Identification and Comparative Pathogenicity of *Pythium* species from Wheat Roots and Wheat-Field Soils in the Pacific Northwest

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ABSTRACT

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Ten species and varieties of *Pythium*, two unidentified, were recovered from wheat roots and wheat-field soils in eastern Washington and northern Idaho. Of these, *P. ultimum* var. *ultimum, P. u.* var. *sporangiiferum, P. aristosporum, P. volutum, P. torulosum, P. irregulare, P. sylvaticum* complex and *Pythium* sp."D" (unidentified) are homothallic, and *P. heterothallicum* and *Pythium* sp. "E" (unidentified) are heterothallic. Of 302 isolates from a single site, 165 were homothallic, 137 were heterothallic, and seven species were identified. Pathogenicity was tested on Daws wheat at 15 C with either mycelial inoculum in a mixture of cornmeal, sand, and vermiculite, or with oospores added to fumigated soil (500 and 1,000 oospores per gram). All 10 species and subspecies were pathogenic. *P. aristosporum* and *P. volutum* were the most pathogenic and caused seed decay, severe root rot, and root browning. *P. ultimum* (both varieties), *P. sylvaticum* complex, and *P. irregulare* were next most pathogenic and caused seed decay, root rot, and root necrosis. *P. torulosum, P. heterothallicum, Pythium* sp. "E" and *Pythium* sp. "D" were least pathogenic and caused mild root necrosis and little or no seed decay. In general, the *Pythium* species resulted in less seedling emergence; shorter roots, shoots, and first leaves; and less dry weight of roots and shoots compared with the healthy plants from the controls.

Additional key words: soilborne pathogens.

Pythium root rot occurs commonly on wheat in eastern Washington and adjacent northern Idaho, and it may be the factor responsible for the plant growth suppression and nutrient deficiencylike symptoms that were eliminated with soil fumigation (3,4). The disease can be important on direct-drilled (no-till) wheat (4). However, the species responsible for root damage under Pacific Northwest conditions are generally unknown.

The first studies of *Pythium* species pathogenic to wheat were conducted in Canada and England about 50 yr ago (26,28). Middleton (19) reported ten *Pythium* species associated with wheat in the United States, and Sprague (24,25) added five species to this list. The most recent monograph of *Pythium* (22) lists 21 species on cereals and/or grasses in the United States. In the Pacific Northwest, P. *iwayami* and *P. okanoganense* cause snow rot of wheat leaves beneath snow and ice (13–17), and *P. irregulare* (24), *P. aristosporum* (16), and *P. ultimum* (3,23,24) cause root rot. Except for these reports, little is known of the identity and relative pathogenicity of Pythium species associated with root rot of wheat in eastern Washington and adjacent Idaho. This study was undertaken to identify *Pythium* species from wheat roots and to compare their pathogenicity under controlled conditions.

MATERIALS AND METHODS

Media. The media used were homemade cornmeal agar (CMA); Difco cornmeal agar (dehydrated) (DCMA); Difco cornmeal agar with dextrose (DCMA-D); V-8 juice agar (V8A); V-8 juice-cholesterol broth (V8CB); potato carrot agar (PCA); a 1:1 blend of potato carrot agar and cornmeal agar (PCA-CMA); potato carrot agar with cholesterol added at 0.03 g/L (PCCA); Mircetich's (20) pimaricin-vancomycin agar medium with rose bengal at 0.01 g/L (MPVM); MPVM with rifampicin at 0.1 g/L instead of rose bengal (MPVM-R); and water agar plus rifampicin at 0.1 g/L (WA-R).

To prepare CMA, 60 g freshly ground yellow popcorn kernels were wrapped with three layers of cheesecloth, boiled in 1 L of distilled water (DW), and allowed to simmer for 1 hr. The suspension was then strained through two layers of cheesecloth and DW was added to make 1 L (22). V-8 juice agar was prepared by mixing 200 ml of V-8 juice and 3 g of powdered $CaCO_3$ with 800 ml DW. V-8 juice-cholesterol broth was prepared by adding 2.5 g $CaCO_3$ to 200 ml of V-8 juice and then centrifuging for 30 min; the cleared liquid was amended with an ethanol-solution of cholesterol (1.5% cholesterol in 95% ethanol) to provide 0.03 g/L medium (1). To prepare potato carrot agar, 20 g of fresh potatoes and 20 g of fresh carrots were chopped and boiled for 10 min in 1 L of DW, filtered through cheesecloth, and then DW was added to the filtrate to make 1 L (22). Unless mentioned otherwise, all solid media contained agar at 15 g/L of the broth and were autoclaved at 121 C for 20 min.

Isolation of *Pythium* species from the field. *Plants*. Seedlings, young plants, and adult wheat plants were dug from fields around Pullman, WA, and Moscow, ID, at different times during the growing season (mostly November, December, April, May, and June) and brought immediately to the laboratory in plastic bags . Root systems were placed on a No. 35 (60-mesh) screen and adhering soil clumps were washed away with running tap water. Roots with fine soil particles still attached were then transferred to a white enameled pan containing tap water, and the soil particles were removed with a soft brush. Root systems were then washed further in five successive changes of sterile DW before plating. In some cases, the roots were washed in three successive changes of sterile DW in 125-ml Erlenmeyer flasks with 15–20 glass beads (3–4 mm diameter) and a magnetic stirrer. Roots were cut into pieces 1–3 cm long in the water, blotted on clean soft tissues (to remove free water), and then 8–10 pieces of roots (including tips and bases) were plated on MPVM, MPVM-R, and WA-R. After 24–48 hr at 20 C, emerging hyphal tips were transferred to DCMA. Hyphal tips

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from roots on WA-R were transferred to the MPVM-R to eliminate nonpythiaceous fungi which were inhibited by the pimaricin and PCNB.

