Pathogenicity of *Pythium* species causing seed rot and
damping-off in soybean under controlled conditions
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pourriture de racine et la fonte des semis chez la fève de soja
en conditions contrôlées

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Résumé de l’article
Les espèces de *Pythium* provoquent la pourriture de racine (PR) et la fonte des
semis (FS) chez la fève de soja dans le monde entier. Dans une étude
précédente, des espèces de *Pythium* ont été isolées à partir de plants de fève de
soja infectés en Ontario et au Québec, mais leur pouvoir pathogène n’a pas été
évalué. Dans la présente recherche, le pouvoir pathogène de 24 isolats de huit
espèces de *Pythium* a été évalué relativement à leur capacité de provoquer la
PR et la FS dans des serres; l’effet de la température sur leur capacité de
provoquer la PR a également été étudié. Il y avait des différences significatives
entre les huit espèces de *Pythium* pour la PR et la FS. À 25°C, *P. ultimum*
détenait le plus grand pouvoir pathogène, provoquant 97,0 % de PR et 46,4 %
de FS, en moyenne, chez les deux cultivars utilisés. *Pythium aphanidermatum*
détenait le deuxième plus grand pouvoir pathogène, provoquant 88,5 % de PR
et 41,8 % de FS. Des deux cultivars utilisés dans ces essais, ‘Beechwood’ était
significativement plus susceptible que ‘Nattawa’ à la PR et à la FS. La
température a eu un effet significatif sur la PR. Pour les quatre températures
evaluées (4°C, 12°C, 20°C et 28°C), *P. ultimum* détenait un important pouvoir
pathogène, alors que *P. arrenomanes, P. coloratum* et *P. dissotocum* étaient
les moins pathogènes. L’influence de la température était plus prononcée chez *P.
aphanidermatum*, qui montrait un pourcentage élevé de PR avec une
augmentation de la température, et chez *P. irregulare, P. macrosporum* et *P.
sylvaticum*, qui ont montré une diminution de PR avec une augmentation de la
température.
Pathogenicity of *Pythium* species causing seed rot and damping-off in soybean under controlled conditions

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*Pythium* species cause seed rot (SR) and damping-off (DO) in soybean worldwide. In a previous study, a number of *Pythium* species were isolated from infected soybean plants across Ontario and Quebec, but their comparative pathogenicities to soybean were not examined. In the present research, 24 isolates from eight *Pythium* spp. were evaluated for their pathogenicity in causing soybean SR and DO in a greenhouse environment. The effect of temperature on the ability of these isolates to cause SR was also studied. There were significant differences among the eight *Pythium* spp. for both SR and DO. When tested at 25°C, *Pythium ultimum* was the most pathogenic species, causing 97.0% SR and 46.4% DO, on average, in the two soybean cultivars used. *Pythium aphanidermatum* was the second most pathogenic species, resulting in 88.5% SR and 41.8% DO. The two species resulted in significantly greater SR and DO than the other six species tested and were considered highly pathogenic. Of the two cultivars used in these trials, 'Beechwood' was significantly more susceptible than 'Nattawa' to both SR and DO. Temperature had a significant influence on SR caused by *Pythium* spp. At all four temperatures tested (4°C, 12°C, 20°C and 28°C), *P. ultimum* was highly pathogenic, while *P. arrenomanes*, *P. coloratum* and *P. dissotocum* were the least pathogenic. The interactions between temperature and *Pythium* spp. were more pronounced for *P. aphanidermatum*, which showed an increased percentage of SR with an increase in temperature, and for *P. irregulare*, *P. macrosporum* and *P. sylvaticum*, which showed a decreased percentage of SR with an increase in temperature.

Keywords: damping-off, *Glycine max*, pathogenicity, *Pythium* spp., seed rot, soybean.

[Pouvoir pathogène d’espèces de *Pythium* provoquant la pourriture de racine et la fonte des semis chez la fève de soja en conditions contrôlées]

