Snow Rot of Winter Wheat in Washington

P. E. Lipps and G. W. Bruehl

Research Assistant and Professor, respectively, Department of Plant Pathology, Washington State University, Pullman, WA 99164. Portion of a M.S. thesis of the senior author submitted to the faculty of Washington State University.

We acknowledge the support of the Washington State Wheat Commission.

Scientific Paper 4952, Project 0142, College of Agriculture Research Center, Washington State University, Pullman, WA 99164.

Accepted for publication 27 February 1978.

ABSTRACT

LIPPS, P. E., and G. W. BRUEHL. 1978. Snow rot of winter wheat in Washington. Phytopathology 68: 1120-1127.

Pythium aristosporum, P. iwayamai, P. ultimum, and an unidentified *Pythium* sp. (*Pythium* sp. "d") were isolated from dead wheat plants after snow and ice melt in eastern Washington. Leaves and crowns were rotted, and leaf sheaths at the base of tillers were browned. Plants with snow rot occurred in the same fields as plants affected by snow molds, but they were confined to areas where water had collected during snow and ice melt. Spraque winter wheat (which is moderately resistant to snow mold) was susceptible to snow rot, indicating that resistance to snow rot and to snow mold is not correlated. The *Pythium* spp. were isolated from plant tissues incubated at 1 C. At 0.5 C, *P. iwayamai* was pathogenic to Nugaines winter wheat when wet absorbent cotton was used to simulate snow on saturated and drained rooting medium. *Pythium aristosporum* was pathogenic with or without the cotton cover under saturated conditions but only moderately pathogenic under drained conditions. *Pythium ultimum* was nonpathogenic to plants under snow rot conditions, but it invaded root and leaf tissues without inciting snow rot. *Pythium* sp. "d" was isolated most frequently from plants associated with ice, but pathogenicity tests were not attempted. *Pythium iwayamai* killed 50% of the plants after 56 days under snow rot conditions at 0.5 C, but failed to incite root rot in the greenhouse at 8-15 C. *Pythium aristosporum* killed 50% of the plants after 72 days under snow rot conditions, and also caused severe root rot at 8-15 C. *Pythium iwayamai* and *P. aristosporum* grew more rapidly at 0.5 C than did *Typhula idahoensis* or *Fusarium nivale*. On agar media adjusted to varying osmotic potentials, *P. aristosporum* grew most rapidly at −1.3 bars (the highest water potential tested). *Pythium ultimum* and *P. iwayamai* grew most rapidly at −3 to −6 bars. Growth of the *Pythium* spp. was restricted at −28 bars. *Pythium iwayamai* produced zoospores at 0-15 C, but none at 20 C. Although *P. aristosporum* produced few zoospores at 5-15 C, it produced none at 0 or 20 C; direct germination of sporangia occurred at 0-20 C. *Pythium iwayamai* (and probably *Pythium* sp. "d") are considered true snow rot pathogens; *P. aristosporum* and *P. ultimum* are not.

In the spring of 1976, dead or diseased winter wheat (*Triticum aestivum* L.) plants were collected from 21 fields in Douglas, Lincoln, Okanogan, Spokane, Stevens, and Whitman Counties of eastern Washington in fields where water from snow or ice melt had stood or run. The snow cover, though persistent through most of the winter, was inadequate for profuse development of snow molds [caused by *Typhula idahoensis Remsberg and Fusarium nivale* (Fr.) Ces.]. Nevertheless, Sprague wheat, which is moderately resistant to snow molds, as well as other locally grown cultivars, was killed in these wet sites. Four *Pythium* spp. were isolated from tissues of these rotted plants.

Iwayama (8) described a snow rot of cereals caused by a *Pythium* sp. in Japan in 1929. In 1935, Ito (7) provided a Latin description and named the snow rot fungus *P. iwayamai* S. Ito. Hirane (5) described five additional *Pythium* spp. capable of causing snow rot. Snow rot in Japan was favored by high soil fertility, poor drainage, and deep snow (4).

Because snow cover is requisite for pathogenesis, growth at near-freezing temperatures is essential. Ekstrand (3) found that the growth rate of the snow mold fungi increased with incubation time at low temperatures. Bruehl and Cunfer (1) investigated the effect of water potential on growth rates of the snow mold fungi and reported that growth diminished markedly at osmotic potentials below −6 bars at 0.5 C.

This investigation was conducted to identify the *Pythium* spp. associated with dead wheat plants and to determine their role in the foliar rot of wheat plants under snow. Their growth at near-freezing temperatures was compared with that of the snow mold fungi and an experiment also was conducted to determine the influence of temperature on zoospore production.

MATERIALS AND METHODS

Collection and identification of the Pythium spp.—Diseased plants were dug from low-lying areas from 5 February to 5 May 1976 and transported to the laboratory in open plastic bags. When available, snow was packed into bags to keep the plants cool and to prevent drying, otherwise no attempt was made to keep plants cool and moist.

Plants were washed briefly in tap water, then placed in

00032-949X/78/000 199$03.00/0

cheesecloth bags and rinsed in cold running tap water for 24 hr. Diseased tissue was cut into pieces 1 cm long, pressed between paper towels to remove free water, and then four pieces of either leaf, crown, or root were placed in separate petri dishes of 2% water agar. After 7-10 days at 1 C, hyphal tips were isolated. For identification, the fungi were grown on Difco cornmeal agar (CMA) at 10 C and then maintained as stock cultures.

