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Pouvoir pathogène d'espèces de *Pythium* provoquant la 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 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.

Pathogenicity of *Pythium* species causing seed rot and damping-off in soybean under controlled conditions

Lai Wei¹, Allen G. Xue²✉, Elroy R. Cober², Carolyn Babcock², Jinxiu Zhang², Shuzhen Zhang¹, Wenbin Li¹, Junjiang Wu³, and Lijun Liu³

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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.

Mots clés : fève de soja, fonte des semis, *Glycine max*, pourriture de racine, pouvoir pathogène, *Pythium* spp.

1. Key Laboratory of Soybean Biology, Chinese Ministry of Education, Northeast Agriculture University, Harbin 150030, China
2. Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada (AAFC), Ottawa (Ontario), Canada K1A 0C6; corresponding author e-mail: allen.xue@agr.gc.ca
3. Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China

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 seedling 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 Dernoeden (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. macrosporium* Vaartaja & Plaats-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 × 28 cm × 8 cm) consisting of 72 cells (4.5 cm × 4.5 cm × 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 for each experiment 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 $P \leq 0.05$, based on the analyses of transformed data. The assumption of normality based on Shapiro Wilk's test was examined using the Univariate Procedure of SAS and the random and homogeneous distribution of residuals was assessed using the Plot Procedure (SAS Institute Inc. 2004). 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 χ^2 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 $P \leq 0.05$ 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. × cultivar interactions (Table 1). The effects of *Pythium* spp. × 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. irregulare* and *P. macrosporum*, and significant cultivar × 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)

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

^a 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 = $P > 0.05$.

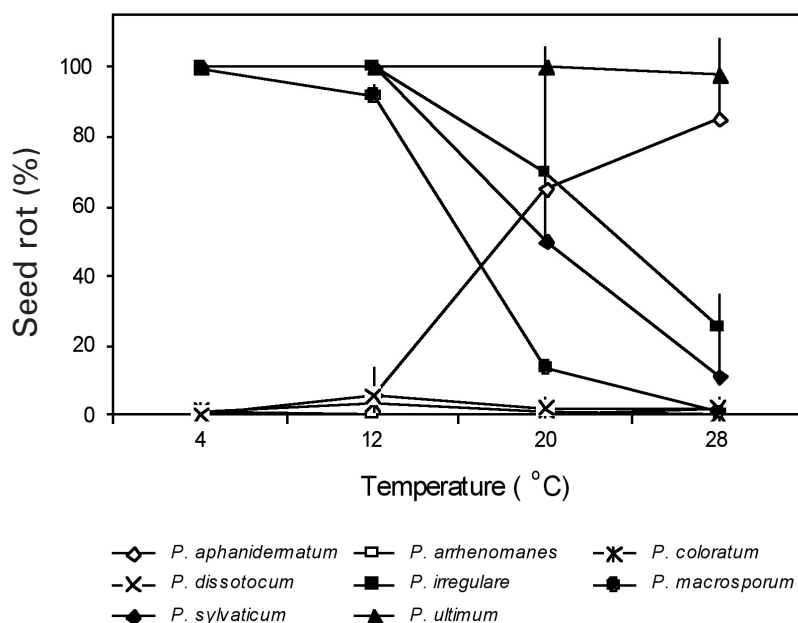


Figure 1. Quantitative differences in percentage of soybean seed rot caused by eight *Pythium* spp. as affected by temperature 7 d after inoculation. The percentage of seed rot for each species is the mean of two to five isolates and 40 seeds per isolate in each of the two experiments. Vertical bars 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^a

<i>Pythium</i> spp.	Isolate ^c	SR (%) ^b			DO (%) ^b		
		Beechwood	Nattawa	Mean	Beechwood	Nattawa	Mean
<i>P. aphanidermatum</i>	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
<i>P. arrenomanes</i>	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
<i>P. coloratum</i>	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
<i>P. dissoticum</i>	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
<i>P. irregulare</i>	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
<i>P. macrosporum</i>	BR 479	21.4 b	22.9 a	22.1 b	20.7 a	23.3 a	22.0 a
	DOAM 230396	57.8 a	25.0 a	41.4 a	31.9 a	18.9 a	25.4 a
<i>P. sylvaticum</i>	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
<i>P. ultimum</i>	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
Species average							
	<i>P. aphanidermatum</i>	85.9 b	91.0 b	88.5 b	55.7 a	27.9 ab	41.8 a
	<i>P. arrenomanes</i>	28.1 e	20.8 d	24.5 e	25.4 b	18.5 c	22.0 b
	<i>P. coloratum</i>	29.2 e	20.8 d	25.0 e	26.3 b	19.4 c	22.8 b
	<i>P. dissoticum</i>	31.8 de	23.4 d	27.6 de	26.3 b	20.7 c	23.5 b
	<i>P. irregulare</i>	60.7 c	37.8 c	49.2 c	29.9 b	22.6 bc	26.3 b
	<i>P. macrosporum</i>	39.6 d	24.0 d	31.8 d	26.3 b	21.1 bc	23.7 b
	<i>P. sylvaticum</i>	62.5 c	35.4 c	49.0 c	28.0 b	22.4 bc	25.2 b
	<i>P. ultimum</i>	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
	Nattawa			44.0 b			23.4 b

^a Data are means of two trials. SR and DO expressed in percentage were angular-transformed before the analysis of variance was performed.