Les espèces de *Pythium* provoquent la pourriture de racine (PR) et la fonte des semis (FS) chez la fève de soja dans le monde entier. Dans une étude précédente, des espèces de *Pythium* ont été isolées à partir de plants de fève de soja infectés en Ontario et au Québec, mais leur pouvoir pathogène n’a pas été évalué. Dans la présente recherche, le pouvoir pathogène de 24 isolats de huit espèces de *Pythium* a été évalué relativement à leur capacité de provoquer la PR et la FS dans des serres; l’effet de la température sur leur capacité de provoquer la PR a également été étudié. Il y avait des différences significatives entre les huit espèces de *Pythium* pour la PR et la FS. À 25°C, *P. ultimum* détenait le plus grand pouvoir pathogène, provoquant 97,0 % de PR et 46,4 % de FS, en moyenne, chez les deux cultivars utilisés. *Pythium aphanidermatum* détenait le deuxième plus grand pouvoir pathogène, provoquant 88,5 % de PR et 41,8 % de FS. Des deux cultivars utilisés dans ces essais, ‘Beechwood’ était significativement plus susceptible que ‘Nattawa’ à la PR et à la FS. La température a eu un effet significatif sur la PR. Pour les quatre températures évaluées (4°C, 12°C, 20°C et 28°C), *P. ultimum* détenait un important pouvoir pathogène, alors que *P. arrenomanes*, *P. coloratum* et *P. dissotocum* étaient les moins pathogènes. L’influence de la température était plus prononcée chez *P. aphanidermatum*, qui montrait un pourcentage élevé de PR avec une augmentation de la température, et chez *P. irregulare*, *P. macrosporum* et *P. sylvaticum*, qui ont montré une diminution de PR avec une augmentation de la température.

INTRODUCTION

*Pythium* spp. are capable of causing plant diseases individually, but several species are frequently isolated from a single plant (Dorrance et al. 2004). Typical symptoms of infection by *Pythium* spp. 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 (Rosso et al. 2008; Yang 1999).

*Pythium* root rot of soybean, commonly referred to as *Pythium* complex, is found in all soybean-producing regions of the world (Yang 1999). *Pythium* complex is a serious problem for soybean seedling establishment in the USA, and disease severity increases with cool and moist conditions, minimum tillage and earlier planting (Broders et al. 2007).

Previous studies have shown that a number of *Pythium* spp. might be pathogenic to soybean (Brown and Kennedy 1965; Thomson et al. 1971; Zhang and Yang 2000). Van der Plaats-Niterink (1981) reported that *Pythium* species, including *P. aphanidermatum* (Edson) Fitzp., *P. irregulare* Buisman, *P. oligandrum* Drechsler, *P. ultimum* Trow, *P. vexans* de Bary, and group HS (hyphal swellings), were associated with soybean roots. Bates et al. (2008) demonstrated that these species were pathogenic in soybean. In addition, Kirkpatrick et al. (2006a) reported that 47% of 208 selected isolates of *Pythium* spp. were pathogenic to soybean and were moderately to highly aggressive, based on plant emergence and root discoloration. Zhang and Yang (2000) showed that the population of *Pythium* spp. collected from corn-soybean rotation fields contained high frequencies of isolates pathogenic to both crops.

There is no information on the different levels of aggressiveness among isolates within a *Pythium* sp. to soybean. Studies in other crops have shown that isolates within a *Pythium* sp. can vary in aggressiveness (Chagnon and Bélanger 1991; Hendrix and Campbell 1973; McCarter and Littrell 1970; Zhang and Yang 2000). Moorman and Kim (2004) demonstrated that several *P. irregulare* isolates were highly pathogenic to geranium (*Pelargonium × hortorum*), while others were relatively weakly pathogenic.

Temperature is another important factor affecting the pathogenicity of *Pythium* spp. *Pythium debaryanum* Auct. Non R. Hesse and *P. ultimum* greatly reduced soybean seed germination below 24°C (Thomson et al. 1971). *Pythium aphanidermatum* reduced seed germination only above 20°C (Ben-Yephet and Nelson 1999; Thomson et al. 1971), and *P. irregulare* caused cucumber damping-off only from 20 to 24°C (Ben-Yephet and Nelson, 1999). Abad et al. (1994) reported that isolates of *P. volutum* Vanterp. & Truscott from North Carolina were more aggressive on turf grass from 28 to 32°C than at 16°C, while Feng and Dernoen (1999) reported that *P. volutum* isolates from Maryland were more aggressive on bentgrass at 18°C than at 28°C.

The method commonly used for assessing the pathogenicity of *Pythium* spp. in causing seed rot involves placing seeds directly on a *Pythium* culture growing in a Petri dish (Broders et al. 2007; Brown and Kennedy 1965; Dorrance et al. 2004; Thomson et al. 1971; Zhang and Yang 2000). Pre-emergence damping-off is commonly assessed using a pot assay, in which inoculum is mixed with soil or another growing medium and seeds are subsequently planted in the medium (Ali-Shtayeh et al. 2003; Bates et al. 2008; Broders et al. 2007; Kirkpatrick et al. 2006a, 2006b; Zhang and Yang 2000). Alternatively, a plug of *Pythium* isolate can be placed directly on the hypocotyl to induce infection (Rosso et al. 2008; Thomson et al. 1971).

