# Pathogenicity of *Pythium* Species in Causing Seed Rot and Damping-off of Soybean

## Annual Report
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*Pythium* seed rot and seedling blight caused by *Pythium* species are serious problems of soybean seedling establishment in Ontario and Manitoba where majority of Canadian soybean is grown. Typical symptoms of infection by *Pythium* species include soft and decayed seed before germination, pre- or post-emergence damping-off in the seeding stage, and hypocotyl discoloration and root rot in advanced growth stages (Fig. 1). The disease severity increases with cool and moist conditions, minimum tillage, and earlier planting. Previous research indicates that *Pythium* species are capable of causing soybean diseases individually, but frequently several species are isolated from a single plant. There is little information in aggressiveness among isolates within a *Pythium* species and difference in pathogenicity among *Pythium* species to soybean. The objectives of this research were to compare the pathogenicity of 24 isolates from eight *Pythium* species in causing seed rot and damping-off of soybean and to determine the influence of temperature on seed rot caused by the eight *Pythium* species.

Twenty four isolates from eight *Pythium* species were obtained from Canadian Collection of Fungal Cultures (CCFC) at the Eastern Cereal and Oilseed Research Centre (ECORC), Agriculture and Agri-Food Canada (AAFC) and used in the study (Table 1). Soybean cultivars Beechwood and Nattawa were used in the experiments to evaluate the comparative pathogenicity of the eight *Pythium* species and cultivar PS 50 was used in the experiments to determine the influences of temperature on the pathogenicity. Both Beechwood and PS 50 were considered susceptible and Nattawa was moderately resistant to root rot under the field conditions. Seeds of these soybean cultivars were provided by the soybean breeding program at the ECORC, AAFC.

Seed rot tests were conducted in a plant pathology lab using Petri dishes containing water agar with the Pythium fungus grown on a 5-$mm^2$ V8 agar plug in the center of each plate. Damping-off tests were conducted in a greenhouse environment using planting trays filled with Pro-mix soil and inoculated with a layer of *Pythium* inoculum prepared in sand-cornmeal medium. The effect of temperature on pathogenicity of the eight *Pythium* species in causing seed rot was examined under 4°C, 12°C, 20°C and 28°C provided by growth cabinets.

There were significant differences among the eight *Pythium* species in both seed rot and damping-off (Table 1). *P. aphanidermatum* and *P. ultimum* were the most pathogenic species, causing seed rot by 84.6% and 94.9%, and damping-off by 28.8% and 21.7%, respectively; *P. irregulare* and *P. sylvaticum* were intermediate, having seed rot of 35.7% and 37.2%, and damping-off of 5.2% and 3.6%, respectively; *P. arrenomanes*, *P. coloratum*, *P. dissotocum*, and

*P. macrosporum* were least pathogenic, causing seed rot ranging from 7.8 to 16.6% and damping-off of 0.4 to 3.1%.

Temperature had significant influences on seed rot by the *Pythium* species At all four temperatures (4°C, 12°C, 20°C, and 28°C) used, *P. ultimum* was highly pathogenic while *P. arrenomanes, P. coloratum, and P. dissotocum* were least pathogenic. The temperature by *Pythium* species interactions were more obvious for *P. aphanidermatum* which showed an increased seed rot values with the increase of temperature and for *P. irregulare, P. macrosporum, and P. sylvaticum* which showed a decreased seed rot values with the increase of temperature (Fig. 2).

This study demonstrated that *P. aphanidermatum, P. irregulare, P. macrosporum, P. sylvaticum,* are temperature dependent in causing soybean seed rot. Of these temperature dependent pathogenic species, only *P. aphanidermatum* and *P. irregulare* had been previously recognized. *P. aphanidermatum*, although was highly pathogenic at or above 20°C (Tables 1), showed little or no pathogenicty to soybean at 4°C and 12°C (Fig. 2). These results suggest that *P. aphanidermatum* is not likely responsible for soybean root rot and damping-off in short-season soybean growing regions of Eastern Ontario and Manitoba where the soil temperature is often below 20°C during the crop emergence and the early seedling development stage.