Soils. Two methods were used to recover *Pythium* spp. from soils: baiting, whereby wheat seedlings were first grown in the soil and then washed and plated as described above; and dilution plating. Soil from the surface 15 cm was crumbled into small particles by hand while still moist, dried slowly to approximately 2–5% moisture, and then sieved through 0.5 and 0.25 mm (35 and 60-mesh, respectively) screens. Soil retained on the 0.25-mesh screen was used.

For baiting, plastic tubes with drain holes at their bottoms (Conetainers, Ray Leach Conetainer Nursery, 1787 N. Pine St., Canby, OR 97013), 16.5 cm deep by 2.5 cm in diameter (top) were used. Each tube was two-thirds filled with autoclaved vermiculite and then 10 g of soil was added as a layer. Two surface-sterilized wheat seeds (cultivar Daws) were planted 5–8 mm deep in the soil and the soil then was covered with 1.5 cm of autoclaved vermiculite. Ten tubes were used for each soil sample. Tubes containing fumigated soil sandwiched between autoclaved vermiculite served as controls. The tubes were irrigated initially with 20 ml DW each, placed in a growth chamber (12 hr at 10 C with dark and 12 hr at 15 C with light), and watered by adding 3–5 ml of DW to each tube every 2–3 days. The seedlings emerged after 15 days, were washed, and the *Pythium* species isolated as described.

For direct isolation from soil, 2 g of soil was suspended in 100 ml of 0.2% water agar and then 1 ml of suspension was spread with a glass rod on the surface of MPVM. After 24–48 hr at 20 C (in the dark), the soil was washed from the agar surface with running water. Hyphal tips from the colonies were transferred to MPVM and/or MPVM-R (to secure a pure culture of the *Pythium* species) before being transferred to DCMA, CMA, or V8A for identification.

Induction of growth and reproduction in culture. *Mycelial growth.* Diameters of colonies in 100-mm-diameter petri dishes on both DCMA and CMA at 20 or 25 C were measured every 24 hr to determine growth rates. Isolates with similar growth rates and colony morphology were grouped and then representative isolates were selected for further study.

Sporangia and zoospores. To induce formation of sporangia, two to three plugs (1 cm in diameter) from a colony growing on either DCMA or V8A were placed in a petri dish in a shallow layer of water, and then several pieces of boiled (10 min) grass leaf (*Poa annua* L.) 1–2 cm long were placed in physical contact with mycelium in the plug. The water consisted of one part sterilized (autoclaved) pond water and one part sterile DW and was changed every 24 hr or as necessary to favor the production of sporangia and discharge of zoospores. Different baits were used for isolates that did not produce sporangia or zoospores on the grass leaf fragments; these included boiled carrot disks (1 cm in diameter), boiled hemp seeds, and boiled or fresh wheat roots and leaves, in combination with different incubation temperatures between 0 and 20 C.

Oogonia, antheridia, and oospores. In general, agar media (CMA, DCMA, DCMA-D, and V8A) or plant materials (grass leaf, carrot disks, or hemp seeds) were used to induce formation of oogonia, antheridia, and oospores. For isolates that formed oospores rarely or not at all, V8CB was used. If the isolate still failed to produce oospores, tests were made for heterothallism.

Tests for heterothallism. Pairings were made among similar isolates on PCA, PCA-CMA (22), and (in some cases) PCCA. Agar plugs (6 mm in diameter) of each pair of isolates were placed at opposite sides of a petri dish and incubated in the dark at 20 C. The zone where the advancing mycelia interacted was observed daily for 2 wk or longer. Agar plugs from this zone were stained in lactofuchsin (0.01% acid fuchsin in 50% lactic acid) on a microscopic slide, softened over a flame, and then covered with a cover slip. Pairings were also made with heterothallic isolates of known identity, obtained from the American Type Culture Collection (ATCC). Each unidentified isolate was transferred to a petri dish together with two identified, compatible strains from ATCC, arranged in a triangular pattern. The occurrence of fertile oospores on the lines of contact between mycelium of either tester strain was used as a basis for positive identification.

Measurements of the various reproductive structures were made on a glass slide either in water or after staining with lactofuchsin. A minimum of 50 measurements were made of each structure. Identification to species or variety was based on monographs or keys of Matthews (18), Middleton (19), Waterhouse (29,30), Hendrix and Papa (11), and Plaats-Niterink (22).

Pathogenicity to wheat. *Mycelial inoculum.* A mixture of cornmeal (5 g), sand (485 g), and water (120 ml) in 1,000-ml Erlenmeyer flasks was autoclaved at 121 C for 45 min, inoculated with agar disks (6 mm in diameter) from a culture of the test isolate, and then shaken or stirred periodically for 10 days to insure uniform colonization. One part of this mycelial inoculum was then diluted with three or seven parts (v/v) of autoclaved vermiculite (referred to hereafter as ¼ and ⅛, respectively) and the mixture was used as a rooting medium for wheat (3). Noninfested cornmeal-sand mixed with vermiculite served as a control. The inocula included five isolates of *P. ultimum* Trow. var. *ultimum* Drechsler; two of *P. u.* var. *sporangiiferum* Drechsler; nine of *P. irregulare* Buisman; two of *P. torulosum* Coker and Patterson; one each of *P. aristosporum* Vanderpool, *P. volutum* Vanderpool and Truscott, *P. sylvaticum* complex, and *Pythium* sp. "D"; and one male and one female isolate each (mixed together) of *P. heterothallicum* Campbell and Hendrix and *Pythium* sp. "E". Four clear plastic cups (300 ml) were filled with each inoculum:vermiculite mixture per isolate and inoculum concentration. Ten seeds of cultivar Daws were planted 1.5 cm deep per cup. All cups were watered initially with 60 ml DW and then maintained at an alternating 12 hr at 10 C (without light) and 12 hr at 15 C (with light). After seedling emergence, the plants were watered every 3–5 days, or as necessary for normal growth, with full-strength Hoagland's solution (macronutrients only).