The objectives of this study were to compare the pathogenicity of 24 isolates from eight *Pythium* spp. in causing seed rot (SR) and damping-off (DO) in soybean and to determine the influence of temperature on SR.

MATERIALS AND METHODS

*Pythium* isolates and soybean cultivars

Twenty-four isolates from eight *Pythium* spp., including *P. aphanidermatum* (3 isolates), *P. arrenomanes* Drechs. (4), *P. coloratum* Vaartaja (2), *P. dissotocum* Drechs. (2), *P. irregulare* (4), *P. macrosporum* Vaartaja & Plaäts-nit. (2), *P. sylvaticum* W.A. Campbell & J.W. Hendrix (2), and *P. ultimum* (5), were obtained from the Canadian Collection of Fungal Cultures (CCFC) located at the Eastern Cereal and Oilseed Research Centre (ECORC) of Agriculture and Agri-Food Canada (AAFC) and used in the study. These *Pythium* isolates were either recovered from the soil or unknown species of field and horticultural crops from different geographic regions of British Columbia (13), Alberta (2), Manitoba (2), Ontario (3) and Quebec (4) from 1973 to 2002. The isolates had been preserved in liquid nitrogen since their deposition at the CCFC and were cultured at 28°C on V8 agar (100 mL V8 juice, 0.6 g CaCO₃, and 20 g agar L⁻¹) both in slants and Petri dishes for use in the present study. Pure cultures of these isolates were confirmed by internal transcribed spacer (ITS) sequencing for species identity at the CCFC Research Laboratory. Cultures were maintained on V8 agar at 4°C and transferred every 3 mo for a maximum of three transfers during the course of this study.

Soybean cultivars Beechwood and Nattawa were used in the experiments to evaluate the comparative pathogenicity of the eight *Pythium* spp., and cultivar PS50 was used to determine the influence of temperature on their pathogenicity. ‘Beechwood’ and ‘PS50’ are considered susceptible and ‘Nattawa’ is moderately resistant to root rot under field conditions (E.R. Cober, AAFC, pers. comm.). Seeds of these soybean cultivars were provided by the AAFC soybean breeding program.

Seed rot (SR) tests

A 5-mm² V8 agar plug of *Pythium* isolate was placed at the centre of a 9-cm Petri dish containing 20 mL water agar. Petri dishes were kept at 22°C for 3 d, then 10 seeds of soybean cultivar were added to each plate. Seeds were spaced equally, approximately 2-cm apart, in each Petri dish. The seeds were previously surface sterilized in 0.25% sodium hypochlorite.
solution (The Clorox Company, Oakland, CA, USA) for 1 min, then rinsed in sterile distilled water. The Petri dishes were incubated at 25°C with a 12 h photoperiod at a light intensity of 250 mol.m⁻².s⁻¹ for 7 d, and the number of rotted seeds per plate was recorded. Four plates that were inoculated with sterile V8 agar plugs for each of the two soybean cultivars were included as controls for the existence of possible extraneous airborne or seedborne inoculum. SR was calculated based on four replicate Petri dishes for each isolate by cultivar combination in each experiment. The experiment was arranged in a two-factor (species and cultivar) nested design, with isolates nested within species and repeated once.

**Damping-off (DO) tests**

Six 1-cm² plugs of V8 agar with 3-d-old cultures of each *Pythium* isolate were placed in 500 mL flasks containing 200 mL of sand (U.S. Silica Company, Berkeley Springs, WV, USA), 11.2 mL of corn meal, and 80 mL of deionized water that had been autoclaved for 40 min and then autoclaved again 24 h later to prepare a large quantity of inoculum for greenhouse inoculation. The isolates were allowed to colonize the sand-cornmeal medium at room temperature for 9 d prior to being used for inoculation. The flasks were shaken every other day to ensure uniform colonization.

The soybean seeds were surface sterilized as previously described, then soaked in sterile distilled water for approximately 6 h. Seeds were kept moist and at room temperature for 2 d until germination.

Plants were grown in planting trays (58 cm x 28 cm x 8 cm) consisting of 72 cells (4.5 cm x 4.5 cm x 5 cm). The cells were each filled with a base layer of 30 g of Pro-mix soil (Plant Products Ltd., Brampton, ON, Canada), followed by a layer of 2.5 g of *Pythium* inoculum. Two germinated soybean seeds per cell were planted directly on the inoculum and covered with an additional 2 g of Pro-mix soil. Four replicate planting trays for each isolate by cultivar combination were used in each experiment. The trays were placed in the greenhouse with a 16-h photoperiod and temperature of 24°C during the day and 18°C at night. Trays were watered once per day to maintain soil moisture. The number of seedlings that emerged and survived after emergence was recorded 10 d after planting.

