Temperature dependency of *Pythium* and *Globisporangium* spp. in causing soybean damping-off in Japan

Xiaodong You and Motoaki Tojo

**Abstract**

*Pythium aphanidermatum*, *P. coloratum*, *P. myriotylum*, and *Globisporangium irregulare* (syn. *P. irregulare*), *G. spinosum* (syn. *P. spinosum*), and *G. ultimum* var. *ultimum* (syn. *P. ultimum* var. *ultimum*) are the causal pathogens of soybean damping-off in Japan. However, their temperature preferences have not been systematically investigated. In this study, we compared the soybean pathogenicity of these six species in low (15°C day/12°C night) and high (28°C day/25°C night) temperatures, under laboratory conditions (12h day/12h night). In the low-temperature treatment, *G. irregulare*, *G. spinosum*, and *G. ultimum* var. *ultimum* were highly pathogenic to the soybeans, whereas the other three species tested were weakly pathogenic. In the high-temperature treatment, *P. aphanidermatum*, *P. myriotylum*, *G. spinosum*, and *G. ultimum* var. *ultimum* were highly pathogenic, whereas *G. irregulare* was weakly pathogenic to soybean. *P. coloratum* showed no pathogenicity to the soybeans when compared to the non-inoculated control in the high-temperature treatment. These results demonstrate that the pathogenicity of *P. aphanidermatum*, *P. myriotylum*, and *G. irregulare* will be more temperature-dependent than those of *P. coloratum*, *G. spinosum*, and *G. ultimum* var. *ultimum*.

**Key words:** *Pythium*, *Globisporangium*, soybean, damping-off

**Introduction**

The genera *Pythium* and *Globisporangium* are important groups of soil-borne plant pathogens, present in almost every agricultural soil. These pathogens attack the roots of numerous economically important crops, including soybeans, reducing their yield and quality. Since *Pythium myriotylum* was first reported as a pathogen of soybeans in Japan (Watanabe, 1977a), *P. aphanidermatum*, *P. coloratum*, *G. irregulare*, *G. spinosum* and *G. ultimum* var. *ultimum* have also been identified as the causal agents of soybean damping-off (Kodama et al., 2010; You and Tojo, 2017; You et al., 2019a). Soybean damping-off caused by *Pythium* and *Globisporangium* is highly temperature-dependent (Wei et al., 2010). However, a systematic comparison of their pathogenicities to soybeans in Japan has never been conducted. The objective of this study was to compare the pathogenicities of the six *Pythium* and *Globisporangium* spp. in low- and high-temperature conditions to establish appropriate control strategies in Japan.

**Materials and Methods**

**Pythium isolates and soybean cultivar**

The isolates used in this study were *P. aphanidermatum* strain D1, *P. coloratum* strain D2 and *G. ultimum* var. *ultimum* strain D7 from Sakai, Osaka, *G. irregulare* strain KN1 and *G. spinosum* strain KN4 from Kawachinagano, Osaka, and *P. myriotylum* strain OPU894 from Toyama, Toyama. All of the isolates were recovered from damping-off soybean seedlings grown in converted paddy fields. The species identified based on morphology and sequencing of the ribosomal ITS region (You and Tojo, 2017; You et al., 2019a; You et al., 2019b). The GenBank Accession Nos. of the ITS regions are shown in Table 1.

Soybeans (*Glycine max. Merr.* cv. EzoMidori) were used in this experiment to evaluate the comparative pathogenicity of the six *Pythium* and *Globisporangium* spp. **Pathogenicities of the six Pythium and Globisporangium spp. to soybeans in low- and high-temperature conditions**

Each isolate was cultured on autoclaved bentgrass seeds at 25°C in darkness for one week; 2.5 g of the colonized...
seeds were thoroughly mixed with 1 L of commercial nursery soil (Takii Co. Ltd., Kyoto, Japan) using a mortar and pestle, and 200 ml of the mixture was put in a ceramic pot. Eight soybean seeds were sown per pot and incubated in one of the two temperature treatments; a low temperature growth chamber at 15°C (12 h day)/12°C (12 h night) or a high temperature growth chamber at 28°C (12 h day)/25°C (12 h night). Non-inoculated pots were used as controls. Before the experiment, the seeds were surface sterilized in 0.05% sodium hypochlorite (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) for 5 minutes, then rinsed in sterile distilled water. Each pot was watered daily with tap water. After 14 days, the pathogenic aggressiveness of the strains was determined using a 0–3 scale as in John et al. (2010), with some modifications: 0 = healthy, seed germinated without visible infection; 1 = germinated with short, discolored roots; 2 = died after germination; and 3 = died before germination. The aggressiveness of the Pythium or Globisporangium isolates on the soybean seeds was calculated as a disease index from the following equation according to Abdel-Monaim et al. (2011), with some modifications:

\[
\text{Disease index} = \frac{\sum (1A + 2B + 3(C-D))}{3(T-D)} \times 100
\]

where \(A\), \(B\), and \(C\) are the number of plants corresponding to the numerical grade 1, 2 and 3, respectively, \(D\) is number of un-germinated seeds in the non-inoculated pots, and \(3T\) is the total number of plants \((T)\) multiplied by the maximum disease grade 3, where \(T = A + B + C\).