Media used in taxonomic studies included CMA, Difco lima bean agar (LBA), Difco potato-dextrose agar (PDA), and Miller's (12) V-8 juice agar (V8A). When possible, the fungi were identified from single-zoospore cultures grown on CMA. Dimensions recorded for the various fungal structures were based on a minimum of 100 measurements.

Growth in culture.—Agar plugs cut with a No. 3 cork borer (7-mm diameter) from the periphery of cultures actively growing on CMA at 10 C were placed in the center of a 100 × 15-mm plastic petri dish containing 20 ml of the culture medium. Immediately after inoculation, dishes were put into plastic bags and secured with rubber bands to minimize water loss. Then the dishes were placed in the constant-temperature chambers in the dark. Two perpendicular measurements of the colony diameter were taken from each dish. Recorded data were the means of the replicate colony diameters minus the 7-mm diameter of the inoculum plug. These methods were used in the following growth studies.

Twenty-four-hr growth rates of the *Pythium* spp. were recorded from the increase in colony diameter of the second 24-hr period of growth on CMA with three replicates.

Growth curves for *P. iwayamai, P. aristosporum* Vanterpool, the Wa. 5999-5 isolate of *T. idahoensis,* and the Wa. 74-61 isolate of *F. nivale* were studied on CMA at 0.5 C. Colony diameters were taken from three replicates every 48 hr for 20 days.

The effect of water potential on growth was determined by adding sucrose or KCl (14) to a basal medium of CMA. Osmotic potentials from −1.3 bars in the basal medium (2), to −28 bars (solutes added to basal medium) were examined at 5 and 0.5 C with five replicates at each temperature. Colony diameters were recorded for *P. iwayamai* and *P. ultimum* after 4 and 8 days at 5 and 0.5 C, respectively. *Pythium aristosporum* was measured after 7 days and *T. idahoensis* after 14 days at 5 C; both fungi were measured after 18 days at 0.5 C.

The effect of temperature on zoospore production.—Single-zoospore isolates were transferred from 2- to 3-mo-old CMA cultures to LBA or V8A. Five inoculum plugs cut from the periphery of colonies with a No. 5 cork borer (1-cm diameter) were placed into 20 ml of sterile distilled water at room temperature and then incubated at 0, 5, 10, 15, and 20 C in the dark. After 24 hr, the water in each dish was replaced with sterile distilled water previously incubated at each temperature. The number of motile plus encysted zoospores was counted with a haemocytometer 48 hr after zoospore release began. The means of three counts per dish of five dishes per fungus at each temperature were recorded.

Pathogenicity trials.—Seeds of Nugaines winter wheat (C. I. 13968) were submerged first in 70% ethanol for 5 min and then in 6% sodium hypochlorite for 5 min, rinsed three times in distilled water, planted in autoclaved sand in 237-ml (8-oz) styrofoam cups (one seed/cup) and incubated outdoors until seedlings attained the three-leaf stage. Plants were fertilized by watering with 10% Hoagland's solution No. 2 (6). Seedlings were hardened either at 3 C for 3 wk with 8 hr of light at 2,691 lux (250 ft-c) per day or were hardened outdoors from 16 October to 5 December 1976. Seedlings were used as transplants or were inoculated directly.

Inoculum consisted of field isolates of *P. iwayamai, P. aristosporum,* or of *P. ultimum* grown on a cornmeal-sand mixture (15 g cornmeal, 485 g sand, and 120 ml distilled water autoclaved 45 min at 121 C). Jars inoculated with different *Pythium* spp. were incubated at 10 C and shaken occasionally to insure uniform colonization of the medium. The 3-wk-old inocula were mixed with autoclaved sand and then the propagule counts were made by the dilution plate technique (13). Hardened seedlings were transplanted to infested sand, or the sand was applied uniformly over the surface of the rooting medium. An autoclaved cornmeal-sand culture of one of the *Pythium* spp. was used as the control.

Combinations of treatments were used to determine the effect of saturated soil and snow cover on disease development. Treatments consisted of flooding the plants with tap water to saturate the rooting medium or draining cups by perforating the bottoms and covering plants with a pad of wet absorbent cotton to simulate snow cover or with no covering. One seedling transplanted into 5% (w/w) infested sand in a 237-ml styrofoam cup was used for each replicate. Inoculated seedlings were maintained at 0.5 C in the dark for 85 days. Chamber-hardened seedlings were transplanted to sand infested with *P. iwayamai, P. aristosporum, or P. ultimum* in the first trial (five replicates per fungus per treatment). Isolates obtained from diseased plants were compared with original isolates. Only the species which killed plants in the first trial were used to infest sand to which outdoor-hardened seedlings were transplanted (10 replicates per fungus per treatment) to confirm pathogenicity.

Plants were taken from the low-temperature chamber and placed on greenhouse benches (8-15 C). The cotton cover was removed and thereafter plants were watered with 10% Hoagland's solution to facilitate recovery, and survivors were recorded after 2 wk.

The effect of incubation period on disease severity was studied using outdoor-hardened seedlings inoculated with 20 cc of 5% (w/w) infested sand applied uniformly to the surface of the rooting medium. Plants were flooded, covered with wet cotton, and incubated at 0.5 C in the dark. Ten replicates inoculated with either *P. iwayamai* or *P. aristosporum* were taken from the chamber at successive 8-day intervals, starting after 40 days of incubation. The number of surviving plants was recorded after the 2-wk recovery period.