Four planting trays that were inoculated with sterile sand-cornmeal medium for each of the two soybean cultivars were included as controls for the presence of possible extraneous soilborne inoculum. The calculation of the percentage of DO and the experimental design were the same as the ones described earlier for SR tests, and the experiment was repeated once.

**Effect of temperature**

The effect of temperature on the pathogenicity of the eight *Pythium* spp. in causing SR was examined at 4°C, 12°C, 20°C and 28°C in growth cabinets. The PS50 soybean seed used in this experiment was surface sterilized as described earlier for the SR tests. Seed placement and seed rot assessment method also were the same. For each isolate and temperature combination, four replicate Petri dishes were assessed for SR 7 d after plating in each experiment.

Petri dishes were arranged in a completely randomized design in each growth cabinet, and the experiment was repeated once.

**Statistical analyses**

Residuals for each parameter were examined for normality and homogeneity of variances. An angular transformation of percent reductions in SR and DO was used in the analysis of variance to stabilize variances (Snedecor and Cochran 1980). Treatment means of the untransformed data were presented and separated by Fisher’s least significant difference (LSD) test at a probability level of *P* < 0.05. Data from the repeated experiments were analyzed separately and in a combined analysis using SAS/STAT® mixed models (Littell et al. 1996) with experiments and interactions with experiments considered as random effects. Heterogeneity of variances among experiments was checked for each parameter with a likelihood ratio test (LRT) comparing the difference of -2 log likelihood of a homogeneous and heterogeneous variance model with the χ² distribution with 2 degrees of freedom. The significance of interactions with experiments were also examined with LRT by comparing models with and without selected interactions (Wolfinger 1993). Non-significant interactions were pooled with error. Contrasts were used to compare the two isolates within each species of *P. coloratum*, *P. dissotocum*, *P. macrosporum* and *P. sylvaticum*, and significance is at the probability level *P* ≤ 0.05 unless otherwise indicated, based on the analyses of transformed data.

**RESULTS**

Pathogenicity of *Pythium* spp.

Significant differences (*P* < 0.01) were observed in both SR and DO among the eight *Pythium* spp. between the two soybean cultivars and in *Pythium* spp. x cultivar interactions (Table 1). The effects of *Pythium* spp. x cultivar interactions, although statistically significant, were relatively small and represented less than 5% of the total treatment effects for both SR and DO. As a result, the pathogenicity of *Pythium* spp. was calculated based on each soybean cultivar and the mean of the two cultivars, and vice versa for the differences in susceptibility to *Pythium* spp. between the two cultivars. Significant differences among isolates within species were observed in SR for *P. aphanidermatum*, *P. arrenomanes*, *P. irregularare* and *P. macrosporum*, and significant cultivar x isolate interactions were observed for *P. dissotocum* and *P. macrosporum*.

The eight *Pythium* spp. showed different levels of pathogenicity to soybean, with SR ranging from 28.1 to 95.3% in ‘Beachwood’ and from 20.8 to 98.8% in ‘Nattawa’, and DO ranging from 25.4 to 58.1% in ‘Beachwood’ and from 18.5 to 34.6% in ‘Nattawa’ (Table 2). On average for the two cultivars, *P. ultimum* had the greatest SR (97.0%) and DO (46.4%), followed...
Table 1. Mean squares from the analysis of variance for the effect of *Pythium* species, isolates within species, soybean cultivar, and their interactions in seed rot (SR) and damping-off (DO)

