Characterization of *Pythium* spp. from Three Ohio Fields for Pathogenicity on Corn and Soybean and Metalaxyl Sensitivity

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**Abstract**

*Pythium* spp. were baited with corn and soybean seed from soils collected from three locations in Ohio where soybean and corn stand establishment was a concern. Five species, *P. catenulatum*, *P. irregulare*, *P. paroecandrum*, *P. splendens*, and *P. torulosum*, were recovered and a subset of these isolates was then tested for pathogenicity on corn and soybean seed and sensitivity to the seed treatment fungicide metalaxyl. There was a range of both pathogenicity and sensitivity to metalaxyl within and among the *Pythium* spp. recovered from the three locations. A more thorough evaluation of the *Pythium* populations that exist in grain production fields in the north central region of the US is needed to facilitate the development and deployment of broader based seed treatment products.

**Introduction**

*Pythium* spp. can cause seedling blights and root rots of a number of field crops including soybean and corn. Fourteen *Pythium* species have been implicated in causing corn seed and seedling blight (3). In Ohio, earlier studies recovered *P. arrhenomanes* Drechsler, *P. graminicola* Subramanian, *P. dissotocum* Drechsler, and *P. torulosum* Coker & F. Patterson from corn roots (6,14). There was a range of aggressiveness on corn roots among the isolates evaluated in these studies (3,6,14).

*Pythium aphanidermatum* (Edson) Fitzp., *P. irregulare* Buisman, *P. myriotylum* Drechs., *P. paroecandrum* Drechs., *P. spinosum* Sawada, *P. sylvaticum* W. A. Campbell & J. W. Hendrix, *P. torulosum*, and *P. ultimum* Trow were isolated from dying soybean seedlings or baited from soils in two studies in Iowa (15,19). *P. ultimum* was attributed to soybean losses in the soybean cultivar Essex in Virginia (9) and from soybeans grown under irrigation in Arkansas (10). In Minnesota, *P. ultimum* and *P. debaryanum* Auct. non R. Hesse were the most prevalent species causing damping-off of soybean (2).

*Pythium* spp. can cause disease individually, but frequently several species are isolated from a single plant. For example, *P. irregulare* can cause root rot on corn and may provide infection courts for other corn pathogens to colonize tissues later in the season (11). In addition, *Pythium* spp. recovered from infected corn or soybean seed or seedlings, or directly from soil have a range of pathogenicity and aggressiveness when tested in pathogenicity assays (2,6,19).

*Pythium* seed and seedling blights of soybean and corn are currently managed with the use of fungicide seed treatments. The primary active ingredients used on corn and soybean seed are metalaxyl and mefenoxam while previously captan was used for corn. Metalaxyl is an acylalanide fungicide which inhibits ribosomal RNA synthesis in the Peronosporales (4). *Pythium* spp. isolated from carrots (18), sugar beet seedlings (1), and wheat (5) exhibited a range of sensitivity to metalaxyl. This variability was thought to be responsible for the lack of disease control detected in certain locations.
During spring of 2000, approximately 40% of the soybean acreage in northwest Ohio required replanting. The majority of this loss was attributed to *Phytophthora sojae* Kaufmann and Gerdemann; however, questions were raised as to whether *Pythium* spp. could also be contributing to these losses. *Pythium* spp. were also recovered from 90% of the soil samples from two intensively sampled fields where the *Phytophthora sojae* population was high (7). Based on these reports, the objectives of this study were to characterize the *Pythium* populations in three fields with a history of stand establishment problems for the species present, pathogenicity, and metalaxyl sensitivity.