Oospore inoculum. To produce oospore inoculum, a 0.5-mm-diameter agar plug from the periphery of a 5-day-old culture was transferred into a petri dish containing 15 ml of V8CB (1), and the dishes then were incubated at 20 C in the dark for 3 wk. Mycelial mats with oospores were washed three times with sterile DW, suspended in 100 ml of sterile DW, comminuted in a Waring blender at low speed for 2 min, and then filtered through two layers of cheesecloth and one layer of lens paper to remove the mycelium (1). The resultant oospore suspension was added to a Palouse silt loam (PSL) that had been sieved through a 0.25-mesh screen and fumigated (methyl bromide) to eliminate the natural population of *Pythium*. The oospore suspension was introduced as a spray as the soil was poured into a tray (21). Each infested soil was mixed thoroughly and then incubated in plastic bags for 7–10 days at 20–25 C. The soils were then spread on clean paper, air-dried to 4–7% water content (w/w), and then stored in plastic bags at 5 C for up to 60 days prior to use.

The oospore concentration was estimated for each infested soil by counting colonies on soil dilution plates of MPVM (20). The inoculum of each isolate was adjusted to 500 or 1,000 propagules per gram by dilution of the infested soil with sieved fumigated soil. Autoclaved vermiculite was added to the soil at 10% (v/v) to improve drainage. Eight isolates of *P. irregulare* and one each of *P. u.* varieties *ultimum* and *sporangiiferum*, and *P. torulosum* were tested as oospores in fumigated soil. The infested soils were added to 300-ml clear plastic cups, 250 g per cup, four cups for each infested soil. Ten surface-sterilized seeds of cultivar Daws were planted in each pot, watered with 80 ml DW per pot, and incubated as described above. After emergence, the plants were watered every 2–3 days with DW, and occasionally fertilized with half-strength Hoagland's solution (macronutrients only).

Measurement of plant responses to Pythium species. The number of emerging plants were counted, and then (4 wk after planting) all plants were harvested and the roots were washed on a wire screen with running water. Lengths of the shoots, roots, and first true leaves were measured, and the roots were examined for decay and necrosis and observed microscopically for oospores. *Pythium* species was reisolated from the infested roots, identified, and compared with the original isolates.

RESULTS

***Pythium* species isolated from wheat roots and wheat field soils.** Eight species and varieties were identified among more than 350 isolates obtained directly from wheat roots or baited from soil. These were: *P. u.* var. *ultimum*, *P. u.* var. *sporangiiferum*, *P. sylvaticum* complex, *P. irregulare*, *P. aristosporum*, *P. volutum* Vanterpool and Truscott, *P. torulosum*, and *P. heterothallicum*. Two other species, "D" and "E," are still unidentified. Of all isolates, *P. heterothallicum* and *Pythium* sp. "E" were the only heterothallic species. Each of the species and varieties were isolated directly from dilution plates on the MPVM medium except *P. aristosporum* and *P. volutum*, which did not grow in MPVM. One isolate of each of the 10 species has been deposited in the American Type Culture Collection.

Seven *Pythium* species or varieties were identified from a total of 302 isolates from a single site, a long-term tillage × rotation experiment maintained by the University of Idaho north of Moscow, ID. Of the 302 isolates, 165 were homothallic and 137 were heterothallic. Those that were recognized included: *P. u.* var. *ultimum*, *P. u.* var. *sporangiiferum*, and *P. sylvaticum* combined (5%); *P. irregulare* (42%); *P. heterothallicum* (14%); *Pythium* sp. "E" (32%); and *Pythium* sp. "D" (7%).

P. ultimum was the species most frequently isolated on MPVM soil dilution-plates. Morphology and growth rate (34 mm/24 hr at 25 C) of the colonies of *P. u.* var. *sporangiiferum* on DCMA or CMA was similar to that of *P. u.* var. *ultimum*. However, some isolates of *P. u.* var. *sporangiiferum* with a slow growth rate (28 mm/24 hr at 25 C) showed a vague chrysanthemum pattern and less aerial mycelium. In water cultures, sporangia of *P. u.* var. *sporangiiferum* formed within 2 days around the margins of grass blades, and zoospores were released during the next 2–3 days. Zoospores were released at 7–22 C with the optimum temperature at 15 C; 6–20 zoospores formed per vesicle. Oogonia, antheridia, and oospores were indistinguishable from those of *P. u.* var. *ultimum*. The sizes and shapes of sporangia, oogonia, antheridia, and oospores of *P. u.* var. *sporangiiferum* isolated from wheat roots matched the description given by Plaats-Niterink (22) and were similar to those of ATCC isolate 13647. Sporangia of the wheat isolates had thicker walls than the ATCC isolate; therefore, they retained their shape better after zoospore release. The discharge tubes from the wheat isolates were longer than those of the ATCC isolate.

Growth of the isolates of *P. torulosum* was in a distinct radiate or vague rosette pattern on CMA and its rate was 12 mm/24 hr at 20 C. In liquid cultures, boiled-grass blades, autoclaved carrot disks, and V8A were the most suitable substrates for production of sporangia and zoospores by *P. torulosum*. Sporangia and zoospore discharge were observed within 24–36 hr after the first irrigation of a colonized substrate. Abundant oospores were produced in water cultures as well as in V8C broth. Colony development on MPVM (soil dilution plates) was slow; colonies were not evident macroscopically until 48 hr after the plates were seeded.

