Identification and Pathogenicity of Three Species of *Pythium* Newly Isolated from Diseased Wheat and Barley Just After Thawing in Japan

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Abstract

Three species of *Pythium* (*P. okanoganense*, *P. vanterpoolii*, *P. volutum*) were newly identified as pathogens of *Pythium* snow rot of wheat and barley in Japan. From the results on frequency of isolation and pathogenicity, it can be concluded that *P. okanoganense* is an important pathogen, like *P. iwayamai* and *P. paddicum* reported previously by other authors, but both *P. vanterpoolii* and *P. volutum* are minor.

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Key words : *Pythium* spp., *Pythium* snow rot, wheat, barley.

Introduction

A snow rot of cereals caused by *Pythium* sp. was first reported in Japan⁵⁾. A Latin description of the fungus was then provided and it was named *P. iwayamai* S. Ito³⁾. Besides this fungus, three additional *Pythium* spp. (*P. graminicola* Subr., *P. horinouchiense* Hirane, *P. paddicum* Hirane) were described as causal organisms¹⁾, but two other species remained unidentified as minor pathogens¹⁾.

The *Pythium* snow rot in Japan is favored by high soil fertility, poor drainage and snow cover, and no effective control measures are available so far, especially when the snow cover remains for a long period.

This study was conducted to identify three species of *Pythium*, in addition to the above four species already identified^{1,3)}, which are associated with rotted winter wheat and barley plants under snow and to determine their role in *Pythium* snow rot. Part of this work has already been reported elsewhere^{2,12)}.

Materials and Methods

Collection and identification of the *Pythium* spp. Diseased wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) plants (1,285 plant samples) were collected from 157 different fields in Fukui Prefecture between 1982 and 1984. A diseased leaf

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was cut into pieces (5×5 mm) and some of these were disinfected by dipping into 80% ethanol for 1 sec, followed by surface sterilizing with sodium hypochlorite solution (available chlorine: 0.5%) for 3 min. Isolations were made on plain agar or Bacto-cornmeal agar (Bacto-CMA) after rinsing the leaf pieces 3 times in sterile distilled water and removing the excess water by filter papers. After 7 days incubation at 2-3 C, hyphal tips were isolated, followed by subculturing on cornmeal agar (CMA; 20 g cornmeal, 15 g agar, 1,000 ml distilled water) at about 15 C.

Zoospores were induced by the method described by Waterhouse¹⁸⁾. Media used in taxonomic studies included Bacto-CMA supplemented or not with 5,000 ppm wheat germ oil (Japan Impex Co., Ltd., Tokyo), Bacto-lima bean agar and Schmitthenner's synthetic agar. Dimensions recorded for the different fungal structures were based on a minimum of 100 measurements. Identification was usually made by following the Keys of Plaats-Niterink⁹⁾ or Waterhouse¹⁸⁾.

Temperature-growth relations of *Pythium* spp. in the authors' collection was determined as described by Middleton⁸⁾.

Pathogenicity trials. Pathogenicity tests were performed by using essentially the same method as described previously¹¹⁾. Seeds of the snow endurance wheat cultivar: Nanbu komugi and the susceptible: Nōrin No. 61 were submerged in 0.1% HgCl₂ solution for 30 min, rinsed in running tap water for 1-2 hr, then allowed to germinate in a moist chamber at 20 C for 1 day. Thirty seedlings/cultivar were grown in a row in autoclaved sandy loam soil in a plastic box (32×25×7 cm) under glasshouse conditions, with two rows in the same box for different cultivars (Oct. 18-Nov. 5, 1983). Seedlings were fertilized once every 7-10 days by watering with 200-300 ml of about 50% Knop's solution and used for the pathogenicity tests without a hardening treatment.

Pythium isolates (2-4/species) were grown on a wheat bran-sand mixture (2 g wheat bran, 100 g sand and 25 ml distilled water autoclaved at 121 C for 30 min) at 15 C for about 1 month. During incubation, the cultures were mixed thoroughly once to ensure uniform colonization of the medium, then used as an inoculum.

The leaves of seedlings at the 2-3 leaf stage were bent to contact with a thin layer of inoculum which had been applied previously onto the soil surface (100 g/800 cm²) in the plastic box, then covered with a pad of wet absorbent cotton to stimulate snow cover. After putting the lid, the box with inoculated seedlings was removed to a reservoir in a cold room (0-1 C) to feed water through a hole at the bottom of the box and kept for 40 days in the dark, then returned to the glasshouse (Dec. 14, 1983). The cotton cover was removed and plants were watered once with Knop's solution (200-300 ml/box) to facilitate recovery. The number of surviving plants was recorded after the 10-day recovery period. The same experiments were also performed using 2 barley cultivars: Benkei mugi and Miyuki ōmugi.