In contrast to *P. aphanidermatum*, *P. macrosporum* that was considered weakly pathogenic and *P. irregulare* and *P. sylvaticum* that were moderately pathogenic at 26°C, were highly pathogenic to soybean at low temperatures, causing >90% seed rot at both 4°C and 12°C (Fig. 2), suggesting that these species are likely responsible for the seed decay, seedling blight and root rot of short-season soybeans. The high level of pathogenicity of *P. macrosporum* to soybean at the low temperatures has not been previously reported. This species was detected using a *Pythium* DNA array hybridization method from diseased soybean roots during an extensive survey for root rot pathogens in commercial fields of soybean in eastern Ontario and Quebec. The high levels of pathogenicity of the two *P. macrosporum* isolates to soybean observed in this research suggest that the species could have a significant negative impact on soybean stand in Eastern Ontario and Manitoba where soil temperature is below 20°C during the crop emergence and early stages of plant growth. Further studies with a large number of *P. macrosporum* isolates from soybean and various host plants are needed to better understand the *P. macrosporum* isolates × soybean cultivar interaction as affected by temperature.

Significant differences among isolates within a *Pythium* species were found in seed rot only for *P. aphanidermatum, P. arrenomanes, P. irregulare*, and *P. macrosporum* (Table 1). The presence of different levels of aggressiveness among isolates within the pathogenic *Pythium* species has practical implications that must be considered when screening and breeding soybean for *Pythium* root rot resistance. It is important that aggressive isolates be used because isolates of low aggressiveness may not discriminate among lines of different levels of resistance. A mixture of several different isolates may be used in screening for resistance.

Soybean cultivar resistance to *Pythium* species has recently been identified in the United States, making resistance breeding possible and a viable strategy for managing Pythium seed rot and damping-off. Of the two soybean cultivars used in the pathogenicity experiment of the present study, Nattawa was significantly more resistant than Beechwood (Fig. 3). The cultivar reactions were in agreement with previous field observations. Although the cultivar × *Pythium* species interaction were significant for both seed rot and damping-off, the differential responses of the two cultivars were less apparent to the highly pathogenic and moderately pathogenic species (Table 1). These results indicate that soybean may share common genes for resistance to these pathogenic species and that breeding for resistance to one *Pythium* species may also give enhanced resistance to other *Pythium* species Further research is needed to confirm the presence and heritability of resistance genes in Nattawa and their usefulness in future cultivar development.

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**Table 1.** Variation among isolates and species of eight *Pythium* species in causing seed rot and damping-off on two soybean cultivars[a]