Colonies of *P. aristosporum* on CMA and DCMA were submerged with radiate patterns and grew at 13 mm/24 hr at 20 C. Sporangia consisted of inflated filaments, digitate or lobate, simple or complex, and they germinated either by zoospores from a long slender discharge tube (up to 100 μm long) or by germ tubes. In general, germination was direct at 20–22 C and indirect at 10–15 C. Abundant sporangia and oogonia with antheridia but little mycelium formed around grass leaves in water cultures within 2–3 days. Only a few normal mature oospores developed either in solid media (CMA and DCMA) or in water cultures. The isolate studied in detail was identical to one from wheat roots provided by G.W. Bruehl.

Colonies of *P. volutum* on CMA and DCMA were submerged with a vague radiate pattern when young, became pale yellow or brown and granular-appearing when older, and they grew at 18 mm/24 hr at 20 C. Sporangia were not observed on the media or substrates tested. Oospores were smooth and globose when produced in culture or in water, elongate when produced inside host tissue, and usually aplerotic or free within oogonia but sometimes were plerotic. Two or three oospores sometimes formed within an oogonium, but usually only one matured.

Colonies of *P. heterothallicum* on CMA and DCMA grew at 16 mm/24 hr at 20 C. They were submerged and formed short aerial mycelium with a slight chrysanthemum pattern that was more obvious in the male than in the female isolates. On PCA, the colonies were submerged with a faint chrysanthemum pattern. Sporangia and zoospores were not produced under any of the conditions tested. Hyphal swellings (possibly chlamydospores) formed abundantly on PCA. Oogonia and oospores formed abundantly only near a contact line in paired cultures of compatible isolates.

When two compatible strains from wheat roots were paired in a petri dish with the male isolate, ATCC 18197, the zone where the male and female wheat isolates interacted was diffuse, whereas the zone between the ATCC male and the wheat female remained sharp. No reaction line was observed between male isolates. Well-developed oospores were detected from both zones of contact. A female isolate, ATCC 18198, was also tested, but this isolate apparently had lost the mating capability and did not provide the white line of mating reaction when paired with either the male ATCC or wheat isolates.

Pairings of these wheat isolates (male and female) with *P. catenulatum* (ATCC 10950), *P. intermedium* (ATCC 36445), *P. splendens* (ATCC 14557), and two isolates of *P. sylvaticum* (ATCC 18195 and 18196) failed to produce mating reactions on any of the media tested. A broad white pattern occurred on PCCA at the zone of contact between a female wheat isolate and *P. splendens* (ATCC 14557), but oospores were not formed.

Colonies of *P. irregulare* on CMA and DCMA were submerged, radiate with vague chrysanthemum patterns and limited aerial mycelium, and grew 25 mm/hr at 20 C. Colonies on V8A formed abundant short, dense, aerial mycelium in no special pattern. Hyphal swellings developed, but sporangia were not observed. Oospores were mostly aplerotic but occasionally plerotic or nearly so. The most unique characteristic of the isolates of *P. irregulare* from wheat roots was a failure to produce zoospores. Typically, isolates produced terminal, irregular-shaped oogonia with several wall projections that varied in length and had monoclinous stalked antheridia. One isolate produced many short projections on the walls of globose or subglobose, terminal, or intercalary oogonia. Another isolate produced abundant hyphal swellings (that germinated by one to five germ tubes) and few oospores in either solid media or water culture, but it produced more oospores within grass leaves. Most isolates produced a few small hyphal swellings while some produced abundant larger swellings. The colonies on MPVM were similar to but smaller than those of *P. ultimum* (both varieties).

Most isolates of *P. irregulare* from wheat exhibited less aerial mycelium on CMA and DCMA than did isolate 1120 from ATCC. The ATCC isolate produced more oogonia with projections (more than 50%) and more aplerotic oospores than did the wheat isolate.

Colonies of *P. sylvaticum* complex on CMA and DCMA formed cottony aerial mycelium with a radiate pattern and grew at 28 mm/24 hr. Sporangia did not form, but chains of two to four globose hyphal swellings formed readily. Oogonia formed in single culture and were indistinguishable from hyphal swellings. Antheridia (three to six per oogonium) were inflated, branched, diclinous, and surrounded the oogonium. Oospores were aplerotic or nearly plerotic. Growth and asexual reproduction were similar to ATCC isolates 18195 and 18196 of *P. sylvaticum*, none of which produced zoospores. Pairings of the wheat isolates with *P. sylvaticum* (ATCC 18195 and 18196), *P. intermedium* (ATCC 36445), *P. heterothallicum* (ATCC 18197 and 18198), *P. catenulatum* (ATCC 10950), and *P. splendens* (ATCC 14557) yielded no mating reactions on PCA, PCA-CMA, or PCCA at 20 C.