The ability of the *Pythium* spp. to incite root rot on seedlings grown in the greenhouse at 8 to 15 C was tested. Seedlings were transplanted into 15-cm diameter plastic pots lined with plastic bags that contained autoclaved sand infested with either *P. iwayamai* or *P. aristosporum* (5%, w/w). Plants were fertilized by watering with 10% Hoagland's solution every 10 days. Half of the pots were provided with drainage holes made in the plastic liner; the others were kept saturated by adding tap water daily. Plants were maintained in the greenhouse for 50 days (five

plants per pot, five pots per treatment). The number of tillers per plant, the fresh weight of the tops and roots, and plant height from crown base to leaf tip were recorded.

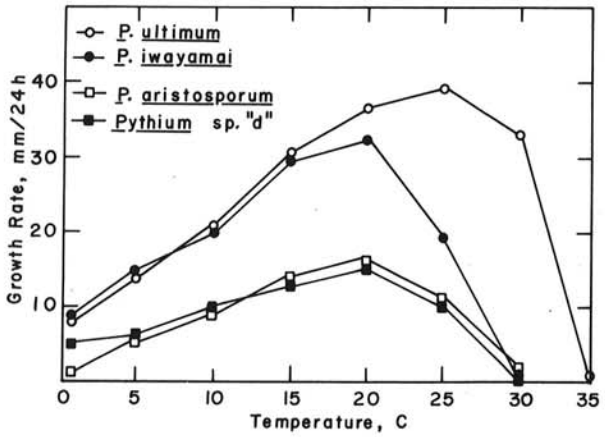

Fig. 1. Growth rates of *Pythium ultimum, P. iwayamai, P. aristosporum,* and *Pythium* sp. "d" on Difco cornmeal agar at 0-35 C. Growth rate based on the increase in colony diameter during the second 24-hr period at each temperature.

RESULTS

Field symptoms and signs.—As plants emerged from under a thick snow cover, older leaves had large, dark-green, water-soaked patches. Younger or more predisposed leaves were distorted, watersoaked, dark-green, and flaccid. Leaves in direct contact with the soil surface were more extensively rotted than those in an upright position. Plants died if the fungus invaded the growing point of the plant or tiller. After snow melt, the soft-rotted tissues dried to a brown or light-tan color. Basal leaf sheaths were dark-brown and filled with oospores. Root tissues were invaded only when run-off water washed soil from around the crowns. Plants could not be pulled from the soil because of the rotten crowns.

Field conditions.—Snow rot and snow mold both depend on extended periods of snow cover. Snow rot and snow mold occur in the same field, but in different sites because of varying soil moisture levels. Snow rot is favored in saturated sites, whereas snow mold develops in better drained sites. Snow rot may occur in spots as large as several hectares, a few square meters, or may involve only a few plants in a single row.

Isolation.—Attempts to isolate fungi from plants collected from beneath snow cover yielded many rapidly growing fungal colonies, whereas attempts to isolate them