**Soil Sample Collection, Isolation, and Identification**

Three fields were chosen in northwest Ohio with a history of soybean stand establishment problems in Defiance, Sandusky, and Wood counties. The soil type for both Defiance and Wood County fields was Hoytville clay while the Sandusky County field type was a Brookstone silty clay loam. From each field, 10 to 25 soil samples over a 2- to 5-ha area were collected for a total of approximately 30 to 40 liters of soil. The soil from each field was air dried, ground in a burr mill, mixed, and divided into 15-cm-diameter pots and stored at room temperature until assays were made. The pots were saturated for 24 hours, allowed to drain, placed in plastic bags and incubated for two weeks at room temperature at 18.3°C (65°F). Pots were planted with 15 seeds per pot of either corn hybrid Pfister 2295 or soybean cultivar Sloan, and three pots with each host plant were placed in growth chambers set at 12.8°C (55°F) or 18.3°C (65°F). Three and five days after planting corn and soybean seeds, respectively, pots were placed in 10-liter (2.5-gal) buckets of water in which the water level was approximately 1 cm above the soil surface in the pots. These were then placed in the growth chamber for 24 hours to saturate soil. Pots were removed from the buckets and allowed to drain for 24 hours in the growth chamber. Soybean and corn seeds were then removed from the soil and washed under running tap water with soap (Tide, Proctor & Gamble, Cincinnati, OH) until the visible soil was removed. Seeds were cut in half with a sterile knife and placed between 3 to 5 layers of sterile paper toweling. A rolling pin was used to roll over the paper toweling to remove excess water from seeds. The seeds were then placed on PIBNC media (16). Hyphal tip transfers were made from mycelia growing from the seeds onto cornmeal agar within 2 to 3 days and stored at 15°C (60°F) until used. Isolates were transferred onto water agar, lima bean 10 agar (10 g lima beans, 15 g agar per liter) or onto sterile grass blades floated in sterile water for identification (17). All *Pythium* species were identified based on morphological characteristics of hyphae, antheridia, oospores, and sporangia using the keys of Middleton (12) and Waterhouse (17).

Five species, *P. catenulatum*, *P. irregularare*, *P. paroecandrum*, *P. splendens* H. Braun, and *P. torulosum* (Fig. 1), were recovered from soils in Ohio using both corn and soybean seed as bait but the number of isolates and the percentage of each species was different for each of the three field locations. There were 129, 85, and 53 isolates recovered from soils collected in Defiance, Sandusky, and Wood County, respectively. *P. torulosum*, *P. splendens*, and *P. irregularare* represented 40, 38, and 22% of the isolates recovered from the Defiance County soil (Fig. 2). *P. splendens* was the predominant species (72%) recovered from both soybean and corn seed from the Sandusky soil, while *P. catenulatum* and *P. splendens* comprised 20 and 14% of the isolates from Wood County soil, respectively. More isolates were recovered from corn seed than soybean seed in Defiance and Sandusky County soils.
Fig. 1. *Pythium* spp. recovered from three Ohio fields with a history of stand establishment problems: (A) oogonium of *P. catenulatum* with multiple diclinous and monoclinous antheridia; (B) irregularly swollen sporangia of *P. catenulatum* produced in water cultures; (C) oogonium of *P. irregulare* with a single irregular wall projection and a diclinous antheridium; (D) oogonium of *P. paroecandrum* with single, flatly applied, sessile antheridium; (E) common terminal, spherical sporangium of *P. splendens*; and (F) a single, stalked antheridium attached to an oogonium of *P. torulosum* containing an immature plerotic oospore.
Pythium spp. recovered from these three locations were different than those reported from Arkansas, Iowa, Minnesota, and previous studies in Ohio. *P. ultimum* was not recovered in this sampling nor was it recovered from diseased plant samples collected from these locations when these fields were first implicated as having stand establishment problems (data not shown).

**Pathogenicity assays.** A petri dish assay, similar to that used previously for evaluations of pathogenicity and aggressiveness of *Rhizoctonia* and *Pythium* on soybean (13,19), was used. A 5-mm colonized lima bean agar plug of the *Pythium* isolate was placed in the center of a 15-mm petri plate containing 20 ml water agar. After 3 days, 10 seeds of either corn or soybean previously surface disinfested for 1 min in 10% Clorox (The Clorox Company, Oakland, CA) and rinsed with sterile distilled water were equally placed 3 mm from the edge of the plate. Plates were examined for fungal colonization of seeds after 2 and 7 days. At 7 days, the number of seeds that were killed by *Pythium* or germinated per plate was recorded. The pathogenicity assays were completed 3 times for 39, 27, and 31 *Pythium* isolates from Defiance, Sandusky, and Wood County soils, respectively. The isolates from each county were compared in the same assay. Seeds colonized by seed-borne pathogens were not included in the counts. The percentage of seeds killed by the *Pythium* spp. was transformed, arcsin of the square root of the percentage killed, prior to statistical analysis. Analysis of variance for the isolates within each location was completed using the General Linearized Model in SAS (SAS Institute, Cary, NC).