Colonies of the unidentified *Pythium* sp. "E" were submerged and exhibited chrysanthemum or mixed radiate and chrysanthemum patterns and grew at 11 mm/24 hr at 20 C. Sporangia were globose, subglobose, or limoniform (Fig. 1, A to N), hyaline when young and golden brown when mature, mostly

single (Fig. 1A to E and K to N), sometimes catenulate (2–4) and basipetally developed (Fig. 1G to J), terminal and intercalary, and 24–37 × 18–31 (avg. 30.5 × 24) μm in diameter. Sporangia germinated either directly or indirectly (Fig. 1M and N). Discharge tubes were 23–35 × 5–7 μm, and sometimes slightly constricted at the base. Empty sporangia retained their shapes after zoospore release (Fig. 1M and N). Vesicles were slightly larger than sporangia and contained 10–15 zoospores each. Encysted zoospores were 9–10 μm in diameter and germinated by a single germ tube (Fig. 1O). Oogonia, usually 20–24 (avg. 24.2) μm in diameter, formed in paired cultures of compatible isolates on PCA; they were smooth, globose or subglobose, terminal (Fig. 2, A-E), and intercalary (Fig. 2F and G). Antheridia (1–4) were diclinous, bulbous, crook-necked, made apical contact with the oogonial wall (Fig. 2A to G), entwined the oogonium, and branched near them (Fig. 2B). The antheridial cells were 13–16 × 6–8 m. Oospores were aplerotic (Fig. 2E to G), sometimes nearly plerotic (Fig. 2D), single or double, usually 18–19 (avg. 18.9) μm in diameter, and contained a single oil globule 4–13 (avg. 8.3) μm in diameter and a reniform refringent body 3–6 × 2–3 μm. The oospore wall was 0.5–2.0 μm thick.

Owing to its slow growth rate on MPVM, colonies of *Phythium* sp. "E" were not observed on dilution-plates until after 48 hr of incubation, They exhibited a dense and submerged radiate pattern on MPVM. Numerous sporangia and zoospores were produced within 3–5 days after colonized baits (boiled grass leaf, carrot disk or hemp seed) or culture plugs were transferred into water in a petri dish and irrigated frequently with sterile DW.

Oospores (Fig. 2) were never produced by any single culture of this species on several substrates or on media containing cholesterol (PCCA and V8CB). Pairings of 36 isolates in all possible combinations were therefore made on both PCA and PCA-CMA and incubated at 20 C in the dark. Most pairings on PCA exhibited either slight or no mating reaction. Seven isolates (four male and three female) selected from this first test were paired in a second test on both PCA and PCCA at 10 and 20 C; slight reactions were observed only on PCA at 20 C. A thin, cottony mycelium and a few oogonia with antheridia were evident 1–2 mm under the surface of the agar in the zone of reaction. Intercrossings of *Pythium* sp. "E" with *P. catenulatum* (ATCC 10950), *P. heterothallicum* (ATCC 18197 and 18198), *P. sylvaticum* (ATCC 18195 and 18196), *P. splendens* (ATCC 14557), *P. intermedium* (ATCC 36445), and *P. macrosporum* (CBS 574.80 (+)) failed to produce mating reactions and oospores.

Colonies of the unidentified *Pythium* sp. "D" on CMA and DCMA were submerged, exhibited a sharp chrysanthemum pattern, and grew at 8 mm/24 hr at 20 C. Sporangia and zoospores did not form. Hyphal swellings were globose or subglobose, 10–25 μm in diameter, or limoniform to ellipsoidal, 14 × 11 μm in size, sometimes irregularly shaped, terminal, intercalary, or catenulate. Oogonia and antheridia rarely formed in water cultures; they were smooth, globose, mostly intercalary, and 20–28 (avg. 23.5) μm in diameter. Antheridia (one to two per oogonium) were monoclinous or diclinous, sessile or hypogynous. Oospores were completely plerotic, 19–26 (avg. 21.9) μm in diameter with walls 1.0–2.5 μm thick, and formed in grass-leaf or wheat-root tissues.

Disease symptoms caused by *Pythium* species from wheat. All 10 species or varieties of *Pythium* from wheat were pathogenic to various degrees on cultivar Daws wheat. The symptoms included: seed decay or seed rot; root rot, browning, and necrosis; distortion and shortening of the first true leaf; and stunting of the roots and shoots.

Seed decay. At the ¼ dilution of mycelial inoculum in sand and vermiculite, the isolates of *P. aristosporum, P. volutum, P. u.* var. *ultimum, P. u.* var. *sporangiiferum, P. irregulare*, and *P. sylvaticum* complex resulted in 60–100% seed decay (Table 1). At the ⅛ dilution of inoculum, *P. aristosporum, P. u.* var. *ultimum*, and *P. u.* var. *sporangiiferum* resulted in 13–39% fewer emerged seedlings compared to the control. *P. torulosum, P. heterothallicum, Pythium* sp. "E" and *Pythium* sp. "D" caused little or no failure of emergence at either inoculum concentration.

About 10% seed decay occurred in fumigated PSL infested with oospores (500 propagules per gram) of an isolate of *P. torulosum* (Table 2). Similar results were obtained with eight isolates of *P. irregulare* (*unpublished*). In contrast, 50–90% seed decay occurred in soil infested with oospores of *P. u.* var. *ultimum* (500 per g) and *P. u.* var. *sporangiiferum* (1,000 per gram) (Table 2).

Root rot, browning, and necrosis. *P. u.* var. *ultimum*, *P. u.* var. *sporangiiferum, P. aristosporum, P. volutum, P. sylvaticum*

