New Disease Reports

First report of soybean damping off caused by *Pythium aphanidermatum* and *P. coloratum* in Japan

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Received: *07 Oct 2017.* ***Published:*** *17 Oct 2017.* ***Keywords:*** *former rice paddy, soil-borne pathogens*

In Japan more than 80% of soybean crops (*Glycine max*) are cultivated in fields converted from rice paddies. However, the soil moisture levels of these fields are generally high due to poor drainage which promotes the growth of soil-borne pathogens, such as *Pythium* spp. (Kato *et al*., 2013). In June 2016, severe damping-off was found on soybean (cv. Ezomidori) grown in a field converted from a rice paddy in Osaka, Japan. Approximately 10% of all soybean plants were destroyed by the disease.

A total of ten *Pythium* isolates were obtained from twenty diseased soybean seedlings (Fig. 1) and identified as *Pythium aphanidermatum*, *P. coloratum* and *P. ultimum*, based on their morphological characteristics (van der Plaats-Niterink, 1981). *Pythium ultimum* has been reported as a pathogen of soybean in Japan (Kodama *et al*., 2010). *Pythium aphanidermatum* and *P. coloratum* have been reported as pathogens of soybean in Iowa and North Dakota, USA (Rizvi & Yang, 1996; Zitnick-Anderson & Nelson 2015), but have never been reported in Japan. Detailed descriptions are provided here on representative isolates of *P. aphanidermatum* (D1) and *P. coloratum* (D2). Their morphological features corresponded with the descriptions of van der Plaats-Niterink (1981) (Table 1, Figs. 2-3). The ITS regions of isolates D1 and D2 were amplified and sequenced with primers ITS4 and ITS5 (White *et al*., 1990). The sequence analysis determined that isolates D1 (GenBank Accession No. MF769579) and D2 (MF769577) showed 100% identity with *P. aphanidermatum* isolate CBS118.80 (HQ665084) and 99.0% identity with *P. coloratum* isolate CBS154.64 (AY598633), respectively.

Pathogenicity was studied using isolates D1 and D2 in pot experiments. *Pythium* species were cultured on autoclaved bentgrass seeds at 25°C for one week. One gramme of the culture was thoroughly mixed with 100 g of commercial nursery soil (Aisai-1, Katakura Chikkarin Co. Ltd., Tsuchiura, Japan) using a mortar and pestle. The infested soil was put in a plastic pot (inner diameter 7 cm, inner depth 6 cm). Five seeds of soybean (cv. Ezomidori) were sown per pot and incubated in a growth chamber at 25°C (12h day) / 20°C (12h night) with 73 mol/m2/s (measured at plant levels) of light intensity and irrigated daily with tap water. Non-infested soil was used as a control. The percentage of seedling emergence was determined seven days after sowing. The experiments were repeated five times using one pot per repetition. Both isolates D1 and D2 caused severe damping-off on soybean. In the non-infested soil 92% of the seedlings emerged (Fig. 4A), whereas only 0 and 24% of the seedlings emerged in the soil infested with D1 and D2, respectively (Fig. 4B and C). *Pythium aphanidermatum* and *P. coloratum* were re-isolated from the damaged plants grown in the soil infested with isolates D1 and D2, respectively.

To our knowledge, this is the first report of *P. aphanidermatum* and *P. coloratum* causing damping-off on soybean in Japan.

Acknowledgements

We thank Ms. Lorien E. Radmer for her critical previewing of the manuscript.

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Figure 1

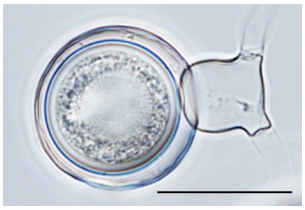

Figure 2

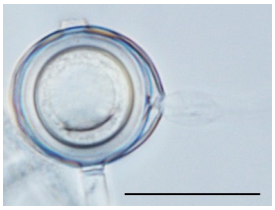

Figure 3

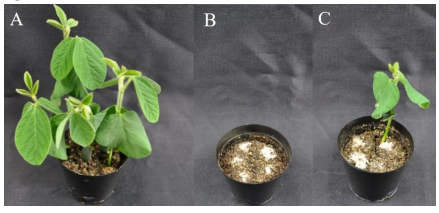

Figure 4

To cite this report: You X, Tojo M, 2017. First report of soybean damping off caused by *Pythium aphanidermatum* and *P. coloratum* in Japan. *New Disease Reports* **36**, 14. http://dx.doi.org/10.5197/j.2044-0588.2017.036.014

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