The ANOVA indicated a highly significant test × isolate interaction for the three times the agar plate bioassay was completed. The isolates that caused high or low levels of disease were consistent across the three experiments, but isolates that caused 20 to 70% seed rot in the plate assays were variable. The average percentage of rotted seeds across isolates was 20.9, 33.7, and 41.7% for the *Pythium* isolates from the Defiance location for Test 1, 2, and 3; respectively. However, in spite of the variability, the general trends were similar across the three experiments and the results from the second experiment are shown for illustration. Very few of the isolates tested were highly aggressive on both corn and soybean seed, but the degree of variability differed by location (Table 1). The *P. splendens* isolates from Sandusky killed 100% of the soybean seed and 13 of the 16 isolates killed 40 to 60% of the corn seed. In contrast, 3 of the 5 *P. splendens* isolates from Wood County were not pathogenic on soybean or corn
while 11 of 15 were highly pathogenic on soybean. Additionally only 7 of 15 *P. splendens* isolates from Defiance County were moderately pathogenic on corn. All of the isolates of *P. torulosum* were non-pathogenic on soybean and only a few isolates were moderately pathogenic on corn from all three locations. *P. catenulatum* isolates were mainly moderately pathogenic on both corn and soybean from both Defiance and Wood counties.

Table 1. *Pythium* spp. and levels of pathogenicity\(^a\) on both corn and soybean seeds from three locations in Ohio.

| *Pythium* spp. | No. of isolates | Corn | Soybean | | | | |
|---|---|---|---|---|---|
| | | Low | Moderate | High | Low | Moderate | High |
| Defiance | | | | | | | |
| *P. catenulatum* | 3 | 2 | 1 | 3 | | | |
| *P. irregulare* | 8 | 6 | 2 | 6 | 2 | | |
| *P. splendens* | 15 | 8 | 7 | 4 | 11 | | |
| *P. torulosum* | 5 | 3 | 2 | 5 | | | |
| Sandusky | | | | | | | |
| *P. splendens* | 16 | 13 | 3 | 16 | | | |
| *P. torulosum* | 2 | 2 | | | | | |
| Wood | | | | | | | |
| *P. catenulatum* | 12 | 1 | 11 | 1 | 10 | 1 | |
| *P. irregulare* | 6 | 5 | 1 | | 6 | | |
| *P. paroecandrum* | 2 | 1 | 1 | | 1 | | |
| *P. splendens* | 5 | 3 | 2 | 3 | 1 | 1 | |
| *P. torulosum* | 2 | 1 | 1 | | 2 | | |

\(^a\) Levels of pathogenicity were determined on the percentage of corn or soybean seed rotted in an agar plate assay. Zero to 29, 30 to 69, and 70 to 100% of the seeds rotted were classified as low, moderate, and high levels of pathogenicity, respectively. The assays were repeated three times. Data presented is from the second assay.

This is the first report of *P. catenulatum* as a pathogen of corn and soybean seed. This species was first identified from water containing plant debris in North Carolina and Missouri (12). Only eight host plants are listed for *P. catenulatum* in the USDA Systematic Botany and Mycology Database including *Oryza sativa*, an unknown grass, and *Phaseolus vulgaris* (8). This study corroborates earlier studies where differences in aggressiveness and pathogenicity among *Pythium* isolates were reported (2,6,19). Additionally, Brown and Kennedy (2) reported 9 to 100% emergence in pot assays among *P. ultimum* isolates recovered from dying soybean seed and seedlings. More recently, *Pythium* spp. recovered from fields in a corn-soybean rotation ranged from not pathogenic, to varying levels of aggressiveness on both corn and soybeans, to high levels of aggressiveness on both crops (19).

