 hr of darkness and inoculated with a 5-mm mycelial disk.
[b] Data are means from two experiments of five replicate plants each. Disease severity scale: 0 = healthy, 1 = slight, 2 = moderate, 3 = severe, and 4 = dead or dying.
[c] Within each column, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

species was much less pathogenic at warmer temperatures. *P. aristosporum* and *P. dissotocum* caused moderate root pruning, with reddish brown discoloration of roots but not of hypocotyls. As temperature increased, disease severity tended to increase with *P. aristosporum* and *P. catenulatum*, although in general, the newly isolated *Pythium* spp. were not temperature-sensitive. The reverse was true for *P. ultimum* and *P. irregulare*. *P. aristosporum* was the most pathogenic of the four new *Pythium* spp. at all three temperatures; *P. catenulatum* caused the least disease.

When all four isolates were inoculated onto single plants, disease severity was greater than with each isolate separately, indicating that a synergistic or additive effect on disease severity was maybe occurring.

Although these new *Pythium* spp. have been isolated throughout the bean-growing region of the Central Sands, the total extent of their distribution is unknown. Attempts at quantitative epidemiology of *Pythium* populations involved in the bean root rot complex as it exists in the Central Sands may be difficult if additive or synergistic effects occur under field conditions.

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