The Influence of Temperature and Water Potential on Asexual Reproduction by *Pythium* spp. Associated With Snow Rot of Wheat

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ABSTRACT

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Zoospores were released by sporangia of *Pythium iwayamai* on colonized wheat leaf disks in deionized water at 1, 5, 10, and 15 C, but not at 20 C. *P. okanoganese* released zoospores from sporangia on colonized leaf disks at 1, 5, and 10 C, but not at 15 or 20 C. Fewer zoospores were released by *P. okanoganense* at 5 or 10 C than at 1 C, whereas *P. iwayamai* released relatively high numbers of zoospores at 1, 5, and 10 C. Both fungi released more zoospores over a 15-day period at 1 C than at higher temperatures. Empty sporangia with terminal evacuation tubes (indicating indirect germination) were observed on leaf disks maintained for 5 days in soil at 0.5 C, but only at 0 bars matric water potential. *P. iwayamai* and *P. okanoganense* released zoospores from 21 and 9% of their sporangia, respectively, in flooded soil (water to a depth of 1 mm above the soil surface), and from 4 and 1% of the sporangia, respectively, in saturated soil. At 1 C, sporangium production on wheat leaf disks was limited to osmotic water potentials (Ψ_0) above −8 bars, and zoospore release was limited to osmotic water potentials above −0.5 bars. Both *Pythium* spp. produced more sporangia and released more zoospores in snow melt water (Ψ_0 = −0.035 bars) than in any other solution tested. Neither *Pythium* spp. released zoospores in two soil extracts (Ψ_0 = −0.251 or −0.933 bars) nor in $MgSO_4$ solutions (Ψ_0 = −0.046 bars). More zoospores were released in dilute solutions of NaCl (Ψ_0 = −0.150 bars) and $NaNO_3$ (Ψ_0 = 0.143 bars) than in deionized distilled water (Ψ_0 = −0.002 bars). In nature, *P. iwayamai* and *P. okanoganense* probably produce zoospores only when sporangia at the soil surface are immersed in water from melted snow at near freezing temperatures.

The snow-rot disease of winter wheat (caused by *Pythium* spp.) occurs only in areas where water collects or drains during snow melt. The association of dead plants with water indicates that propagules of the *Pythium* spp. are disseminated in the snow-melt water (2,6). In the field, zoospores are probably released into water trickling down the drill rows at near freezing temperatures.

The importance of water potential on zoospore production by pathogenic Phycomycetes has been stressed in recent literature (1,3,4,8,10,14,15). Matric water potential was more restricting to zoospore release than osmotic water potential for both *Phytophthora* (8,10,15) and *Aphanomyces euteiches* (3). The effect of water potential on zoospore production by *Pythium* was not previously investigated, but observations indicate that saturated or flooded conditions favor zoospore release (13,14).

The purpose of this study was to investigate the effect of temperature and the osmotic and matric forces of water potential on sporangium production and zoospore release by two *Pythium* spp. associated with snow rot of winter wheat.

MATERIALS AND METHODS

The IMI 209669 isolate of *P. iwayamai* Ito (6) and the IMI 209671 isolate of *P. okanoganense* (5) were used in all studies. Both fungi were grown on Difco corn meal agar (DCMA) and incubated at 10 C until colony growth reached 5 cm in diameter. Disks, 7 mm in diameter, excised from the youngest blades of winter wheat, *Triticum aestivum* L. 'Nugaines', were placed around the margin of the growing colonies for 24 hr at 10 C.

Temperature effects. Five colonized wheat leaf disks were placed in each of 25 glass petri dishes containing 20 ml of deionized water (DW). The dishes were distributed into five groups which were incubated at either 1, 5, 10, 15, or 20 C. At 24-hr intervals for 15 days, the leaf disks were transferred to fresh DW precooled to one of the above temperatures. The number of zoospores in each dish was counted every 24 hr with a haemacytometer and the mean of five dishes with three counts per dish at each temperature was recorded. The experiment was performed twice.