^b 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 $P = 0.05$.

^c 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^a

Source of variance	DF	Mean square ^b
Trial	1	9.7
Temperature (T)	3	10225.1 **
Error A	3	41.5
<i>Pythium</i> spp. (S)	7	123656.7 **
Isolate (<i>Pythium</i> spp.) I (S)	16	1197.1 **
<i>P. aphanidermatum</i>	(2)	7350.5 **
<i>P. arrenomanes</i>	(3)	28.3
<i>P. coloratum</i>	(1)	47.8
<i>P. dissoticum</i>	(1)	1.0
<i>P. irregulare</i>	(3)	521.6 *
<i>P. macrosporum</i>	(1)	23.5
<i>P. sylvaticum</i>	(1)	2678.2 **
<i>P. ultimum</i>	(4)	13.3
T x S	21	14692.6 **
T x I (S)	48	324.8 **
<i>P. aphanidermatum</i>	(6)	1453.9 **
<i>P. arrenomanes</i>	(9)	22.6
<i>P. coloratum</i>	(3)	19.5
<i>P. dissoticum</i>	(3)	15.2
<i>P. irregulare</i>	(9)	346.0 *
<i>P. macrosporum</i>	(3)	59.3
<i>P. sylvaticum</i>	(3)	1035.4 **
<i>P. ultimum</i>	(12)	13.3
Error B	668	65.9

^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. dissotocum* 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 pathogenic at 25°C (Table 2). In addition, this study demonstrated that *P. aphanidermatum*, *P. irregulare*, *P. macrosporum* and *P. sylvaticum* are temperature dependent in causing soybean SR. Of these temperature-dependent pathogenic species, only *P. aphanidermatum* and *P. irregulare* had previously been recognized as pathogens of soybean (Ben-Yephet and Nelson 1999; Thompson *et al.* 1971). *Pythium aphanidermatum*, 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. irregulare* can have different optimal temperatures for pathogenicity. In addition, *P. irregulare* is known for its variable morphological and genetic characters, and several distinct groups and a new species (*P. cryptoirregulare*) 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 reported previously. *Pythium macrosporum* has been isolated in several countries, including Canada, Germany, Japan, the Netherlands and the United States (Allain-Boule *et al.* 2004; Uzuhashi *et al.* 2008; Van der Plaats-Niterink 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 (Van Os *et al.* 1999), and carrot (Allain-Boule *et al.* 2004). This species was detected in diseased soybean roots using a *Pythium* DNA array hybridization 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 × soybean cultivar interactions.

There were significant differences among isolates within *P. aphanidermatum*, *P. arrenomanes*, *P. irregulare* 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 pathogenicity responses of *Pythium* species can be isolate-specific. The presence of different levels of aggressiveness 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 resistant than Beechwood (Table 2). The cultivars' reactions were in agreement with previous field observations. Although the cultivar × *Pythium* spp. interactions were significant for both SR and DO (Table 1), the differential responses of the two cultivars to the highly pathogenic species were less apparent (Table 2). These results indicate that soybean may share common genes for resistance with these pathogenic species and that breeding for resistance 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.

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REFERENCES

- Abad, Z.G., H.D. Shew, and L.T. Lucas. 1994. Characterization and pathogenicity of *Pythium* species isolated from turf grass with symptoms of root and crown rot in North Carolina. *Phytopathology* 84 : 913-921.
- Ali-Shtayeh, M., A.M.A. Salah, and R.M. Jamous. 2003. Ecology of hymexazol-insensitive *Pythium* species in field soils. *Mycopathologia* 156 : 333-342.
- Allain-Boule, N., C.A. Lévesque, C. Martinet, R.R. Bélanger, and R.J. Tweddell. 2004. Identification of *Pythium* species associated with cavity spot lesions on carrots in eastern Quebec. *Can. J. Plant Pathol.* 26 : 365-370.
- Barasubiye, T., K.A. Seifert, A.U. Tenuta, S. Rioux, T.R. Anderson, and C.A. Lévesque. 2005. Application of DNA methods to identify and detect *Pythium*, *Phytophthora*, and *Fusarium* spp. associated with soybean root rot in eastern Ontario and Québec. *Can. J. Plant Pathol.* 27 : 464-465.