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>SR</th>
<th>DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial</td>
<td>1</td>
<td>153.5</td>
<td>2314.5</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>808.9 **</td>
<td>6632.4 **</td>
</tr>
<tr>
<td>Error A</td>
<td>1</td>
<td>715.1</td>
<td>1972.6</td>
</tr>
<tr>
<td><em>Pythium</em> species (S)</td>
<td>7</td>
<td>25000.1 **</td>
<td>2155.0 **</td>
</tr>
<tr>
<td>Isolate within <em>Pythium</em> species (I (S))</td>
<td>16</td>
<td>375.8 **</td>
<td>71.3</td>
</tr>
<tr>
<td><em>P. aphanidermatum</em> (2)</td>
<td></td>
<td>1151.8 **</td>
<td>262.6</td>
</tr>
<tr>
<td><em>P. arrenomanes</em> (3)</td>
<td></td>
<td>145.6 *</td>
<td>25.6</td>
</tr>
<tr>
<td><em>P. coloratum</em> (1)</td>
<td></td>
<td>47.1</td>
<td>140.3</td>
</tr>
<tr>
<td><em>P. dissoticum</em> (1)</td>
<td></td>
<td>0.0</td>
<td>4.2</td>
</tr>
<tr>
<td><em>P. irregulare</em> (3)</td>
<td></td>
<td>587.2 **</td>
<td>20.1</td>
</tr>
<tr>
<td><em>P. macrosporum</em> (1)</td>
<td></td>
<td>1169.9 **</td>
<td>29.3</td>
</tr>
<tr>
<td><em>P. sylvaticum</em> (1)</td>
<td></td>
<td>119.7</td>
<td>0.2</td>
</tr>
<tr>
<td><em>P. ultimum</em> (4)</td>
<td></td>
<td>43.4 *</td>
<td>76.2</td>
</tr>
<tr>
<td>C x S</td>
<td>7</td>
<td>1284.9 **</td>
<td>377.3 **</td>
</tr>
<tr>
<td>C x I (S)</td>
<td>16</td>
<td>92.9 *</td>
<td>91.6</td>
</tr>
<tr>
<td><em>P. aphanidermatum</em> (2)</td>
<td></td>
<td>31.1</td>
<td>171.7</td>
</tr>
<tr>
<td><em>P. arrenomanes</em> (3)</td>
<td></td>
<td>41.4</td>
<td>110.4</td>
</tr>
<tr>
<td><em>P. coloratum</em> (1)</td>
<td></td>
<td>7.3</td>
<td>6.1</td>
</tr>
<tr>
<td><em>P. dissoticum</em> (1)</td>
<td></td>
<td>170.1 *</td>
<td>240.6</td>
</tr>
<tr>
<td><em>P. irregulare</em> (3)</td>
<td></td>
<td>15.0</td>
<td>65.6</td>
</tr>
<tr>
<td><em>P. macrosporum</em> (1)</td>
<td></td>
<td>902.8 **</td>
<td>230.8</td>
</tr>
<tr>
<td><em>P. sylvaticum</em> (1)</td>
<td></td>
<td>0.6</td>
<td>8.3</td>
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<tr>
<td><em>P. ultimum</em> (4)</td>
<td></td>
<td>43.4</td>
<td>27.1</td>
</tr>
<tr>
<td>Error B</td>
<td>334</td>
<td>47.1</td>
<td>74.3</td>
</tr>
</tbody>
</table>

* Seed rot and damping-off expressed in percentage were angular-transformed before the analysis of variance was performed; ** = P < 0.01; * = P < 0.05; no asterisk = P > 0.05.

Figure 1. Quantitative differences in percentage of soybean seed rot caused by eight *Pythium* spp. as affected by temperature 7 d after inoculation. The percentage of seed rot for each species is the mean of two to five isolates and 40 seeds per isolate in each of the two experiments. Vertical bars represent standard deviations.
### Table 2. Variations among isolates and species of eight *Pythium* spp. causing seed rot (SR) and damping-off (DO) in two soybean cultivars