Matric water potential effects. A Palouse silt loam (PSL) (16.2% sand, 60.0% silt, 22.8% clay; pH 6.6) was wet-sieved through a 2-mm (mesh-size) screen and air dried. River sand was mixed with the PSL (1:1, v/v) (PSL-sand) to modify the texture and moisture retention characteristics (Fig. 1). Moisture retention characteristics were determined by using tensiometers constructed of Büchner funnels with fritted glass plates of coarse porosity (Kimble 28400) and hanging water columns described by Pfender et al (10). Soil

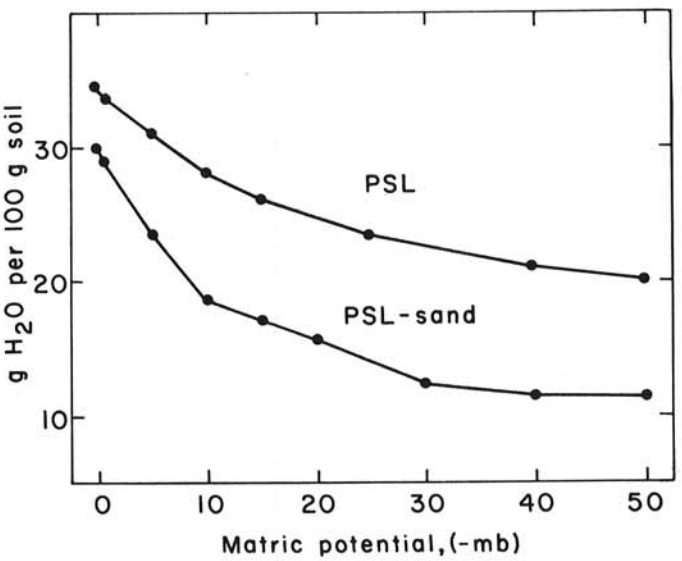

Fig. 1. The relationship between water content and matric water potential for Palouse silt loam (PSL) and a mixture of PSL and river sand (PSL-sand) obtained by wetting the soils in tensiometers.

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was placed on the porous tension plates to a depth of 2 mm. Colonized leaf disks were placed directly on the soil surface and covered with an additional 1-mm layer of soil. After the soil was wetted at low tension, the matric potential was controlled by adjusting the height of the hanging water column (8). Tensiometers were covered with aluminum foil to reduce water loss by evaporation.

Matric water potential studies were conducted at 0.5 C in the dark. Colonized leaf disks were placed in tensiometers when the fungus was present as mycelium only, thus sporangial development occurred on leaf disks while at the various water potentials. Leaf disks were retrieved from tensiometers after 5–10 days and immediately placed in 10-ml vials containing 1 drop of lactophenol per 5 ml of DW. Leaf disks were mounted in lactophenol on glass slides and examined microscopically to determine the mean number of full and empty sporangia viewed in a ×100 field along the edge of four leaf disks. Empty sporangia with terminal evacuation tubes were presumed to be those that released zoospores by indirect germination. The experiments were repeated at least three times.

Osmotic water potential effects. Five colonized leaf disks were placed in each glass petri dish containing 20 ml of different osmotic solutions and incubated at 1 C in the dark. Twenty-four and 48 hr later, the solutions were decanted and replaced with fresh solutions precooled to 1 C. Forty-eight hours after the last change in osmoticum the number of zoospores per milliliter and the total number of sporangia per ×100 field were determined by the methods above.