The pathogens were re-isolated from the symptomatic roots on NARM medium (Morita and Tojo, 2007) and identification by the formation of sexual organs. The experiments were repeated five times, using one pot per repetition. The pathogenicity of each species between the low- and high-temperature conditions was compared with a Student’s \(t\)-test \((\text{se} < 0.05)\). The other data were evaluated by Tukey’s HSD test \((P < 0.05)\) in IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA).

### Results and Discussion

The Pythium spp. and Globisporangium spp. showed different levels of pathogenicity to the soybean seeds (Figs. 1 and 2). In the low-temperature treatment, \(G.\ irregulare\), \(G.\ spinosum\) and \(G.\ ultimum\ var. \ultimum\) were highly pathogenic, causing 100%, 81% and 86% disease occurrences, respectively. The other three species were weakly pathogenic to the soybean seeds (Figs. 1 and 2A). In the high-temperature condition, \(P.\ aphanidermatum\), \(P.\ myriotylum\), \(G.\ spinosum\) and \(G.\ ultimum\ var. \ultimum\) were highly pathogenic, causing 100%, 78%, 68% and 86% disease occurrences, respectively. \(G.\ irregulare\) showed weakly pathogenic to soybean (Figs. 1 and 2B). \(P.\ coloratum\) was not pathogenic to the soybean seeds when compared to the non-inoculated control in the high-temperature group (Figs. 1 and 2B). Furthermore, \(P.\ aphanidermatum\) and \(P.\ myriotylum\) were more pathogenic in the high-temperature condition than in the low-temperature condition, whereas \(G.\ irregulare\) showed opposite reactions (Figs. 1 and 2). These results are consistent with previous studies demonstrating that \(P.\ aphanidermatum\), \(P.\ myriotylum\), and \(G.\ irregulare\) are temperature-dependent in causing soybean damping-

### Table 1. The GenBank Accession Nos. of the ITS regions of the pathogens used in this study

| Species                  | Strain No. | Accession No. |
|--------------------------|------------|---------------|
| \(P.\ aphanidermatum\)   | D1         | MF769579      |
| \(P.\ coloratum\)       | D2         | MF769577      |
| \(P.\ myriotylum\)      | OPU894     | MH707081      |
| \(G.\ spinosum\)        | KN4        | MN720568      |
| \(G.\ ultimum\ var. \ultimum\) | D7       | MN720569      |

Fig. 1. The pathogenicities of the Pythium and Globisporangium spp. to soybeans were compared at low (15°C day/12°C night) and high (28°C day/25°C night) temperatures for 14 days. Non-inoculated pots were used as controls. The bar at the top of each column represents the standard error of the mean \((N = 5)\). The asterisk indicates a significant difference in the pathogenicity of each species between the low and high temperatures \((t\)-test, \(P < 0.05)\). Bars with different letters indicate significant differences according to Tukey’s HSD test \((P < 0.05)\).
Xiaodong You and Motoaki Tojo: Temperature dependency of Pythium and Globisporangium on soybean

off (Watanabe, 1977b; Wei et al., 2010). *G. spinosum* and *G. ultimum var. ultimum* were highly pathogenic and did not differ between temperatures. This result is also consistent with previous work showing that *G. ultimum* var. *ultimum* was highly pathogenic to soybeans at four different temperatures, 4°C, 12°C, 20°C and 28°C (Wei et al., 2010). In this study, *G. spinosum* was highly pathogenic, independent of temperature, although Simizu and Kodama (2010) found that pre-treating the soybean seeds with low temperatures (before sowing) resulted in *G. spinosum* causing more soybean damping-off.

This study compared the temperature-dependent occurrence of soybean damping-off for six pathogens: *P. aphanidermatum*, *P. coloratum*, *P. myriotylum*, *G. irregulare*, *G. spinosum*, and *G. ultimum var. ultimum*. These results will aid the development of appropriate control strategies for this disease.

**Acknowledgments**

We would like to thank Dr. Toshiyuki Morikawa for providing damped-off soybean seedlings for the isolation of *Pythium myriotylum*. X.-D. Y. was supported by the Japan Society for the Promotion of Science (JSPS) through a research fellowship for young scientists (No. 19J10250). This research was supported by JA Bank Osaka Industry-University Cooperation Project, and Geol Cosmetics Co. Ltd.

**References**

Abdel-Monaim, M. E., et al. (2011) Crop Protect. 30: 185–191.
John, R. F., et al. (2010) Crop Protect. 29: 1452–1459.
Kodama, F., et al. (2010) Jpn. J. Phytopathol. 76: 78.
Morita, Y. and M. Tojo (2007) Plant Dis. 91: 1591–1599.
Simizu, M., and F. Kodama (2010) Ann. Rept. Plant Prot. North Japan 61: 43–46.
Watanabe, T. (1977a) Ann. Phytopathol. Soc. Japan 43: 306–309.
Watanabe, T. (1977b) Ann. Phytopathol. Soc. Japan 43: 337.
Wei, L., et al. (2010) Phytoprotection 91: 3–10.
You, X. and M. Tojo (2017) New Dis. Rep. 36: 14.
You, X.D., et al. (2019a) Plant Dis. 103: 2696.
You, X.D., et al. (2019b) Ann. Rept. Kansai Pl. Prot. 61: 9–13.