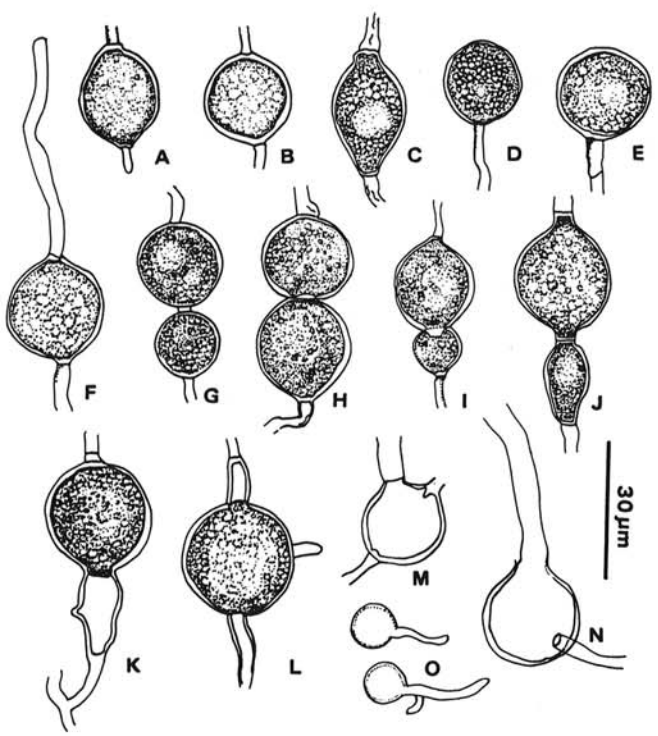

Fig. 1. Camera lucida drawings of *Pythium* sp. "E" grown on potato carrot agar. **A, D, E, and F**, terminal sporangia; **B, C, K, and L**, intercalary sporangia; **G to J**, catenulated sporangia; **M and N**, empty sporangia with discharge tubes; **O**, germinated zoospores.

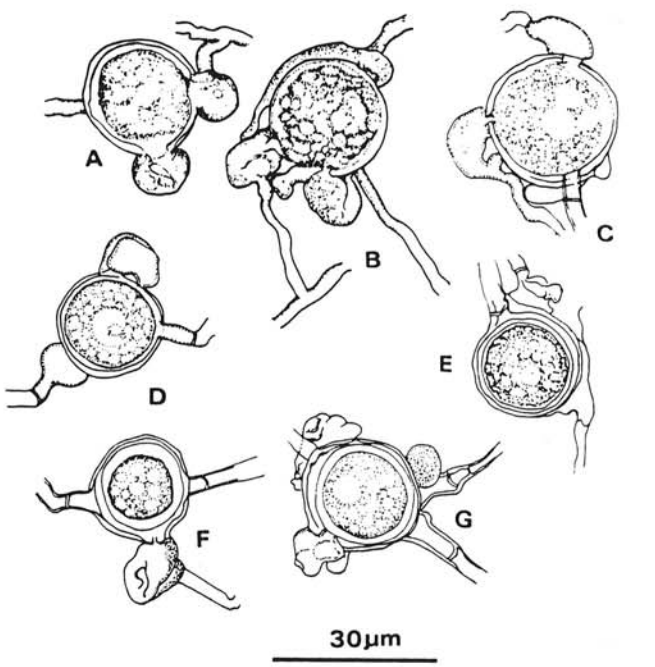

Fig. 2. Camera lucida drawings of oogonia, antheridia, and oospores of *Pythium* sp. "E" grown on potato carrot agar.

complex and *P. irregulare* also caused the most severe root rot or root browning on wheat. *P. aristosporum* was the most pathogenic as mycelial inoculum, and *P. volutum* was the second most pathogenic to roots; both species caused extensive decay of the epidermal cells, cortical tissues, and stele of all roots including the main axes, and the tissue became obviously brown and often dark brown at the root tips. The damage to roots caused by *P. u.* var. *sporangiiferum* and *P. u.* var. *ultimum* was mostly evident as decayed (rounded or blunted) root tips; the area behind the rotted tip remained normal in appearance, and one to several primary or lateral roots grew normally alongside the rotted roots. *P. sylvaticum* complex damaged the root tips but usually less than either of the two varieties of *P. ultimum*.

The symptoms caused by *P. irregulare* were similar to those caused by *P. u.* var. *ultimum* and *P. u.* var. *sporangiiferum*, except that the roots were more distorted. *P. torulosum, P. heterothallicum*, and *Pythium* sp. "E" caused only slight yellowing at the tips of the fine rootlets. *Pythium* sp. "D" caused no obvious symptoms except roots often were yellow compared to the control.

All species tested except *P. aristosporum* and *P. volutum* caused yellow to brown to dark brown necrotic lesions on the root tips or behind them. This symptom was especially prevalent on the fine rootlets. Oospores and/or hyphae were readily observed microscopically in some of the necrotic lesions and *Pythium* species were isolated from them on WA-R, MPVM, or MPVM-R.

Stunting of the roots. With the exception of those exposed to *P. heterothallicum*, the response times of the roots of wheat sown in the 1/8-dilution of mycelium-infested cornmeal sand to each of the *Pythium* species and varieties were significantly shorter than that of the check (Table 1).

Distortion and shortening of the first true leaf. All *Pythium* species or varieties tested as mycelial inoculum in cornmeal sand (1/8 dilution with vermiculite) caused the first true leaf to be significantly ($P = 0.01$) shorter and distorted compared with the control (Table 1). The species that caused root rot, i.e., *P. aristosporum, P. volutum*, and *P. ultimum* (both varieties), also caused the most obvious distortion, bending, and failure of the first true leaf to elongate normally. In contrast, *P. torulosum, P. heterothallicum*, and *Pythium* sp. "E", caused only slight or no bending and had the least affect on length of the first true leaf (Table 1).

The first true leaf was not significantly different from the control when seeds were sown in PSL infested with *P. torulosum* at 500 and/or 1,000 propagules per gram, but it was significantly shorter on plants grown in the same PSL infested with *P. irregulare* at 500 and/or 1,000 propagules per gram (*unpublished*). In another experiment, both varieties of *P. ultimum* but not *P. torulosum* (at either oospore concentration) caused the length of the first leaf to be significantly shorter than that of the control (Table 2).