Osmotic water potentials were computed by determining the electrical conductance (EC in mmhos/cm) of solutions with a conductivity bridge and calculating the osmotic potential in bars by $\Psi_0 = 0.36(EC \times 10^3)$ (11). Osmotica used were $MgSO_4$, $NaNO_3$, $CaCl_2$, KCl, NaCl, and a salt mix of $CaCl_2$: KCl:NaCl in a 1:1:1

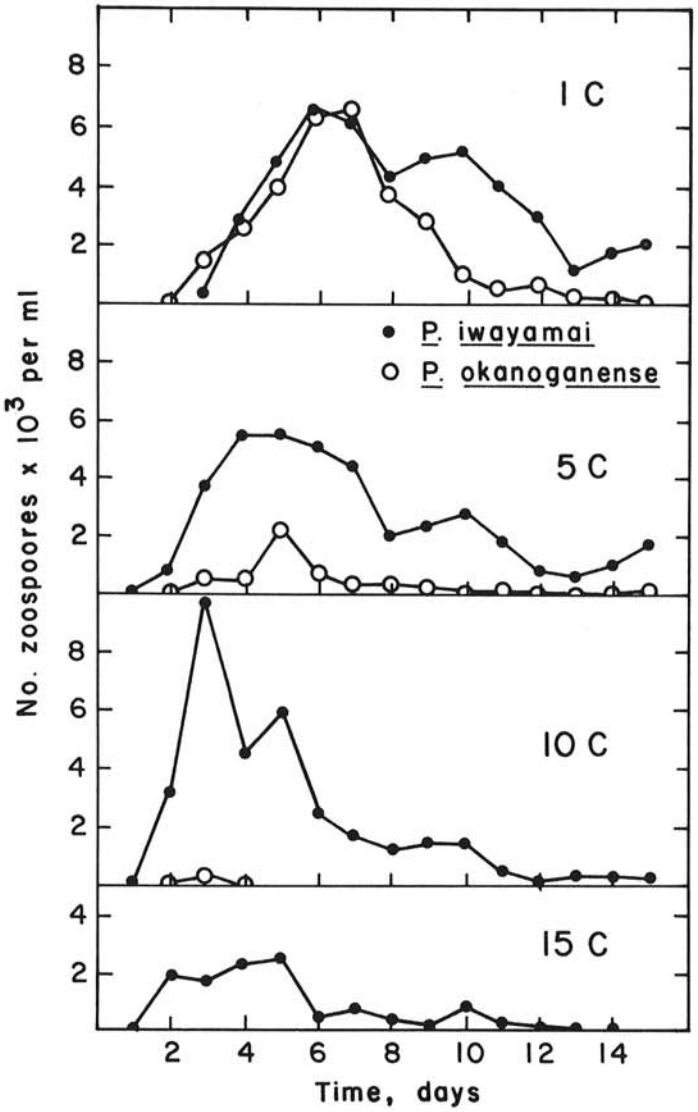

Fig. 2. The effect of temperature on daily zoospore production by *Pythium iwayamai* and *P. okanoganense* from wheat leaf disks in deionized water.

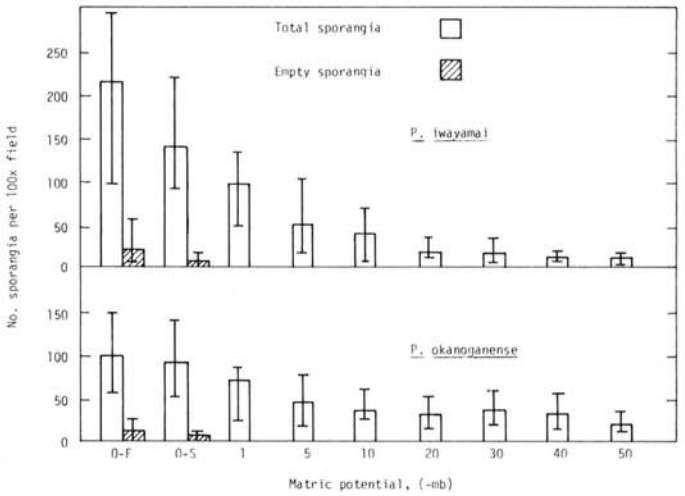

Fig. 3. The effect of matric water potential on sporangium formation and zoospore release by *Pythium iwayamai* and *P. okanoganense* at 0.5 C in the dark. The mean number of sporangia viewed per ×100 microscopic field along the edge of leaf disks after 5 days in tensiometers are represented by the vertical bars; the vertical lines indicate total variation. Empty sporangia with terminal evacuation tubes were interpreted as those which released zoospores. 0-F = flooded to a depth of 1 mm above the soil surface. 0-S = saturated soil; the matric water potential of both is approximately 0 bars.