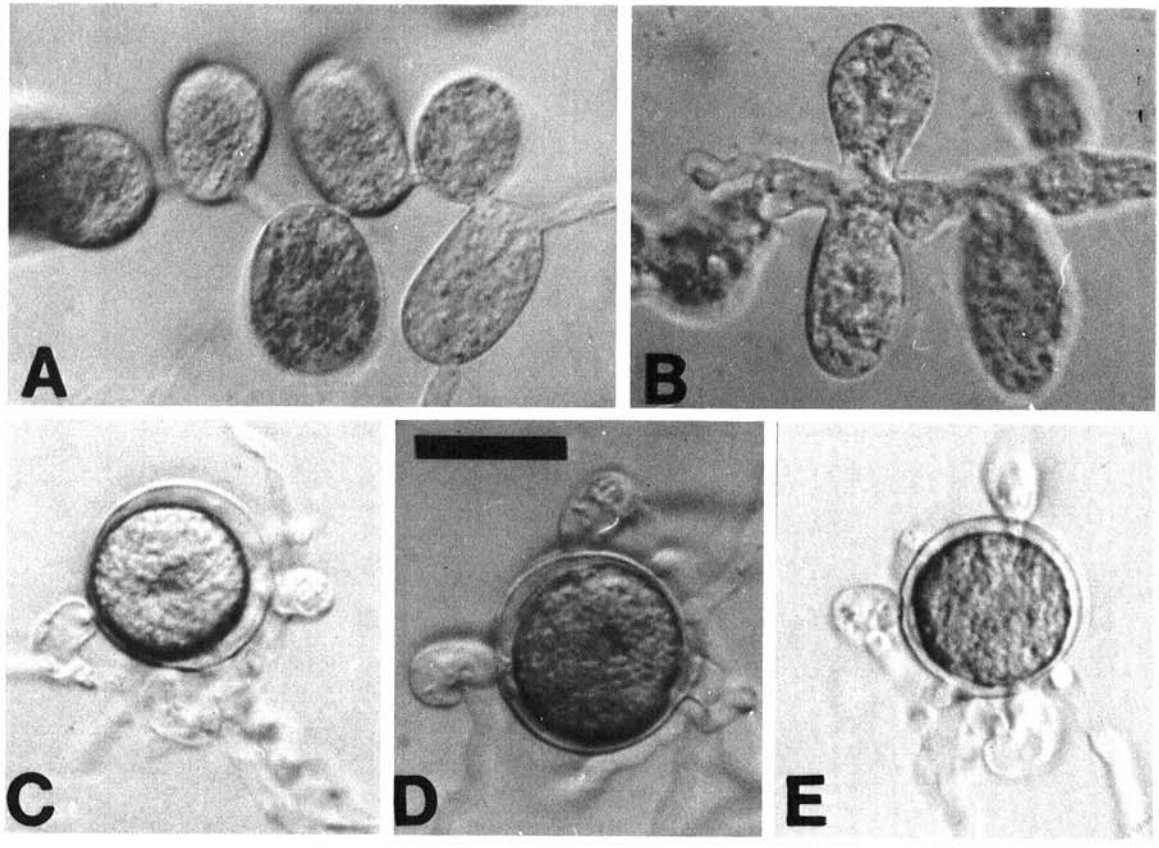

Fig. 2-(A to E). *Pythium aristosporum.* **A)** Sporangium with toruloid enlargements; **B)** Sporangium with digitate lobes; **C, D, E)** Oogonia and antheridia. Bar = 20 μm.

from dried plants collected later in the season produced only a few or sometimes none, even when oospores were detected in the tissues.

Hyphal-tip cultures on CMA at 10 C were identified tentatively as *P. aristosporum, P. iwayamai, P. ultimum,* and an unknown *Pythium* sp. which was designated *Pythium* sp. "d". Low numbers of two other Pythiumlike fungi also were isolated. *Fusarium nivale* was isolated occasionally and *Typhula* spp. were never isolated by the technique used. *Pythium iwayamai* and *Pythium* sp. "d" were isolated more often from leaf tissue than from roots. Leaf and root tissues yielded colonies of *P. aristosporum* or *P. ultimum* with almost equal frequency.

Identification of the Pythium species.—*Pythium aristosporum.*—Radial growth on CMA was most rapid at 20 C and little growth occurred at 0.5 and 30 C over 24 hr (Fig. 1). Oogonia [Fig. 2 (C to E)] were numerous on V8A, but few on CMA, LBA, or PDA. The best medium upon which to study the sexual structures was CMA with autoclaved carrot pieces placed on the agar surface. For production of sporangia and zoospores, V8A proved better than LBA. Sporangia [Fig. 2-(A, B)] either germinated indirectly and produced zoospores in a vesicle or more commonly sporangia germinated directly. Middleton (11) noted that *P. aristosporum* had diclinous and occasional monoclinous antheridia. The Washington isolates had both monoclinous and diclinous antheridia in about equal frequency. All morphologic features reported by Vanterpool (15) were present in our isolates.

The Washington isolates grew at lower temperatures than those reported by Middleton (11). Although the temperature-growth relationship of *P. aristosporum* was considered to be an important taxonomic character (11, 16), we believe this variation could be explained by strain differences. The morphology of the Washington isolates agreed closely with *P. aristosporum* and, therefore, they were considered to be that species.

Pythium iwayamai.—Radial growth on CMA was most rapid at 20 C, but was slower at 30 C than at 0.5 C (Fig. 1). Sexual and asexual structures formed in all media tested; oogonia with large antheridia [Fig. 3-(A to C)] were produced in greater numbers above 15 C and sporangia [Fig. 3-(D to I)] were more prevalent at lower temperatures. More zoospores were produced on LBA than V8A. Encysted zoospores were capable of reemerging by producing a single zoospore in a vesicle.