| | | Seed rot (%) | | | Damping-off (%) | | |
|---|---|---|---|---|---|---|---|
| *Pythium* species | Isolate | Beechwood | Nattawa | Mean | Beechwood | Nattawa | Mean |
| *P. aphanidermatum* | BR 444 | 87.0 b | 94.8 a | 90.9 ab | 59.5 a | 37.9 a | 48.7 a |
| | BR 740 | 76.6 c | 80.2 b | 78.4 b | 50.8 a | 28.4 ab | 39.6 a |
| | BR 910 | 94.3 a | 97.9 a | 96.1 a | 56.9 a | 17.2 b | 37.1 a |
| *P. arrenomanes* | BR 1028 | 31.8 ab | 17.7 b | 24.7 ab | 29.3 a | 18.9 a | 24.1 a |
| | BR 122 | 34.9 a | 27.1 a | 31.0 a | 18.1 b | 21.5 a | 19.8 a |
| | BR 981 | 23.4 ab | 17.7 b | 20.6 b | 27.6 ab | 15.5 a | 21.5 a |
| | BR 985 | 22.4 b | 20.8 ab | 21.6 b | 26.7 ab | 18.1 a | 22.4 a |
| *P. coloratum* | BR 621 | 26.6 a | 19.8 a | 23.2 a | 22.4 a | 16.4 a | 19.4 a |
| | BR 689 | 31.8 a | 21.9 a | 26.8 a | 30.2 a | 22.4 a | 26.3 a |
| *P. dissoticum* | BR 1048 | 35.9 a | 19.8 a | 27.9 a | 30.2 a | 17.2 a | 23.7 a |
| | DAOM 229134 | 27.6 a | 27.1 a | 27.3 a | 22.4 a | 24.1 a | 23.3 a |
| *P. irregulare* | BR 1040 | 59.9 b | 40.6 ab | 50.3 b | 27.6 a | 21.5 a | 24.5 a |
| | BR 1052 | 73.4 a | 52.1 a | 62.8 a | 34.5 a | 23.3 a | 28.9 a |
| | BR 387 | 56.8 b | 31.3 bc | 44.0 bc | 32.7 a | 19.8 a | 26.3 a |
| | BR 901 | 52.6 b | 27.1 c | 39.8 c | 25.0 a | 25.8 a | 25.4 a |
| *P. macrosporum* | BR 479 | 21.4 b | 22.9 a | 22.1 b | 20.7 a | 23.3 a | 22.0 a |
| | DAOM 230396 | 57.8 a | 25.0 a | 41.4 a | 31.9 a | 18.9 a | 25.4 a |
| *P. sylvaticum* | BR 179 | 59.9 a | 32.3 a | 46.1 a | 28.4 a | 21.5 a | 25.0 a |
| | BR 599 | 65.1 a | 38.5 a | 51.8 a | 27.6 a | 23.3 a | 25.4 a |
| *P. ultimum* | BR 1038 | 95.3 a | 95.8 b | 95.6 a | 54.3 a | 26.7 a | 40.5 a |
| | BR 1054 | 95.3 a | 97.9 ab | 96.6 a | 59.5 a | 37.1 a | 48.3 a |
| | BR 144 | 95.3 a | 100.0 a | 97.7 a | 59.5 a | 31.9 a | 45.7 a |
| | BR 600 | 95.3 a | 100.0 a | 97.7 a | 56.9 a | 38.8 a | 47.8 a |
| | DAOM 232337 | 95.3 a | 100.0 a | 97.7 a | 60.3 a | 38.8 a | 49.6 a |

[a]Data were means of two trials. Means followed by the same letter in a column among isolates under each *Pythium* species were not significantly different at $P = 0.05$.

**A**

**B**

**Fig. 1.** Symptoms of soybean seed rot and damping-off caused by *Pythium* species. (A). Seed rot seven-days after inoculation with *Pythium ultimum* (left) and untreated control (right); (B). Pythium damping-off in a flooded field in Ontario.