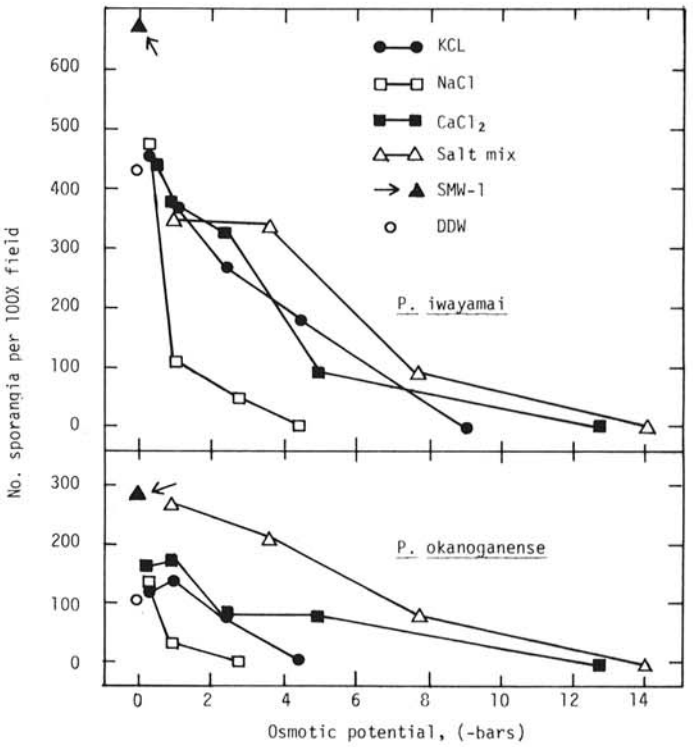

Fig. 4. The effect of osmotic water potential on sporangium formation by *Pythium iwayamai* and *P. okanoganense* at 1 C. The mean number of sporangia viewed per ×100 microscopic field along the edge of leaf disks in osmotic solutions are the data points. Salt mix = 1:1:1 molal ratio of NaCl:KCl:$CaCl_2$. SMW-1 = snow-melt water ($\Psi_0 = -0.035$ bars). DDW = deionized distilled water ($\Psi_0 = -0.002$ bars).

molal ratio. Deionized distilled water (DDW, $\Psi_0 = -0.002$ bars) was the solvent for all solutions. Saturation extracts (11) were prepared from two soils, PSL (soil extract-P) and a loam soil from LaFleur, Okanogan County, WA (42.2% sand, 44.6% silt, 13.2% clay; pH 7.0) (soil extract-0) with osmotic water potentials of −0.251 and −0.933 bars, respectively. Snow melt water was collected near Pullman, WA from two locations; flowing down a drill row of a field planted to winter wheat (SMW-1, $\Psi_0 = -0.035$ bars) and puddled on a lawn (SMW-2, $\Psi_0 = -0.017$ bars). Both were stored frozen until the experiments were conducted.

RESULTS

Temperature effects. *P. iwayamai* released zoospores from sporangia growing on colonized wheat leaf disks after 2 days in water cultures at 5, 10, and 15 C, and after 3 days at 1 C (Fig. 2). No zoospores were released from sporangia developing at 20 C. Sporangia of *P. okanoganense* produced zoospores after 3 days at 1, 5, and 10 C, but none were produced from sporangia at 15 or 20 C. Repetition of the experiment gave the same relative number of zoospores at the various temperatures. At 1 C, *P. iwayamai* and *P. okanoganense* maintained a level of zoospore production above 4,000 spores per milliliter per day for 7 and 4 days, respectively. Fewer zoospores were produced by *P. okanoganense* at 5 or 10 C than at 1 C, whereas *P. iwayamai* produced relatively high numbers of zoospores at 1, 5, and 10 C. Both fungi produced a greater total number of zoospores over the entire 15-day period at 1 C than at higher temperatures.