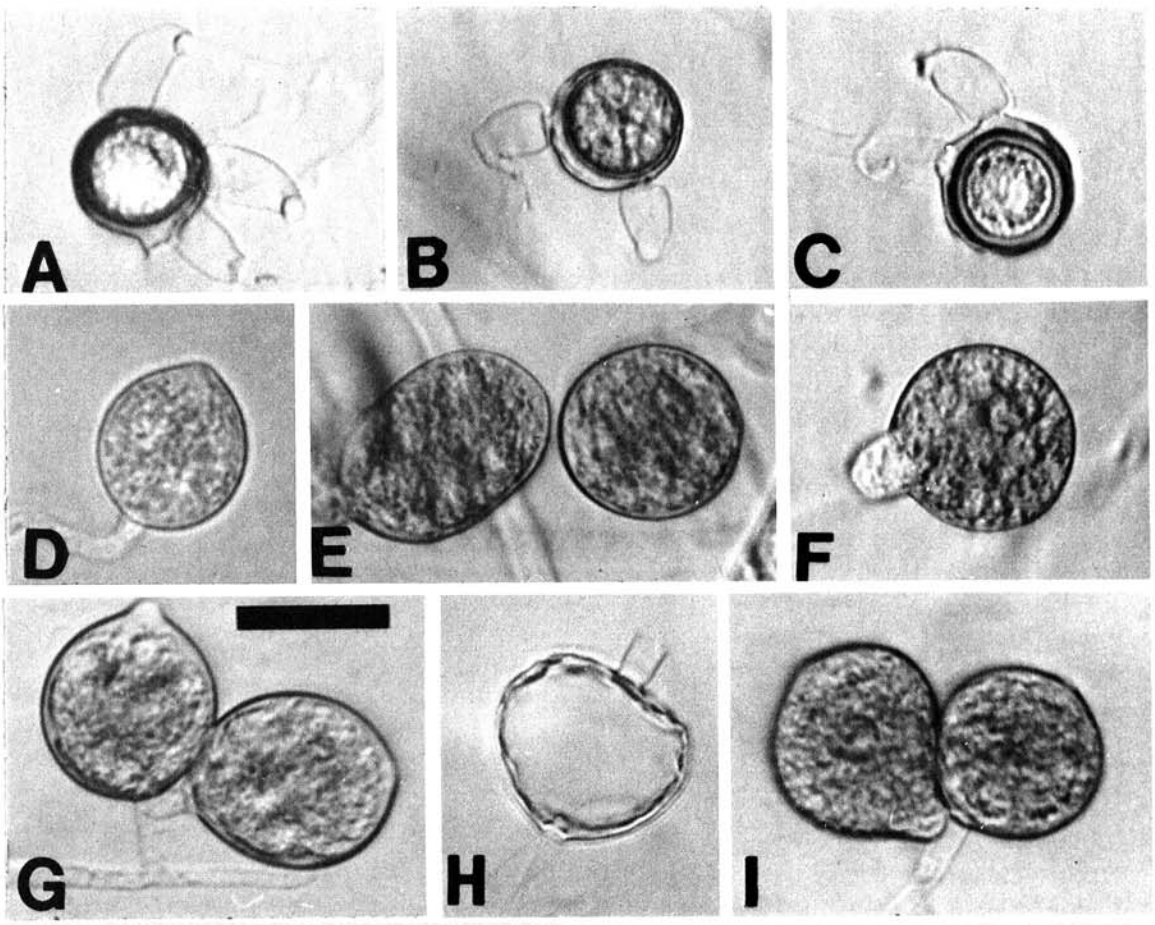

Fig. 3-(A to I). *Pythium iwayamai.* **A, B, C)** Oogonia and developing oospores with three, two, and one antheridium per oogonium, respectively; (D to I) Various shapes of sporangia: **G, I)** Sympodial arrangement of two sporangia; **H)** Empty sporangium with short evacuation tube subsequent to zoospore release. Bar = 20 μm.

Hirane (5) reported that oospores of his strains were aplerotic. Waterhouse (16) noted that photomicrographs in Iwayama's original paper showed aplerotic oospores, contrary to Ito's (7) Latin diagnosis. Oospores of the Washington isolates were aplerotic. Sporangia of the Washington isolates (24-37 × 20-30 μm) fell within the smaller size ranges (28-48 × 26-44 μm) reported by Iwayama (8). The aplerotic oospores, paragynous antheridia, the low temperature requirements, and variously shaped sporangia agreed morphologically with *P. iwayamai*.

Pythium ultimum.—Descriptions of our strains agreed with those given by Waterhouse (16) and Middleton (11) and with that of a culture received from J. M. Kraft, which he had identified to be *P. ultimum*. Radial growth on CMA was most rapid at 25 C and the isolate grew slowly at 35 C and 0.5 C (Fig. 1).

Pythium sp. "d".—This species was isolated most frequently from diseased plants covered with ice. Radial growth at low temperatures was faster than *P. aristosporum* (Fig. 1). Zoospores were produced on V8A in water cultures at 0 C by all isolates. More work is needed to identify this fungus and its pathogenicity has not been experimentally demonstrated.

Our identifications of *P. iwayamai* and of *P. aristosporum* from Washington were confirmed by J. Stamps of the Commonwealth Mycological Institute, Kew, Surrey, England. Single-zoospore cultures of these species and *Pythium* sp. "d" from Washington have been deposited in the CMI collection under the IMI numbers 209669, 209670, and 209671, respectively.

A detailed taxonomic study of *P. iwayamai* and *P. aristosporum* from Washington has been presented (10).

Growth at 0.5 C.—*Pythium iwayamai* and *P. aristosporum* grew more rapidly at 0.5 C than *T. idahoensis* or *F. nivale* (Fig. 4). *Pythium aristosporum* was the slower-growing *Pythium* spp.; its growth rate at 0.5 C approximated that of *T. idahoensis*. Growth rates accelerated with time for all fungi at 0.5 C (Fig. 4).

Growth rates on osmotically-adjusted media.—Because our fungi responded similarly to water potential at 5 and 0.5 C, only the 0.5 C data are presented (Fig. 5). *Pythium iwayamai, P. ultimum,* and *T. idahoensis* grew most rapidly at slightly reduced osmotic potentials (−3 to −6 bars). *Pythium aristosporum* grew most rapidly at the highest water potential tested; e.g., on the basal medium alone (−1.3 bars). The growth of the four fungi was slower at water potentials below −6 bars. After exposure for 25 days to media adjusted to −28 bars, *P. iwayamai* and *P. aristosporum* barely grew from the inoculum plug onto the agar. *Pythium ultimum* grew on agar adjusted to −28 bars with sucrose at 0.5 C and with KCl at 5 C but not at 0.5 C. At both temperatures, *T. idahoensis* grew at all osmotic potentials that were tested.

Zoospore production.—*Pythium iwayamai* (on LBA) and *P. aristosporum* (on V8A) were placed in water prior to sporangium formation. Sporangia were subsequently produced by both species at 0 to 20 C.

Pythium aristosporum produced zoospores on the 3rd day after agar plugs were placed in water at 15 and 10 C, and on the 4th day at 5 C. Over the first 48 hr of zoospore release, the fungus produced relatively few zoospores at either 5 or 10 C, and even fewer at 15 C; one isolate produced more zoospores than the other at 5 C (Fig. 6). No zoospores were produced at 20 or 0 C. Sporangia of *P. aristosporum* germinated directly at every temperature.