<table>
<thead>
<tr>
<th>Pythium spp.</th>
<th>Isolate</th>
<th>Beechwood (%)</th>
<th>Nattawa (%)</th>
<th>Mean (%)</th>
<th>Beechwood (%)</th>
<th>Nattawa (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>BR 444</td>
<td>87.0 b</td>
<td>94.8 a</td>
<td>90.9 ab</td>
<td>59.5 a</td>
<td>37.9 a</td>
<td>48.7 a</td>
</tr>
<tr>
<td></td>
<td>BR 740</td>
<td>76.6 c</td>
<td>80.2 b</td>
<td>78.4 b</td>
<td>50.8 a</td>
<td>28.4 ab</td>
<td>39.6 a</td>
</tr>
<tr>
<td></td>
<td>BR 910</td>
<td>94.3 a</td>
<td>97.9 a</td>
<td>96.1 a</td>
<td>56.9 a</td>
<td>17.2 b</td>
<td>37.1 a</td>
</tr>
<tr>
<td><em>P. arrenomanes</em></td>
<td>BR 1028</td>
<td>31.8 ab</td>
<td>17.7 b</td>
<td>24.7 ab</td>
<td>29.3 a</td>
<td>18.9 a</td>
<td>24.1 a</td>
</tr>
<tr>
<td></td>
<td>BR 122</td>
<td>34.9 a</td>
<td>27.1 a</td>
<td>31.0 a</td>
<td>18.1 b</td>
<td>21.5 a</td>
<td>19.8 a</td>
</tr>
<tr>
<td></td>
<td>BR 981</td>
<td>23.4 ab</td>
<td>17.7 b</td>
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<td>27.6 ab</td>
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<td>21.5 a</td>
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<tr>
<td></td>
<td>BR 985</td>
<td>22.4 b</td>
<td>20.8 ab</td>
<td>21.6 b</td>
<td>26.7 ab</td>
<td>18.1 a</td>
<td>22.4 a</td>
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<tr>
<td><em>P. coloratum</em></td>
<td>BR 621</td>
<td>26.6 a</td>
<td>19.8 a</td>
<td>23.2 a</td>
<td>22.4 a</td>
<td>16.4 a</td>
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<td>30.2 a</td>
<td>22.4 a</td>
<td>26.3 a</td>
</tr>
<tr>
<td><em>P. dissoticum</em></td>
<td>BR 1048</td>
<td>35.9 a</td>
<td>19.8 a</td>
<td>27.9 a</td>
<td>30.2 a</td>
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</tr>
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<td>27.6 a</td>
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<td><em>P. irregularare</em></td>
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<td>40.6 ab</td>
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<td>44.0 bc</td>
<td>32.7 a</td>
<td>19.8 a</td>
<td>26.3 a</td>
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<td></td>
<td>BR 901</td>
<td>52.6 b</td>
<td>27.1 c</td>
<td>39.8 c</td>
<td>25.0 a</td>
<td>25.8 a</td>
<td>25.4 a</td>
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<td>BR 479</td>
<td>21.4 b</td>
<td>22.9 a</td>
<td>22.1 b</td>
<td>20.7 a</td>
<td>23.3 a</td>
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<tr>
<td></td>
<td>DOAM 230396</td>
<td>57.8 a</td>
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<td><em>P. sylvaticum</em></td>
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<td>32.3 a</td>
<td>46.1 a</td>
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<td>21.5 a</td>
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</tr>
<tr>
<td></td>
<td>BR 599</td>
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<td>38.5 a</td>
<td>51.8 a</td>
<td>27.6 a</td>
<td>23.3 a</td>
<td>25.4 a</td>
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<tr>
<td><em>P. ultimum</em></td>
<td>BR 1038</td>
<td>95.3 a</td>
<td>95.8 b</td>
<td>95.6 a</td>
<td>54.3 a</td>
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<td></td>
<td>BR 1054</td>
<td>95.3 a</td>
<td>97.9 ab</td>
<td>96.6 a</td>
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<td>37.1 a</td>
<td>48.3 a</td>
</tr>
<tr>
<td></td>
<td>BR 144</td>
<td>95.3 a</td>
<td>100.0 a</td>
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</tr>
<tr>
<td></td>
<td>BR 600</td>
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<td>100.0 a</td>
<td>97.7 a</td>
<td>56.9 a</td>
<td>38.8 a</td>
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<tr>
<td></td>
<td>DAOM 232337</td>
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<td>97.7 a</td>
<td>60.3 a</td>
<td>38.8 a</td>
<td>49.6 a</td>
</tr>
</tbody>
</table>

**Species average**

- *P. aphanidermatum*: 85.9 b, 91.0 b, 88.5 b, 55.7 a, 27.9 ab, 41.8 a
- *P. arrenomanes*: 28.1 e, 20.8 d, 24.5 e, 25.4 b, 18.5 c, 22.0 b
- *P. coloratum*: 29.2 e, 20.8 d, 25.0 e, 26.3 b, 19.4 c, 22.8 b
- *P. dissoticum*: 31.8 de, 23.4 d, 27.6 de, 26.3 b, 20.7 c, 23.5 b
- *P. irregularare*: 60.7 c, 37.8 c, 49.2 c, 29.9 b, 22.6 bc, 26.3 b
- *P. macrosporum*: 39.6 d, 24.0 d, 31.8 d, 26.3 b, 21.1 bc, 23.7 b
- *P. sylvaticum*: 62.5 c, 35.4 c, 49.0 c, 28.0 b, 22.4 bc, 25.2 b
- *P. ultimum*: 95.3 a, 98.8 a, 97.0 a, 58.1 a, 34.6 a, 46.4 a