Matric water potential effects. Because there were no significant differences in the number of full (nongerminated) or empty (indirectly germinated) sporangia produced at the various matric water potentials in PSL or in PSL-sand mixture, only the data for PSL are presented (Fig. 3). Two water saturation treatments were used; soil was flooded to a depth of 1 mm above the soil surface (0-F) or the soil was maintained at saturation (0-S), both approximately 0 bars matric water potential. All matric water potentials were attained by wetting the soil immediately prior to placing colonized leaf disks in tensiometers.

By 5 days at 0.5 C, both *Pythium* spp. had produced sporangia along the edges of leaf disks at all matric water potentials tested (0 to −50 mb). There was, however, greater variation in the number of sporangia that developed at potentials of 0 to −10 mb than at −20 to −50 mb, with the greatest range in the number of sporangia at 0 bars for both species. *P. iwayamai* released zoospores from 21% of its sporangia in the flooded soil and from only 4% in the saturated soil. *P. okanoganense* released zoospores from 9% of its sporangia in the flooded soil and from only 1% in the saturated soil. No empty sporangia were observed on leaf disks maintained at matric potentials less than or equal to −1 mb. There was no difference in the number of empty sporangia produced after 5 or 10 days in tensiometers and the number of empty sporangia did not increase after the longer period of time for either *Pythium* spp.

Osmotic water potential effects. At 1 C *P. iwayamai* produced more sporangia than *P. okanoganense* on leaf disks at osmotic water potentials (Ψ_0) above −2 bars (Fig. 4). Snow melt water (SMW-1) was more favorable for sporangium production than DDW, or than any of the salt solutions tested. The lower osmotic water potential limit for sporangium formation by *P. iwayamai* in $CaCl_2$ KCl, and the salt mix was −8 to −14 bars and −4.5 bars for NaCl. The lower osmotic water potential limit for sporangium production by *P. okanoganense* in $CaCl_2$ and the salt mix was −12 to −14 bars and −2 and −4.5 bars for KCl and NaCl.

Zoospore production by sporangia that developed on leaf disks in solutions at 1 C was limited to osmotic water potentials above −0.5 bars (Table 1). *P. iwayamai* and *P. okanoganense* both produced more zoospores in snow melt water than in any other solution tested, and *P. iwayamai* produced more zoospores than *P. okanoganense*. Neither of the *Pythium* spp. produced zoospores in the two soil extracts ($\Psi_0 = -0.933$ and −0.251 bars). Of the various osmotica tested, NaCl and $NaNO_3$ were less inhibitory to zoospore production than were KCl, $CaCl_2$ or $MgSO_4$. *P. iwayamai* did not produce zoospores in solutions of $CaCl_2$, $MgSO_4$, or the salt mix, and *P. okanoganense* did not produce zoospores in the $MgSO_4$ solutions. The lowest osmotic water potential at which zoospores were produced was −0.487 bars (salt mix) for *P. okanoganense* and −0.150 bars (NaCl) for *P. iwayamai*.

TABLE 1. The effect of osmotic water potential (Ψ_0) on zoospore production by *Pythium iwayamai* and *P. okanoganense* from colonized wheat leaf disks at 1 C

Solution or osmoticum	Ψ_0 (−bars)	Zoospores (No. × 10^3 ml^{-1})	
		P. iwayamai	*P. okanoganense*
Deionized distilled water	0.002	2.0	1.9
Snow melt water-1[a]	0.035	15.0	6.7
Snow melt water-2	0.017	20.0	6.0
Soil extract-0[b]	0.933	0.0	0.0
Soil extract-P	0.251	0.0	0.0
NaCl	0.150	6.2	5.3
	0.466[c]	0.0	0.1
$NaNO_3$	0.078		3.9
	0.143	4.6	5.3
Salt mix[d]	0.487	0.0	2.8
KCl	0.093	0.4	1.6
$CaCl_2$	0.124	0.0	0.4
$MgSO_4$	0.046	0.0	0.0

[a] Snow melt water-1 and -2 were collected from a snow-covered field sown to winter wheat and a lawn, respectively.