Sproangia of *P. iwayamai* began producing zoospores between the 1st and 2nd day after LBA plugs were placed in water at 5, 10, and 15 C. At 0 C (unfrozen water), zoospore production did not occur until after the 6th day. No zoospores were produced at 20 C. The number of

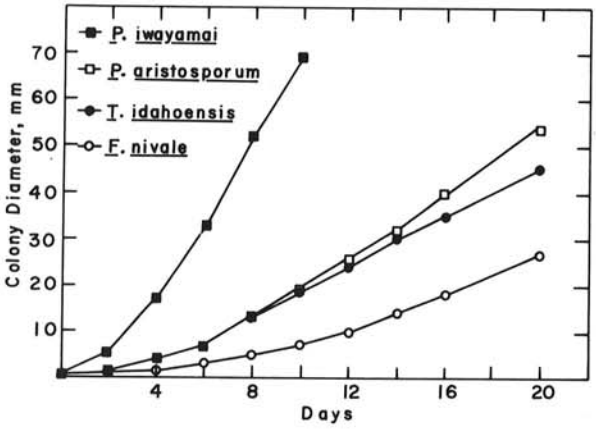

Fig. 4. Growth curves at 0.5 C for *Pythium iwayamai, P. aristosporum, Typhula idahoensis,* and *Fusarium nivale* on Difco cornmeal agar in the dark.

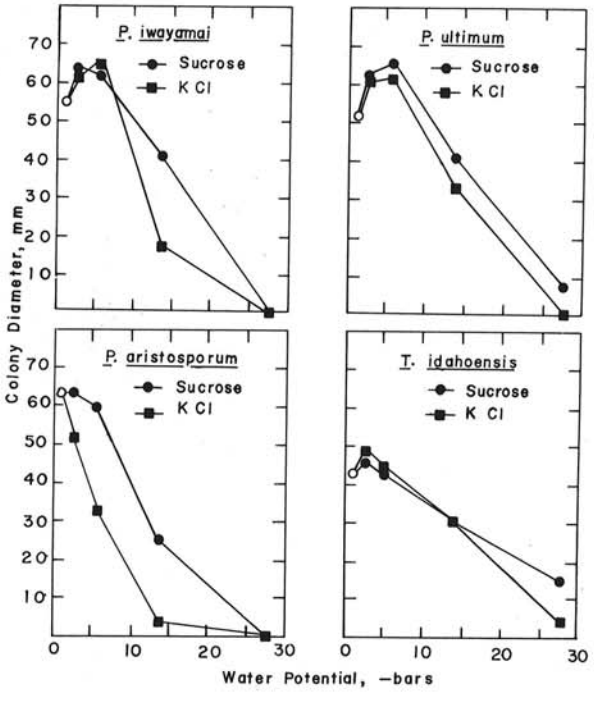

Fig. 5. Growth of *Pythium iwayamai, P. ultimum, P. aristosporum,* and *Typhula idahoensis* on Difco cornmeal agar adjusted to differing water potentials by varying concentrations of sucrose or KCl. Incubation at 0.5 C in the dark, growth based on colony diameter measured for *P. iwayamai* and *P. ultimum* at 8 days, and for *P. aristosporum* and *T. idahoensis* at 18 days.

spores present after the first 48 hr of zoospore production was greatest at 5 C and fewer were produced at either 0 or 10 C. Both isolates released similar numbers of zoospores at each temperature (Fig. 6).

The effect of simulated snow cover and saturated conditions on disease development at 0.5 C.—Inoculated plants under artificial snow rot conditions developed symptoms identical to those of diseased plants in the field. Control plants retained a light-green color during the entire incubation period. The tops of inoculated plants were water-soaked, dark-green, and flaccid. The leaf sheath bases around the crown were browned and rotted. Control plants were growing vigorously within the 1st wk of the recovery period. Plants with snow-rot symptoms did not recover.

Pythium iwayamai and *P. aristosporum* were pathogenic when cotton was used to simulate a snow cover at both moisture levels (Table 1). *Pythium aristosporum* was pathogenic under saturated conditions with no cotton cover, and less so in the drained treatment. Control seedlings and seedlings transplanted to sand infested with *P. ultimum* survived each treatment.

All fungi isolated from test plants were identical with original cultures used to inoculated them. Reinoculation for proof of pathogenicity was performed in trial two using only *P. iwayamai* and *P. aristosporum*. *Pythium ultimum* was isolated from roots and leaf sheaths of inoculated seedlings, but plants were not damaged and did not have snow rot symptoms.

Incubation period.—*Pythium iwayamai* killed two of 10 plants after 40 days of incubation and required 56 days to kill 50% of the plants (Fig. 7). *Pythium aristosporum* killed only one of 10 plants after 56 days, and required 72 days to kill 50% of the plants. None of the inoculated plants survived after 88 days. All noninoculated plants survived the entire period; 88 days at 0.5 C under 1 cm of water.

Root rot.—Control plants produced a mean of 7.7 tillers per plant in the drained rooting medium (Table 2). Seedlings transplanted to sand infested with *P. iwayamai* were 90% as tall as the control and no root browning was detected. Oospores were found in the epidermal and cortical cells of feeder roots. *Pythium aristosporum* caused severe stunting and root rot. The stele and cortical tissues were extensively rotted. Cortical cells sloughed from roots when plants were removed from the rooting medium. Seedlings transplanted to drained sand infested with *P. aristosporum* developed 67% fewer tillers, were 65% as tall, and had root systems with fresh weights only 45% that of healthy plants (Table 2).