**Cultivar average**

- Beechwood: 54.1 a, 34.5 a
- Natawa: 44.0 b, 23.4 b

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1. Data are means of two trials. SR and DO expressed in percentage were angular-transformed before the analysis of variance was performed.
2. Means followed by the same letter in a column among isolates under each *Pythium* species, among *Pythium* spp. under species average, or among soybean cultivars under cultivar average were not significantly different at P = 0.05.
3. Accession numbers for isolates maintained in the Canadian Collection of Fungal Cultures.
Table 3. Mean squares from analysis of variance for the effect of temperature on the pathogenicity of eight Pythium spp. in causing seed rot of soybean.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>Mean square a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial</td>
<td>1</td>
<td>9.7</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>3</td>
<td>10225.1 **</td>
</tr>
<tr>
<td>Error A</td>
<td>3</td>
<td>41.5</td>
</tr>
<tr>
<td>Pythium spp. (S)</td>
<td>7</td>
<td>123656.7 **</td>
</tr>
<tr>
<td>Isolate (Pythium spp.) I (S)</td>
<td>16</td>
<td>1197.1 **</td>
</tr>
<tr>
<td>P. aphanidermatum (2)</td>
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<td>7350.5 **</td>
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<tr>
<td>P. arrenomanes (3)</td>
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<td>28.3</td>
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<tr>
<td>P. coloratum (1)</td>
<td></td>
<td>47.8</td>
</tr>
<tr>
<td>P. dissoticum (1)</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>P. irregulare (3)</td>
<td></td>
<td>521.6 *</td>
</tr>
<tr>
<td>P. macrosporum (1)</td>
<td></td>
<td>23.5</td>
</tr>
<tr>
<td>P. sylvaticum (1)</td>
<td></td>
<td>2678.2 **</td>
</tr>
<tr>
<td>P. ultimum (4)</td>
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<td>13.3</td>
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<td>T x S</td>
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<td>14692.6 **</td>
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<tr>
<td>T x I (S)</td>
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<td>324.8 **</td>
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<tr>
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<td>19.5</td>
</tr>
<tr>
<td>P. dissoticum (3)</td>
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<td>346.0 *</td>
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<tr>
<td>P. macrosporum (3)</td>
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<td>59.3</td>
</tr>
<tr>
<td>P. sylvaticum (3)</td>
<td></td>
<td>1035.4 **</td>
</tr>
<tr>
<td>P. ultimum (12)</td>
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</tr>
<tr>
<td>Error B</td>
<td>668</td>
<td>65.9</td>
</tr>
</tbody>
</table>

a Seed rot data expressed in percentage were angular-transformed before the analysis of variance was performed.

b ** = P < 0.01; * = P < 0.05; no asterisk P > 0.05.

by P. aphanidermatum, which caused 88.5% SR and 41.8% DO. These two species resulted in significantly greater SR and DO than the other six species tested and were considered highly pathogenic. The remaining species resulted in low levels of SR (24.5 to 49.2%) and DO (22 to 26.3%) and were considered weakly pathogenic, even though there were significant differences among these species for SR.

Both the Beechwood and Nattawa cultivars were susceptible, but the former showed more severe SR (54.1%) and DO (34.5%) than the latter, which had 44.0% SR and 23.4% DO when averaged over the eight Pythium spp. (Table 2).

Effect of temperature
There were significant differences among temperatures, Pythium spp., and the temperature × Pythium spp. interaction in SR (Table 3). Significant differences were also observed among isolates within species and temperature × isolate interactions for P. aphanidermatum, P. irregulare and P. sylvaticum. At each of the four temperatures tested, all isolates of P. ultimum were highly pathogenic, causing > 96% SR, while isolates of P. arrenomanes, P. coloratum and P. dissoticum were weakly pathogenic, causing < 7% SR (Fig. 1). The remaining four Pythium spp. caused different degrees of SR depending on the temperature. With the increase in temperature from 4°C to 28°C, the percentage of SR caused by P. aphanidermatum increased while that of P. irregulare, P. macrosporum and P. sylvaticum decreased. Pythium aphanidermatum caused < 1% SR at 4°C and 5.8% SR at 12°C, but 65.4% SR at 20°C and 85.4% SR at 28°C. In contrast, P. irregulare, P. macrosporum and P. sylvaticum each caused > 95% SR at 4°C and 12°C, 18.2 to 69.7% SR at 20°C, and only 8.2 to 25.0% SR at 28°C.