Experiments were repeated several times.

Results

Identification of Pythium spp.

1. *Pythium okanoganense* Lipps (Plate I, Figs. 1-5)

Sporangia terminal, rarely intercalary, globose, subglobose or ovoid (Fig. 1), rarely pyriform (Fig. 2), infrequently proliferating (Fig. 3), 21–32 (mean 26) μm diam., sometimes borne in sympodial succession. Oogonia subglobose, smooth, sometimes with 1, rarely 2 projections, terminal on generally short lateral branches, sometimes bearing short lateral hyphal elements, rarely becoming intercalary after continued outgrowth of a hypha, 19–28 (mean 24) μm diam. Oogonial wall rather unequal in thickness. Antheridia usually 1, sometimes up to 4 per oogonium, making apical blunt contact with the oogonium, antheridial stalks slightly swollen, of monoclinal (Fig. 4) or occasionally diclinal origin at a distance of 3–20 μm , sometimes bearing short lateral hyphal elements. Oospores aplerotic or almost plerotic, 15–22 (mean 18) μm , with a 1.1–2.5 μm thick wall (Fig. 5).

Growth on CMA: min. below 0 C, opt. 22 C, max. below 30 C.

Isolates examined: UOP2=CBS701.83, IFO31941, IMI280168; UOP10=IFO31921, IMI280174; UOP14=IFO31922; UOP336=H-82-72.

Since sporangia of this fungus proliferate occasionally, this character can not be used for species separation among the species having proliferous sporangia such as *P. midletonii*, *P. nagaii*, *P. polytulum* and *P. marsipium*. However, the latter spp. can be readily distinguished from *P. okanoganense* by other morphologic features such as shape of sporangia, catenulate oogonia, size of oogonium, number and origin of antheridia, and width of oospore walls⁹⁾.

P. irregulare, *P. sylvaticum*, *P. ultimum* var. *sporangiiferum* and *P. vexans* are species morphologically similar to *P. okanoganense* and having nonproliferous sporangia. However, an indeterminate number of projection and the size of sporangia of *P. irregulare* distinguish it from *P. okanoganense*. The latter differs from *P. sylvaticum* in having short, frequently inflated antheridial stalk, originating a short distance below the oogonium. The terminal and intercalary conidia common in *P. sylvaticum* are absent from *P. okanoganense*. Although apparently similar to *P. okanoganense*, *P. ultimum* differs in having monoclinal antheridia always originating immediately below the oogonium. The typically bell-shaped antheridial cells in *P. vexans* are never found⁹⁾.

The three species (*P. graminicola*, *P. iwayamai*, *P. paddicum*) reported by Hirane¹⁾ clearly differ in such characters as shape of sporangia and ornamented oogonium. Although sex organs of *P. horinouchiense* are similar to those of *P. okanoganense*¹⁾, comparisons can not be made since no sporangia are described for the former.

The snow rot fungus agrees with *P. okanoganense* described by Lipps⁷⁾ in shape and size of sexual and asexual structures, infrequent proliferation of sporangia, forming sporangia in sympodial succession, aplerotic oospores, thick oospore wall and cardinal temperatures. Thus, this fungus was identified as *P. okanoganense*.

2. *Pythium vanterpoolii* V. Kouyeas & H. Kouyeas (Plate I, Fig. 6; Plate II, Figs. 7–9)

Sporangia often consisting of catenulate complexes of subglobose bodies, terminal or intercalary (Figs. 6, 7). Oogonia smooth, globose, thin-walled, terminal or intercalary, 14–24 (mean 21) μm diam. Antheridia 1–2 per oogonium, club-shaped, monoclinal, rarely diclinal; antheridial stalks originating more than 10 μm below the oogonium (Fig. 8),

soon disappearing after fertilization (Fig. 9). Oospores plerotic, 13-21 (mean 20) μm diam., wall up to 4 μm thick (Fig. 9).

Growth on CMA: min. about 0 C, opt. 25 C, max. 30-35 C.

Isolates examined: UOP3=IFO31923, IMI280169; UOP4=IFO31924, CBS700.83, IMI280170; UOP16=IFO31925, IMI280178; UOP337=H-82-37; UOP338=H-82-40.