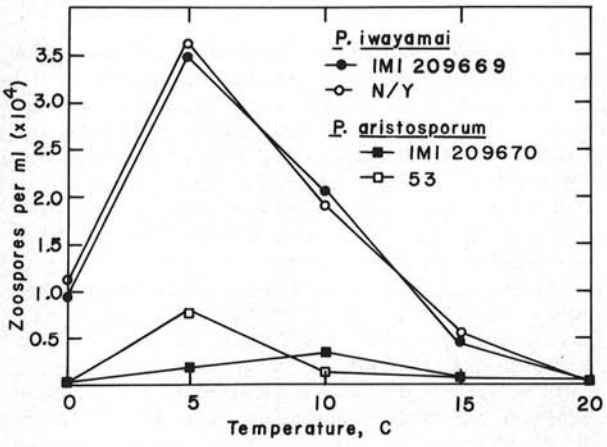

Fig. 6. The effect of temperature on zoospore production by *Pythium iwayamai,* isolates IMI 209699 and N/Y, and *P. aristosporum,* isolates IMI 209670 and 53. Zoospore production in vitro based on the number of spores present 48 hr after the beginning of zoospore release.

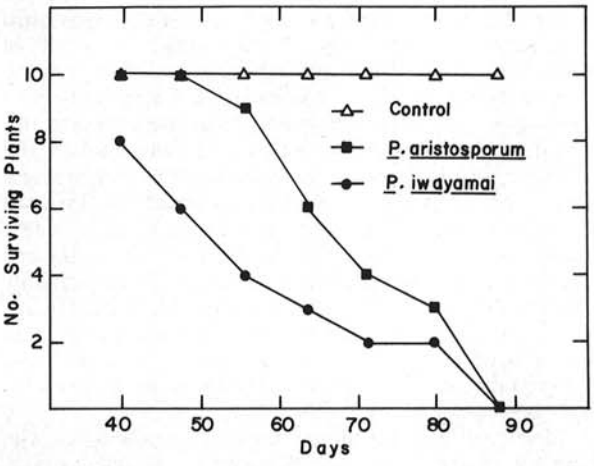

Fig. 7. Survival of wheat seedlings inoculated with *Pythium iwayamai* or *P. aristosporum* after varying periods of incubation at 0.5 C. Plants were inoculated with 5% (w/w) infested sand applied over the rooting medium surface and then incubated in the dark with cotton covers under saturated conditions. Data points are numbers of surviving plants out of ten (one plant/replicate) after a 2-wk recovery period. Calculated propagule density, 615 and 295/g sand for *P. aristosporum* and *P. iwayamai,* respectively.

TABLE 1. Survival of wheat seedlings transplanted to sand infested with *Pythium iwayamai,* or *P. aristosporum* (5% w/w)[a] with or without absorbent cotton covers at two moisture levels

Treatment[b]	Control	*P. iwayamai*	*P. aristosporum*
Cotton cover, saturated	15[c]	0	0
Cotton cover, drained	15	1	0
No cover, saturated	15	15	3
No cover, drained	15	14	8

[a]Calculated propagule density = 295 and 615 sand for *P. iwayamai* and *P. aristosporum,* respectively.
[b]Incubation at 0.5 C in the dark for 85 days. Surviving plants counted after a 2-wk recovery period.
[c]Numbers of surviving plants out of 15 (one plant/replicate) of two trials.

TABLE 2. Root rot development of wheat seedlings transplanted into sand infested with *Pythium iwayamai* or *P. aristosporum* (5%, w/w)[a]

Fungi	Drained sand[b]				Saturated sand			
	Plant height (cm)	Tillers per plant (no.)	Fresh wt Top (g)	Fresh wt Root (g)	Plant height (cm)	Tillers per plant (no.)	Fresh wt Top (g)	Fresh wt Root (g)
Control	32	7.7	3.5	1.9	17	2.6	1.0	1.4
P. iwayamai	29*[c]	7.0	2.9**	1.9	18	2.0*	0.8	1.1
P. aristosporum	21**	2.5**	1.2**	0.9**	15**	1.5**	0.7**	0.8**

[a]The calculated propagule density = 295 and 615/g sand for *P. iwayamai* and *P. aristosporum, respectively.*
[b]Incubation at 8-15 C in the greenhouse for 50 days.
[c]Asterisks * and ** indicate means of five replicates per treatment (five plants/replicate) were significantly different from control treatments at $P = 0.05$ and 0.01, respectively, according to Dunnett's procedure.

Control plants were stunted under the waterlogged conditions. Roots of plants in saturated sand infested with *P. aristosporum* were not as severely rotted or browned as those in the drained treatment. Feeder root development was greater in the control plants than in plants inoculated with either *Pythium* species.

DISCUSSION

Of the six snow rot-causing fungal species described in Japan (4, 5), only *P. iwayamai,* occurred in Washington. *Pythium aristosporum* was described by Vanterpool (15) in his studies of browning root rot of spring wheat in the Canadian prairie; nevertheless, our strains of this species, which were morphologically similar, attacked wheat under sustained low temperatures which are unknown to Canadian spring wheats. *Pythium ultimum* invaded the roots and leaf sheaths of wheat under conditions favorable to snow rot and produced oospores in those structures. Such invasions probably add to the survival of *P. ultimum* with little direct damage to this host. The undescribed *Pythium* sp. "d" was associated primarily with ice, and it is considered important under specialized conditions that have not as yet been determined.