DISCUSSION
Of the eight Pythium spp. evaluated, Pythium aphanidermatum, P. irregulare, P. sylvaticum and P. ultimum had previously been reported to be pathogenic to soybean (Bates et al. 2008; Rizvi and Yang 1996; Thomson et al. 1971; Van der Plaats-Niterink 1981; Yang 1999). However, no studies had examined the comparative pathogenicity of these Pythium spp. in causing SR and DO in soybean. The present research demonstrated that only P. aphanidermatum and P. ultimum were highly pathogenic, causing > 88% SR and > 40% DO,
while the other six species tested were weakly patho-
genic at 25°C (Table 2). In addition, this study demon-
strated that P. aphanidermatum, P. irregulare, P. macrosporum and P. sylvaticum are temperature
dependent in causing soybean SR. Of these tempera-
ture-dependent pathogenic species, only P. aphani-
dermatum and P. irregulare had previously been rec-
ognized as pathogens of soybean (Ben-Yephet and
Nelson 1999; Thompson et al. 1971). Pythium aphani-
dermatum, although highly pathogenic at 25°C, showed little or no pathogenicity at 4°C and 12°C (Fig. 1). These results suggest that P. aphanidermatum is probably not responsible for causing soybean root rot and damping-off in regions with short soybean growing seasons where soil temperatures often are below 20°C during crop emergence and the early seedling development stage. In contrast, P. macrosporum, P. irregulare and P. sylvaticum, which were weakly pathogenic at 25°C, were highly pathogenic at low temperatures, causing > 90% SR at both 4°C and 12°C (Fig. 1). These species may have a greater impact on short-season soybean production than P. aphanidermatum. However, the effect of temperature on P. irregulare observed in the present study is somewhat different from that reported by Ben-Yephet and Nelson (1999), who found that P. irregulare caused cucumber seedling damping-off only at 20°C and 24°C. It is possible that different isolates of P. irregu-
lare can have different optimal temperatures for path-
genicity. In addition, P. irregulare is known for its
variable morphological and genetic characters, and several distinct groups and a new species (P. crypto-
irregulare) within the P. irregulare complex have been
reported in recent taxonomic studies (Garzon et al.
2005, 2007; Matsumoto et al. 2000). It is also possible
that the P. irregulare isolates used by Ben-Yephet and
Nelson (1999) came from more than one species.

The high level of pathogenicity of P. macrosporum
to soybean at low temperatures had not been report-
epd previously. Pythium macrosporum has been iso-
lated in several countries, including Canada,
Germany, Japan, the Netherlands and the United
States (Allain-Boule et al. 2004; Uzuhashi et al. 2008;
Barasubiye 1981; Van Os et al. 1999; Westover and
Bever 2001), and is known to cause root rots in flower bulbs (Westover and Bever 2001), grasses (VanOs et al. 1999), and carrot (Allain-Boule et al. 2004). This species was detected in diseased soybean roots using a Pythium DNA array hybridiza-
tion method during an extensive survey for root rot
pathogens in commercial fields of soybean in eastern
Ontario and Quebec (Barasubiye et al. 2005). The high
levels of pathogenicity of the two P. macrosporum
isolates to soybean observed in this research suggest
that soybean could be a potential host for P. macrosporum, which has been identified as a
pathogen in other plant species. This species could
have a significant negative impact on soybean stands
in eastern Ontario, Quebec and southern Manitoba,
where most of the Canadian short-season soybean is
grown, and where soil temperatures are below 20°C
during crop emergence and early stages of plant
growth. Further studies including a large number of P. macrosporum isolates from soybean and various
host plants are needed to better understand the effect
of temperature on P. macrosporum isolates x soy-
bean cultivar interactions.

There were significant differences among isolates
within P. aphanidermatum, P. arrenomanes, P. irregu-
lare and P. macrosporum in causing SR (Tables 2
and 3). These results are in agreement with those of
Martin and Loper (1999) who found that the patho-
genicity responses of Pythium species can be isolate-
specific. The presence of different levels of aggressi-
eness among isolates within the pathogenic
Pythium spp. 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 with low
aggressiveness may not be able to discriminate
between lines with different levels of resistance;
perhaps a mixture of several different isolates should
be used to screen for resistance.

Soybean cultivar resistance to Pythium spp. has
recently been identified (Bates et al. 2008), making
resistance breeding possible and a viable strategy for
managing Pythium SR and DO. Of the two soybean
cultivars used in the pathogenicity experiment in the
present study, Nattawa was significantly more resis-
tant than Beechwood (Table 2). The cultivars’
reactions were in agreement with previous field
observations. Although the cultivar x Pythium spp.
interactions were significant for both SR and DO
(Table 1), the differential responses of the two culti-
vars to the highly pathogenic species were less appa-
rent (Table 2). These results indicate that soy-
bean may share common genes for resistance with
these pathogenic species and that breeding for resis-
tance to one Pythium species may also give enhanced
resistance to other Pythium spp. Further research is
needed to confirm the presence and heritability of
resistance genes in ‘Nattawa’ and their usefulness in
future cultivar development.

ACKNOWLEDGEMENTS

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REFERENCES