This fungus agreed in general with the description of *P. torulosum*⁹⁾, but sporangia were asymmetrical, having frequently catenulate elements⁶⁾ as compared with the branched toruloid sporangial outgrowths of *P. torulosum*. Thus, this fungus was identified as *P. vanterpoolii*.

3. *Pythium volutum* Vanterpool & Truscott (Plate II, Figs. 10-13)

Sporangia few, inflated with small lobes and toruloid outgrowths (Fig. 10). Oogonia smooth, globose, terminal on short side branches, occasionally intercalary, 20-41 (mean 28) μm diam. Antheridia terminal 1-6 per oogonium, clavate, crook-necked or straight, making apical contact, usually declinuous (Fig. 11), rarely monoclinal, the stalks frequently loosely entwining the oogonial stalk (Fig. 12). Oospores smooth, globose, plerotic, rarely aplerotic, 17-37 (mean 25) μm diam., wall variable, up to 3 μm thick (Fig. 13).

Growth on CMA: min. about 0 C, opt. 25 C, max. 30 C.

Isolates examined: UOP5=CBS699.83, IFO31927, IMI280171; UOP18=IFO31926, IMI280180; UOP39=IFO31928.

The fungus isolated is characterized by its declinuous long antheridial stalks which coil around the oogonial stalk and oogonium. *P. helicum* shows similar characters⁴⁾ but may differ in having monoclinal antheridia. Although the predominantly plerotic oospores of the isolate differ slightly from those of *P. volutum*¹⁵⁾, the fungus may be identified as *P. volutum*, considering the similarity of the other main characters.

Frequency of isolation

Isolates of *P. okanoganense*, *P. volutum* and *P. vanterpoolii* comprised 7, 6 and 4%, respectively, of the total of all *Pythium* spp. detected in Fukui Prefecture. As shown in Table 1, these three species were isolated to an equally low extent regardless of the frequency of cropping. *P. okanoganense* and *P. vanterpoolii* were detected from barley much more frequently than from wheat, whereas *P. volutum* was isolated more often from wheat.

Table 1. Frequency of isolation of three species of *Pythium* from diseased wheat and barley leaves just after thawing^{a)}

<i>Pythium</i> spp.	Wheat			Barley			The sum total
	1 st cropping	Continuous cropping	Total	1 st cropping	Continuous cropping	Total	
No. of field surveyed	37	10	47	85	25	110	157
No. of leaf pieces used	245	209	454	621	210	831	1285
<i>P. okanoganense</i>	2	0	2	21	2	23	25
<i>P. vanterpoolii</i>	1	1	2	4	8	12	14
<i>P. volutum</i>	2	13	15	1	5	6	21
Total	5	14	19	26	15	41	60

a) Isolated between 1982 and 1984.

Table 2. Pathogenicity of *Pythium* spp. to different cultivars of wheat

<i>Pythium</i> spp. (Isolate no.) ^{a)}	Nanbu komugi	Nōrin No. 61
<i>P. okanoganense</i> (UOP 10)	4/30 ^{b)}	0/30
" (" 336)	2/30	0/30
<i>P. vanterpoolii</i> (UOP 3)	30/30	24/30
" (" 16)	24/30	13/30
" (" 337)	29/30	24/28
" (" 338)	29/30	16/30
<i>P. volutum</i> (UOP 5)	21/30	0/30
" (" 39)	27/30	14/30
Uninoculated 1	30/30	30/30
" 2	30/30	30/30

a) UOP 10=IFO 31921, IMI 280174; UOP 3=IFO 31923, IMI 280169; UOP 16=IFO 31925, IMI 280178; UOP 5=CBS 699.83, IFO 31927, IMI 280171; UOP 39=IFO 31928.

b) Ratio of seedlings surviving to those inoculated.

Pathogenicity

Table 2 indicates that *P. okanoganense* was highly pathogenic on both cultivars of wheat, whereas *P. volutum* was less so and *P. vanterpoolii* was weakly or non-pathogenic. Similar results were obtained using 2 barley cultivars: Benkei mugi and Miyuki ōmugi.