**Metalaxyl Insensitivity**

Sensitivity to metalaxyl was measured at 5 and 100 \( \mu g/ml \). Technical grade metalaxyl (Gustafson LLC, Plano, TX) was dissolved in dimethylsulfoxide (DMSO) and added to V8 juice agar after autoclaving. DMSO, at the same volume as treatments, was added to the control. A 5-mm colonized, agar plug of the *Pythium* isolate to be tested was transferred from the actively growing margin of a 3- to 4-day-old culture to the center of the plate. Each isolate was evaluated at 0, 5, and 100 \( \mu g/ml \), using three replicate plates. After 2 days, the length from the plug to the end of the mycelium was measured. Linear growth on metalaxyl-amended media was expressed as a percent of growth on control plates and used in the analysis.
All three locations had *Pythium* isolates that grew on metalaxyl-amended medium at 100 µg/ml. Although the majority of these isolates grew at this concentration, growth was 1 to 30% of the unamended control (Fig. 3). This included 30 of 39, 27 of 27, and 13 of 31 isolates tested for Defiance, Sandusky, and Wood locations, respectively. Only 5 of the 39 isolates tested in Defiance County and 7 of the 31 from Wood County did not grow on metalaxyl-amended medium at the 5-µg/ml concentration. Interestingly, these were all *P. irregulare* and one isolate of *P. paroecandrum* at both locations. There were 5 additional isolates of *P. irregulare* that grew on both concentrations of metalaxyl-amended medium, but growth was slow.

![Fig. 3. Metalaxyl sensitivity at (A) 5 µg and (B) 100 µg of a subset of *Pythium* spp. recovered from soils collected from three locations in Ohio. Metalaxyl was added to V8-juice agar and growth of isolates was measured after 2 days.](image)

A range in sensitivity to metalaxyl has been reported for *Pythium* isolates recovered from carrots (18), sugar beet (1), and wheat (5). *Pythium* spp. that were less sensitive to metalaxyl comprised 25 to 75% of the populations in wheat fields in the Pacific Northwest (5). This variation in levels of sensitivity could account for the development of Pythium root rot in some fields. However, Brantner and Windels (1) suggested that use of metalaxyl as a seed treatment posed a limited risk for development of populations of metalaxyl-insensitive isolates because only a small proportion of the *Pythium* population within sugar beet production fields are exposed to this seed treatment fungicide and other crops in the rotational sequence do not have seed treatment applied. All corn, most soybeans, and a smaller proportion of wheat seed planted in Ohio are treated with metalaxyl or the closely-related compound mefenoxam, which has the same mode of action. In addition, during the 1980s some of the fields in these counties had metalaxyl applied in-furrow to control *Phytophthora sojae* on soybean. At this time, no populations of *P. sojae* with insensitivity to metalaxy have been detected in Ohio (Dorrance, unpublished data). There are a number of seed treatment fungicide compounds with different modes of action that do have some efficacy towards *Pythium* spp. These results suggest that repeated use of one chemical may result in decreased sensitivity of *Pythium* spp. and reduced levels of disease control in the field.

**Conclusion**

*P. catenulatum, P. irregulare, P. paroecandrum, P. splendens,* and *P. torulosum* were recovered from the three fields in Ohio. Isolates of each species varied in number, pathogenicity, and sensitivity to metalaxyl both between and within locations. The species identified in this study are different from those reported in other states from corn and soybean in earlier studies and this is the first report of *P. catenulatum* as a pathogen of corn and soybean seed. The results from this study indicate that repeated use of metalaxyl or mefenoxam alone as a seed treatment to manage *Pythium* spp. may be selecting for
insensitive strains of these species. Variation in species composition and pathogenicity will pose additional challenges for the development of more biologically based seed treatments which may have narrow host ranges. A more thorough evaluation of the Pythium populations that exist in grain production fields in the north-central region is needed to facilitate the development and deployment of broader based biological and chemical seed treatments.

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Literature Cited