Stunting of shoots. Wheat plants infected by *P. aristosporum, P. volutum, P. ultimum* (both varieties), and *P. irregulare* always were shorter than the healthy (check) plants, especially during the first 15–20 days after planting with mycelial inoculum. *P. aristosporum* caused dramatically (up to 56%) shorter shoots compared to the control; *P. volutum, P. u.* var. *ultimum, P. sylvaticum* complex, and *P. irregulare* resulted in shoots shorter than those of the control, although less markedly than did *P. aristosporum* (Table 1).

DISCUSSION

A total of 10 species or subspecies were recognized from among more than 350 isolates of *Pythium* obtained by various methods from wheat roots and wheat-field soils in eastern Washington and northern Idaho. *P. u.* vars. *ultimum* and *sporangiiferum* and *P. irregulare* caused both seed decay and root rot; *P. aristosporum, P. volutum*, and *P. sylvaticum* complex caused seed decay, root rot, and root browning; and *P. heterothallicum, Pythium* sp. "E", *Pythium* species "D", and *P. torulosum* caused milder forms of root necrosis that was usually reflected as slightly shorter shoots and first true leaves. However, all pathogenicity tests were conducted at between 10 and 15 C. Possibly some species would be more pathogenic at lower or higher temperatures, especially if those temperatures were relatively more unfavorable for the plant than for the pathogen (12).

Five of the 10 species and varieties of *Pythium* from wheat roots and wheat-field soils in eastern Washington and northern Idaho have previously been associated with wheat (22), but *P. u.* var. *sporangiiferum, P. sylvaticum* complex, the unidentified *Pythium* sp. "D" (all homothallic), *P. heterothallicum*, and the unidentified *Pythium* sp. "E" (both heterothallic) are first records for wheat. The characters of the two unidentified species do not match those of any species listed by Plaats-Niterink (22) and may be undescribed.

P. u. var. *sporangiiferum* is distinguished from *P. u.* var. *ultimum* by its ability to produce zoospores (5). There are very few records of *P. u.* var. *sporangiiferum* since it was originally described (5). For a long time *P. ultimum* has been known to be a pathogen of wheat, peas, and other crop plants in eastern Washington (11,23,25), and possibly zoospore-producing isolates have been overlooked until now. Both varieties of *P. ultimum* caused seed decay, preemergence seedling death, and root rot and necrosis of roots, especially of root tips.

P. aristosporum was the most pathogenic to wheat roots of all species included in this study. This species was originally described from diseased wheat roots in Canada by Vanterpool (26), and there are several reports of its occurrence on grasses (6,24) and wheat (27) in the United States. Lipps and Bruehl (16) isolated this species from wheat roots in eastern Washington and demonstrated that it causes severe stunting, root rot, and less tillering on wheat under greenhouse conditions at 8–15 C. In our study, *P. aristosporum* was isolated only occasionally from wheat roots and never from soil dilution plates since it does not grow on the MPVM selective medium. Both the morphology of oospores and the 24-hr growth rate of the *P. aristosporum* in our study agrees with that of the isolates reported by Lipps and Bruehl (16).

P. volutum also caused severe root rot or root browning and distorted roots of wheat, although less than did *P. aristosporum* under the same conditions. *P. volutum* has been previously reported to occur on wheat and oats in Canada (28), on wheat in England (26), and on turf in the United States (10). Like *P. aristosporum*, it was rarely isolated from wheat roots and never from soil dilution plates on MPVM. It resembles *P. arrhenomanes* but can be distinguished by its long diclinous antheridial stalk that coils around the oogonial stalk and the oogonium (especially in

TABLE 1. Pathogenicity of representative isolates of *Pythium* species from wheat roots and soil to wheat plants (cultivar Daws)[y]

Pythium species	Emergence[w] at indicated inoculum dilution		Length of plant part (cm)[x]		
	1/4 (v/v)	1/8 (v/v)	Root	Shoot	First leaf
P. aristosporum	1/40	8/40	3.9	11.8	2.3
P. volutum	15/40	30/40	8.2	19.7	3.9
P. torulosum	28/40	39/40	15.4	24.9	5.4
P. ultimum var. *sporangiiferum*	3/40	16/40	19.9	25.0	4.4
P. ultimum var. *ultimum*	1/40	17/40	15.2	21.4	3.6
P. sylvaticum complex	13/40	25/40	19.9	21.6	4.0
P. irregulare	6/40	24/40	11.2	24.1	3.5
P. heterothallicum (male × female)	30/40	37/40	22.4	26.4	5.7
Pythium sp. "E" (male × female)	37/40	38/40	18.4	25.4	5.6
Pythium sp. "D"	34/40	40/40	18.2	25.3	5.6
Control	40/40	39/40	25.1	26.6	7.2
LSD, $P = 0.05$			3.89	3.21	0.7

[x] Grown in infested cornmeal-sand mixture diluted 1/4 or 1/8 (v/v) with autoclaved vermiculite. Plants were maintained in a growth chamber at 10–15 C with 12-hr of light per day for 30 days.

[y] Total number of seedlings emerging out of 10 wheat seeds per pot, four pots per treatment.

[z] Average lengths of roots, shoots, and the first leaves from all emerged plants per pot, four pots per treatment at the 1/8-dilution of inoculum.