[b] Soil extract -0 and -P are saturation extracts from a soil collected in Okanogan Co., WA (pH = 7.1) and Palouse silt loam (pH = 6.58), respectively.

[c] No zoospores were produced in solutions with osmotic water potentials below −0.5 bars and the data are not presented.

[d] Salt mix = 1:1:1 molal of $CaCl_2$:KCl:NaCl.

DISCUSSION

Because snow-rot of wheat occurs beneath snow it is requisite that the causal fungi produce infective propagules at temperatures near 0 C. Both *P. iwayamai* and *P. okanoganense* produced zoospores from wheat leaf disks at near freezing temperatures, with more zoospores produced over a 15-day period at 1 C than at higher temperatures. *P. iwayamai* released zoospores over a wider range of temperatures than *P. okanoganense*. In nature, however, zoospores released at temperatures above 1 C probably have no role in the incidence of the disease.

Zoospore production by *P. iwayamai* and *P. okanoganense* was extremely sensitive to matric and osmotic water potentials in contrast to sporangium formation which was less sensitive to these factors. Zoospore production by both *Pythium* spp. was prevented at matric water potentials below 0 bars and at osmotic water potentials less than −0.5 bars. Although other phycomycetous fungi are also sensitive to reduced matric and osmotic water potentials, *P. iwayamai* and *P. okanoganense* are two of the most sensitive studied to date (3,4,8).

Stanghellini and Burr (14) reported that sporangia and oospores of *P. aphanidermatum* did not produce zoospores in saturated soil, but zoospores were released when the soil was flooded with three times the volume of distilled water necessary to saturate it. Similarly, the snow-rot *Pythium* spp. required either saturated or flooded soil before zoospore release occurred. More empty sporangia were observed in flooded soil when the soil solution was diluted than in saturated soil, although the number of empty sporangia was relatively small in both cases (Fig.3). Neither *P. iwayamai* nor *P. okanoganense* produced zoospores in extracts from two soils (Table 1). Snow melt water was the most favorable medium for zoospore production of all solutions tested. Zoospore production was prevented in $MgSO_4$ solutions at osmotic potentials approximating that of snow melt water (SMW-1, $\Psi_0 = -0.035$ bars, $MgSO_4$, $\Psi_0 = -0.046$ bars). Other osmotica (NaCl, KCl, and $NaNO_3$) were less inhibitory than was $MgSO_4$ at potentials two to three times lower. This indicates that factors other

than matric water potential prevented zoospore release in saturated soil. The osmotic water potential studies demonstrated the sensitivity of the *Pythium* spp. to specific ions (8,15) or other substances in the normal soil solution. They probably release zoospores only when washed with relatively pure water.

P. iwayamai and *P. okanoganese* released more zoospores in dilute solutions of NaCl and $NaNO_3$ than in deionized distilled water. *Phytophthora cinnamomi* produced more sporangia and released more zoospores in nonsterile soil extracts than in sterile distilled water or tap water (9,16). Also, more zoospores were released from zoosporangia of *Aphanomyces* spp. when the sporangia were washed in dilute salt solutions, tap water, or lake water compared with distilled water (3,7,12). Although small amounts of osmotica increased zoospore production, studies are needed to explain the stimulating effect of snow melt water on sporulation by the snow-rot fungi.

Under natural conditions, sporangia of the snow-rot *Pythium* spp. probably release zoospores at the soil surface in running water from snow melt. The aboveground plant parts are the only tissues infected by the snow-rot fungi, except coronal roots when water washes soil from around the crown (6). This evidence supports the theory that sporangia of these fungi produce zoospores at or above the soil surface during flooding in an environment unobstructed for zoospore dissemination (1,13,14), and may explain the restricted occurrence of snow-rot in areas where water from melted snow flowed or accumulated (2,6).

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