Discussion

P. okanoganense, *P. vanterpoolii* and *P. volutum* are newly identified in this paper as the causal fungi of *Pythium* snow rot of wheat and barley in Japan. Moreover, *P. okanoganense* and *P. vanterpoolii* are described for the first time in Japan (See Takahashi¹⁰⁾, Watanabe¹⁷⁾). Although 2 unidentified species have already been isolated by Hirane as a minor snow rot fungus¹⁾, comparisons can not be made between his species and ours since no sexual structures are described in the former. However, four species (*P. graminicola*, *P. horinouchiense*, *P. iwayamai*, *P. paddicum*) identified by Hirane¹⁾ differ from ours in several characters. *P. graminicola* has swollen filamentous sporangia, *P. paddicum* ornamented oogonia. Sporangia of *P. iwayamai* are spherical, ellipsoid, citriform or ovoid and usually papillate compared with the typical oblate-spheroidal and non-papillate sporangia of *P. okanoganense*. Since the sporangia of *P. vanterpoolii* are asymmetrical, frequently with catenulate elements, this differs from any other fungus concerned. *P. volutum* is characterized by its declinuous, long antheridial stalks which coil around the oogonial stalk and oogonium.

For comparison, *P. graminicola*, *P. iwayamai* and *P. paddicum* were simultaneously isolated, together with several unidentified *Pythium* spp. *P. iwayamai* was detected most often (43%), followed by *P. paddicum* (29%), *P. graminicola* (7%), *P. okanoganense* (7%), *P. volutum* (6%), *P. vanterpoolii* (4%) and unidentified *Pythium* spp. (4%). *P. iwayamai* was isolated most frequently from a field under continuous cropping, *P. paddicum* more often from a paddy field after the first wheat or barley crop. No clear differences in *Pythium* spp. and their frequency were found between wheat and barley.

In pathogenicity tests for direct comparison with *P. graminicola*, *P. iwayamai* and *P. paddicum*, *P. okanoganense* was high pathogenic (90-100% disease severity) like *P. iwayamai* (almost 100%) and *P. paddicum* (90-100%), but *P. vanterpoolii* and *P. volutum* were only weakly pathogenic (7-80%). The facts that *P. graminicola* and *P. volutum* were highly pathogenic (80-90%) on the wheat cultivar: Nōrin No. 61 but weakly pathogenic (20-40%) on Nanbu komugi may due to a relatively higher resistance of the latter to snow damage. *P. vanterpoolii* was the weakest pathogen (7-35%) among the *Pythium* isolates tested.

From the results on detection frequency and pathogenicity, it appears that *P. graminicola*, *P. vanterpoolii* and *P. volutum* are minor pathogen of *Pythium* snow rot of wheat and barley in Japan.

"Browning root rot" has been applied as a common name to the disease of wheat and barley under snow in Japan¹³⁾. However, the authors prefer the more appropriate name "*Pythium* snow rot" to distinguish this disease from snow rot of wheat and barley caused by *Typhula ishikariensis*¹³⁾, and from symptoms of other *Pythium* root rots^{14,16)}.

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和 文 摘 要

一谷多喜郎・高松 進・D. J. STAMPS: 融雪直後にオオムギおよびコムギの罹病葉から新たに分離された *Pythium* 菌3種の同定と病原性

既にわが国で報告のある4種のはかに、オオムギとコムギの褐色雪腐病菌として新たに3種を記載した。これらの菌の分離頻度と病原性試験の結果から、*P. okanoganense* は *P. iwayamai*, *P. paddicum* と同じように重要な病原菌であるが、*P. vanterpoolii* と *P. volutum* は重要なものではないと結論された。

Explanation of plates

Plates I, II

Morphology of three species of *Pythium* identified.

All bars on the plates represent 20 μm .

Figs. 1-5. *P. okanoganense*

1. Zoospore release from spherical sporangium. 2. Pyriform sporangium. 3. Proliferating (arrow) sporangium. 4. Oogonium and monoclinal antheridium having slightly swollen (arrow) antheridial stalk. 5. Mature oospores with thick wall.

Figs. 6-9. *P. vanterpoolii*

6. Asymmetrical catenulate sporangia. 7. Empty sporangium with evacuation tube (arrow). 8. Oogonium and monoclinal antheridium. Antheridial stalk originating some distance (more than 10 μm) below the oogonium. 9. Oogonia without antheridia due to disappearing soon after fertilization, and plerotic, mature, thick-walled oospores.

Figs. 10-13. *P. volutum*

10. Empty sporangium (arrow). 11. Diclinous, crook-necked antheridia, making apical contact. 12. Antheridial stalks loosely entwining the oogonial stalk. 13. Plerotic oospores with relatively thin wall.

Plate I

Plate II