- Bates, G.D., C.S. Rothrock, and J.C. Rupe. 2008.** Resistance of the soybean cultivar Archer to *Pythium* damping-off and root rot caused by several *Pythium* spp. Plant Dis. 92 : 763-766.
- Ben-Yephet, Y., and E.B. Nelson. 1999.** Differential suppression of damping-off caused by *Pythium aphanidermatum*, *P. irregulare*, and *P. myiotylum* in composts at different temperatures. Plant Dis. 83 : 356-360.
- Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007.** Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91 : 727-735.
- Brown, G.E., and B.W. Kennedy. 1965.** *Pythium* pre-emergence damping-off of soybean in Minnesota. Plant Dis. Rep. 49 : 646.
- Chagnon, M.C., and R.R. Bélanger. 1991.** Tolerance in greenhouse geraniums to *Pythium ultimum*. Plant Dis. 75 : 820-823.
- Dorrance, A.E., S.A. Berry, P. Browen, and P.E. Lipps. 2004.** Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Plant Health Progress (online) doi: 10.1094/PHP-2004-0202-01-RS.
- Feng, Y., and P.H. Dernoeden. 1999.** *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. Plant Dis. 83 : 516-520.
- Garzón, C.D., D.M. Geiser, and G.W. Moorman. 2005.** Amplified fragment length polymorphism analysis and internal transcribed spacer and cox II sequences reveal a species boundary within *Pythium irregulare*. Phytopathology 95 : 1489-1498.
- Garzón, C.D., J.M. Yáñez, and G.W. Moorman. 2007.** *Pythium cryptoirregulare*, a new species within the *P. irregulare* complex. Mycologia 99 : 291-301.
- Hendrix, F.F., and W.A. Campbell. 1973.** *Pythium* as plant pathogens. Annu. Rev. Phytopathol. 11 : 78-98.
- Kirkpatrick, M.T., J.C. Rupe, and C.S. Rothrock. 2006a.** Soybean response to flooded soil conditions and the association with soilborne plant pathogenic genera. Plant Dis. 90 : 592-596.
- Kirkpatrick, M.T., C.S. Rothrock, J.C. Rupe, and E.E. Gbur. 2006b.** The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90 : 597-602.
- Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996.** SAS System for Mixed Models. SAS Institute Inc., Cary, NC, USA.
- Martin, F.N., and J.E. Loper. 1999.** Soilborne plant disease caused by *Pythium* spp.: ecology, epidemiology and prospects for biological control. Plant Sci. 18 : 111-181.
- Matsumoto, C., K. Kageyama, H. Suga, and M. Hyakumachi. 2000.** Intraspecific DNA polymorphisms of *Pythium irregulare*. Mycol. Res. 104 : 1333-1341.
- McCarter, S.M., and R.H. Littrell. 1970.** Comparative pathogenicity of *Pythium aphanidermatum* and *Pythium myriotylum* to twelve plant species and intraspecific variation in virulence. Phytopathology 60 : 264-268.
- Moorman, G.W., and S.H. Kim. 2004.** Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. Plant Dis. 88 : 630-632.
- Rizvi, S.S.A., and X.B. Yang. 1996.** Fungi associated with soybean seedling disease in Iowa. Plant Dis. 80 : 57-60.
- Rosso, M.L., J.C. Rupe, P. Chen, and L.A. Mozzoni. 2008.** Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. Crop Sci. 48 : 2215-2222.
- SAS Institute Inc. 2004.** SAS/STAT® Users' Guide. Cary, NC, USA. 5121 p.
- Snedecor, G.W., and W.G. Cochran. 1980.** Statistical Methods, 8th ed. The Iowa State University Press, Ames, IA, USA. 503 p.
- Thomson, T.B., K.L. Athow, and F.A. Laviolette. 1971.** The effect of temperature on the pathogenicity of *Pythium aphanidermatum*, *P. debaryanum*, and *P. ultimum* on soybean. Phytopathology 61 : 933-935.
- Uzuhashi, S., M. Tojo, S. Kobayashi, K. Tokura, and M. Kakishima. 2008.** First records of *Pythium aquatile* and *P. macrosporum* isolated from soils in Japan. Mycoscience 49 : 276-279.
- Van der Plaats-Niterink, A.J. 1981.** Monograph of the genus *Pythium*. Stud. Mycol. 21 : 1-239.
- Van Os, G.J., J.P.M. Wijnker, and W.J.M. van Gulik. 1999.** Effects of soil fumigation and flooding on suppression of *Pythium* root rot in ornamental bulb culture. Eur. J. Plant Pathol. 105 : 791-800.
- Westover, K.M., and J.D. Bever. 2001.** Mechanisms of plant species coexistence: roles of rhizosphere bacteria and root fungal pathogens. Ecology 82 : 3285-3294.
- Wolfinger, R. 1993.** Laplace's approximation for nonlinear mixed models. Biometrika 80 : 791-795.
- Yang, X.B. 1999.** *Pythium* damping-off and root rot. Pages 42-44 in G.L. Hartman et al. (ed.), Compendium of Soybean Diseases, 4th ed. APS Press, St. Paul, MN, USA.
- Zhang, B.Q., and X.B. Yang. 2000.** Pathogenicity of *Pythium* populations from corn-soybean rotation fields. Plant Dis. 84 : 94-99.