Snow rot depends upon a prolonged snow cover. The weight of the snow presses leaves to the soil surface. Under artificial conditions, *P. iwayamai* was only virulent when the leaves were weighted down by the wet cotton. In contrast, *P. aristosporum* killed some plants without the cotton cover. With the cotton cover and with only as many as 50% of the propagules per gram of sand as *P. aristosporum, P. iwayamai* caused disease 16 days earlier. At 8-15 C in the greenhouse, *P. aristosporum* was more virulent than *P. iwayamai. Pythium iwayamai* is a pathogen only under a limited environmental range and *P. aristosporum* is better adapted to higher temperatures and to a wider range of environments.

Few isolates were obtained from dead plants that had remained in the field long after snow melt, even though the tissues contained many oospores. These oospores either become dormant rather rapidly, or their germination and growth onto agar media was suppressed by secondary organisms in the morbid tissues. Oospores, in fresh material collected at snow melt and not dried, germinated directly when placed on water agar at 1 C. Isolations should be made promptly from fresh materials if possible.

Even though all four *Pythium* spp. grew more rapidly at 0.5 C than the snow mold fungi (Fig. 1, 4), they required longer to kill wheat. *Pythium iwayamai* and *P. aristosporum* required about 85 days to kill wheat, whereas the snow mold fungi required 40-60 days under favorable conditions. Virulence is not related directly to rate of growth near freezing, especially when such different fungi are compared. Our in vitro temperature studies confirmed the observations of Ekstrand (3) that low-temperature fungi adjust to cold, that over an extended incubation period their growth accelerates. This accelerated growth occurred rapidly in *P. iwayamai* and less rapidly in *P. aristosporum,* the two species studied (Fig. 4).

Kouyeas (9) reported the mimimum water potential for growth of five of seven *Pythium* spp. to be near 97.8% RH at 20 C (−30 bars). His technique estimated the water potential by measuring the matric potential. The minimum water potential for growth of the three low-temperature *Pythium* spp. was near −28 bars as determined with osmotically-controlled media. The *Pythium* spp., like the snow mold fungi (1), grew best at water potentials above −6 bars.

When a sizable snow pack melts slowly, the trickling water must be essentially at 0 C. This water may accumulate in, or run down, the drill rows. *Pythium iwayamai* and *Pythium* sp. "d" produced zoospores in vitro in water at 0 C, *P. aristosporum* did not. *Pythium ultimum* produced no zoospores under any conditions tried. Although we have no proof, we suspect that zoospores are significant in the spread of the fungi that cause snow rot.

Free water for prolonged periods at the soil surface probably inhibits the snow molds, preserving this ecological niche for the *Pythium* spp. The faster-acting snow mold fungi would have destroyed the wheat before the *Pythium* spp., had not the former been inhibited.

LITERATURE CITED

1. BRUEHL, G. W., and B. CUNFER. 1971. Physiologic and environmental factors that affect the severity of snow mold of wheat. Phytopathology 61:792-799.
2. BRUEHL, G. W., B. CUNFER, and M. TOIVIAINEN. 1972. Influence of water potential on growth, antibiotic production, and survival of Cephalosporium gramineum. Can. J. Plant Sci. 52:417-423.

3. EKSTRAND, H. 1955. Overwintering of winter cereals and forage grasses. Summary of results and program for continual investigations. Meded. Vaxtskyddsanst. Stockholm, Sweden 67:1-125.
4. HIRANE, S. 1955. Studies on the control of Pythium snow blight of wheat and barley. Nat. Inst. Agric. Sci., Japn. 60:1-86.
5. HIRANE, S. 1960. Studies on Pythium snow blight of wheat and barley, with special reference to the taxonomy of the pathogens. Trans. Mycol. Soc. Japn. 2:82-87.
6. HOAGLAND, D. R., and D. I. ARNON. 1939. The water-culture method for growing plants without soil. Univ. of Calif., Coll. of Agr., Calif. Agric. Exp. Stn. Circ. 347. 39 p.
7. ITO, S., and Y. TOKUNAGA. 1935. Notae Mycologicae asiae orientalis I. Trans. Sapporo Nat. Hist. Soc. 14:11-33.
8. IWAYAMA, S. 1933. On a new snow-rot disease of cereal plants caused by Pythium sp. Agric. Exp. Stn. Toyama-Ken, Japan (no number):1-20.
9. KOUYEAS, V. 1964. An approach to the study of moisture relations of soil fungi. Plant Soil 20:351-363.
10. LIPPS, P. E. 1977. Pythium snow rot disease of winter wheat in Washington. M.S. Thesis, Washington State University, Pullman, WA. 47 p.
11. MIDDLETON, J. T. 1943. The taxonomy, host range, and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club 20:1-171.
12. MILLER, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
13. MIRCETICH, S. M., and J. M. KRAFT. 1973. Efficiency of various selective media in determining Pythium populations in soil. Mycopathol. Mycol. Appl. 50:151-161.
14. ROBINSON, R. A., and R. H. STOKES. 1955. Electrolyte solutions. Academic Press, New York, NY. 512 p.
15. VANTERPOOL, T. C. 1938. Some species of Pythium parasitic on wheat in Canada and England. Ann. Appl. Biol. 25:528-543.
16. WATERHOUSE, G. M. 1967. Key to Pythium Pringsheim. Commonwealth Mycol. Inst., Mycol. Pap. 109. 16 p.