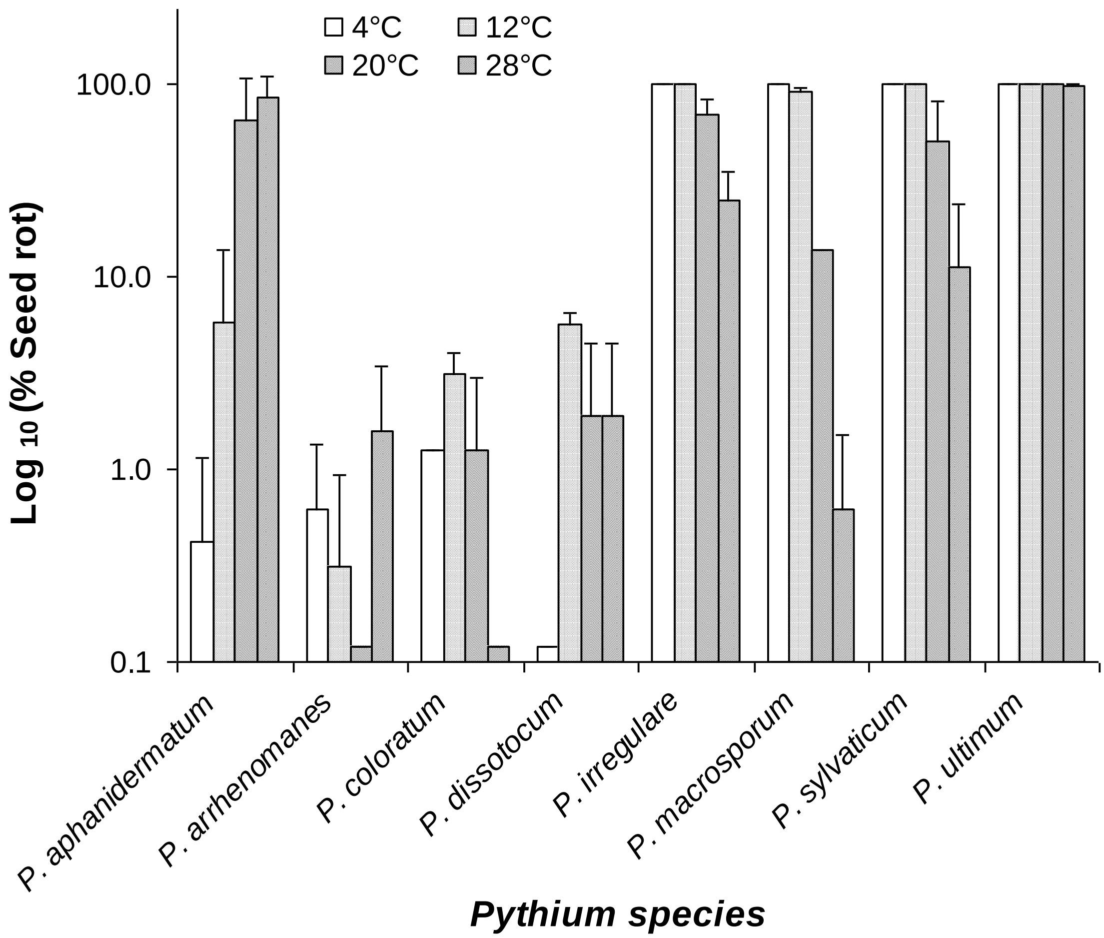


**Fig. 2.** Quantitative differences in percentage of soybean seed rot caused by eight *Pythium* species as affected by temperatures seven days after inoculation. The percent seed rot for each species was the mean of two experiments. Lines above bars represent standard deviations.

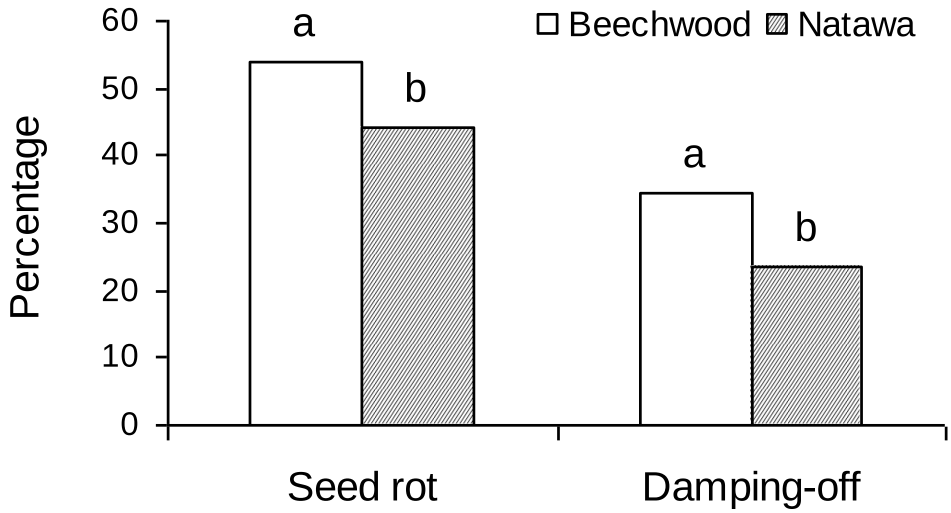


**Fig. 3.** Differences between two soybean cultivars Beechwood and Natawa in susceptibility of Pythium seed rot and damping-off. The percent seed rot and damping-off for each cultivar was the mean of 24 *Pythium* isolates in each of the two experiments. Different letters above bars for each disease represent a significantly difference at $P = 0.05$.