TABLE 2. Effect of three *Pythium* species on seedling emergence; lengths of roots, shoots, and first leaves; and root dry weight of wheat plants grown in soils infested with oospores

Fungi	Oospores[w] (no./g of soil)	Emergence[x] (no.)	Length per plant part (cm)[y]			Root dry weight (mg)
			Root	Shoot	First leaf	
P. ultimum var. *sporangiiferum*	500	16/30	25.7	22.5	4.9	189.9
	1,000	3/30	24.8	23.2	3.7	61.5
P. ultimum var. *ultimum*	500	8/30	28.1	21.7	4.6	74.6
	1,000	3/30	14/8	9.7	2.6	14.9
P. torulosum	500	29/30	22/3	28.2	7.2	363.6
	1,000	24/30	22/1	26.3	7.0	323.3
Control (fumigated soil)	0	30/30	24.7	34.0	7.6	485.3
LSD ($P = 0.05$)			6.93	7.01	2.02	161.9

[w] Plants grown in oospore-infested soils (500 and 1,000 propagules per gram) maintained for 40 days in a growth chamber at 10–15 C with 12 hr of light per day. Propagule density was adjusted by blending oospore-infested PSL with fumigated PSL soil and estimated by soil dilution plate counts on MPVM (20).
[x] Total number of plants emerged out of 10 seeds per pot, three pots per treatment.
[y] Average lengths of roots, shoots, and the first leaves of the plants in each of three pots per treatment.
[z] Mean dry weight of the root systems per pot, three pots per treatment. Roots were air-dried at room temperature for several weeks before measurement.

water culture), and also because it produces aplerotic oospores. The characteristics of our isolates agreed closely with those reported for *P. volutum* by Vanterpool (26), but the growth rates of our isolates were almost twice that reported (19) for this species. Sporangia and zoospores were never found although various substrates and conditions were tried, which agrees with the observations of Vanterpool and Truscott (28) and Sprague (25) for this species.

P. irregulare was first reported by Sprague (23) to cause severe root damage on wheat and oats in the United States. The isolates of *P. irregulare* obtained from wheat in this study were similar to those described by Plaats-Niterink (22), Sprague (25), and Middleton (19) except that our isolates showed a greater variation in the number, size, and shape of projections from the oogonia, and zoospores were never observed.

Vanterpool (26) compared an English strain of *P. torulosum* with a culture of *P. torulosum* isolated from grass roots in the Netherlands (22) and concluded that the two were different. The Netherlands strain exhibited more of a rosette growth habit and the antheridium arose closer to the oogonium than for the English strain. The English strain was slightly to moderately pathogenic to wheat seedlings, in contrast to the Netherlands strain which stimulated growth of grasses. The *P. torulosum* obtained from wheat roots in our study matches the Netherlands' strain based on its rosette pattern of growth, and it matches both strains in that some isolates were slightly to moderately pathogenic to wheat seedlings and others stimulated wheat growth. This species is therefore of doubtful importance as a wheat pathogen, although possibly it can cause damage under some conditions.

The globose or subglobose hyphal swellings of isolates of *P. sylvaticum* complex and the tendency toward heterothallism in these isolates are characteristic of both *P. sylvaticum* and *P. heterothallicum* (7,8). The inflated and branched antheridia that cover the oogonia are similar to traits of *P. heterothallicum* and *P. sylvaticum*, and the occurrence of catenulated hyphal swellings are similar to traits of *P. intermedium*. Our isolates did not mate with known isolates of *P. sylvaticum* (ATCC 18195 and 18196), *P. heterothallicum* (ATCC 18197 and 18198), or *P. intermedium* (ATCC 36445), but based on the similarity of traits, they were placed in the *P. sylvaticum* complex as proposed by Hendrix and Papa (9).

The two unidentified *Pythium* species "D" (homothallic) and "E" (heterothallic) were only slightly pathogenic to wheat at 10–15 C. *Pythium* sp. "D" produced no sporangia and zoospores and only a few oospores on the solid media (CMA, DCMA, and V8A) or in water culture. It showed some tendency toward heterothallism, yet all pairings among isolates failed to produce a consistent mating reaction. *Pythium* sp. "E", on the other hand, was strongly heterothallic and produced no oospores in single cultures under any conditions tested. This species has characteristics of *P. macrosporum* (globose to elongated, nonproliferating sporangia, and oogonia produced in paired cultures) and it forms no hyphal swellings. However, the colony pattern was more obviously chrysanthemum; it grew much slower; had smaller oogonia, oospores, and zoospores; and sizes and shapes of the antheridial cells differed from those of a known isolate of *P. macrosporum* (from Centraalbureau Voor Schimmelcultures (CBS 574.80 (+)) Furthermore, no mating reaction was evident between pairings of *Pythium* sp. "E" (male and female) and *P. macrosporum* (male) on PCA, PCA-CMA, and PCCA. *Pythium* sp. "E" differs from *Pythium* Group 'G' (22) in having oogonia in dual cultures and no proliferating sporangia. It differs from *P. catenulatum* by having globose, subglobose, or elongated sporangia instead of filamentous sporangia.

Heterothallic isolates of *Pythium* comprised 137 of 302 isolates obtained by the baiting method from roots of wheat seedlings grown in naturally infested soil collected from a single site. The site had been in a wheat-pea rotation, and the peas had just been harvested at the time the soil samples were collected. Either these isolates are pathogenic to peas as well as wheat, or they can survive in soil between wheat crops. Isolates obtained from soil dilution-plates on MPVM were mainly homothallic, which suggests that the propagules of the heterothallic species may not have survived the air-drying treatment used to prepare soils for dilution plating.

Undoubtedly more *Pythium* species will be found with further study or by sampling other wheat-growing areas in the Pacific Northwest. Wheat has been grown in a monoculture or rotated with only a few other crops for a long time in eastern Washington and adjacent northern Idaho. The native vegetation of this area was grasses before wheat farming began, and probably many *Pythium* species capable of infecting wheat are indigenous to the area and are adapted to the natural grasses as well as to wheat.

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