Factors Influencing the Incidence of Embryo Infection by *Pythium* spp. During Germination of Wheat Seeds in Soils

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Research conducted by the first author in partial fulfillment of the requirements for the M.S. degree, Washington State University, Pullman.

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Accepted for publication 30 March 1994.

ABSTRACT

Fukui, R., Campbell, G. S., and Cook, R. J. 1994. Factors influencing the incidence of embryo infection by *Pythium* spp. during germination of wheat seeds in soils. Phytopathology 84:695-702.

The incidence of embryo infection (EI) by *Pythium* spp. in soil held at a matric potential (ψ_m) of −10 J/kg was a function of inoculum density (ID) at lower IDs and reached a plateau (EI_{max}) at higher IDs. In field soils, EI_{max} (69.9–89.0%) was achieved at 150–200 propagules per gram. In pasteurized soil reinfested with *P. ultimum* var. *sporangiiferum*, EI_{max} (≅100%) was achieved with only 50 propagules per gram. The incidence of EI was greatest at −10 J/kg, progressively less as soil was drier, and negligible at drier than −200 J/kg. Both ID and ψ_m could be limiting for EI at lower IDs, whereas only ψ_m affected EI when soil contained enough ID for EI_{max}. When the pH levels of two natural soils were adjusted to 4.3–7.6 with H_2SO_4 or $Ca(OH)_2$, the incidence of EI was greatest at pH 5.1–6.7. The treatment of soil with chloramphenicol resulted in a higher incidence of EI in soil adjusted to alkaline pH, suggesting that antagonistic soil bacteria had some involvement in the low incidence of EI in alkaline soil. The incidence of EI was higher as seeds aged and lower when seeds were soaked in water before planting.

Additional keywords: *Pythium* carrying capacity, resident antagonists, seedling blight, soilborne pathogens.

Damage caused by *Pythium* spp. to germinating wheat seeds and fine rootlets is a major factor limiting wheat yield in the fertile Palouse region (southeastern Washington and adjacent northern Idaho) of the Inland Pacific Northwest (10,11). This pathogen is especially damaging to wheat planted in cool, wet seedbeds covered with crop debris (10,12,13) typical of the conservation tillage systems needed to control soil erosion on the rolling topography of the region. Wheat-field soils of this region commonly contain 300–400 *Pythium* propagules per gram in the top 8–10 cm (11). The usual methods for reducing inoculum density of soilborne plant pathogens (e.g., crop rotation and soil fumigation) are neither adequate nor economical for *Pythium* spp. pathogenic to wheat.

Incidence of damping-off caused by *Pythium* spp. can vary greatly among soils and is independent of the amount of primary inoculum present, even when soil moisture is ideal for seed or seedling infection by *Pythium* spp. (5,6,16,19,25). Thus, some *Pythium*-suppressive soils are the type in which the "pathogen becomes established but fails to produce disease" (3). Suppressiveness of soils to *Pythium* spp. may differ with physical and chemical properties of the soil (19,25) and may be the result mainly of competition from soil microorganisms favored by the particular physical and chemical properties of the soil (20,21,23,31). Soils suppressive to *P. ultimum* in the San Joaquin Valley of California have a higher content of sodium chloride compared with conducive soils from the same area; either moist-heat treatment of the soil at 50 C or application of green manure as a source of nutrients eliminated suppressiveness of these soils (25). Suppressiveness of a soil from the South Kohala region of Hawaii to *P. splendens* is related to high calcium content of the soil, but suppressiveness also could be eliminated either by biocide treatments or by addition of nutrients (19).

Our study was conducted to identify the soil factors important in the epidemiology of *Pythium* damage to wheat in the Palouse region of the Inland Pacific Northwest. This information is needed to indicate areas in which wheat may be at highest risk to infection by *Pythium* and to guide development of possible management strategies. Infection of germinating seeds of wheat by *Pythium* spp. is normally nonlethal; i.e., actual seedling blight or damping-off is rare unless the seed is several years old (17). However, *Pythium* spp. infect the embryos of germinating seeds of wheat within the first 24–48 h after they are planted in suitably moist soil (17,18), which may affect the subsequent vigor of wheat. Such embryo infection also offers a sensitive method by which to measure the influence of soil factors on *Pythium* infection of wheat. Therefore, we evaluated the percentage of embryo infection determined by plating as a measure of *Pythium* activity in response to the host (germinating wheat seeds) in different soils.

MATERIALS AND METHODS

Soils. Soil samples were collected from the top 10 cm of 12 different wheat fields in eastern Washington and northern Idaho (Table 1). Each soil sample was a composite of samples taken from at least four places within a 10-m area in each field (for a total of at least 10 kg). These composite samples were individually blended, air dried, passed through a 3-mm mesh, and stored in paper bags in a greenhouse at room temperature until used. The four soils used extensively in this study were 1) a Larkin silt loam (fine-silty, mixed, mesic, ultic agrixerolls), 2) a Lovell silt loam (fine-silty, mixed, frigid, ultic haploxeralfs), 3) a Thatuna silt loam (fine-silty, mixed, mesic, xeric argialbolls), and 4) a Pedigo silt loam (coarse-silty, mixed, mesic, cumlic-haploxerolls)

collected near Fairfield, Washington; Joel, Idaho; and Palouse and Endicott, Washington, respectively.

Wheat seed. One-year-old wheat seed of the cultivar Hill 81 were used in all experiments except the one for the study of the influence of seed age on embryo infection. All seeds were surface disinfested with 25% commercial bleach (5.25% sodium hypochlorite) for 6 min, rinsed thoroughly with running tap water, air dried, and then stored at 7 C for up to 3 mo.

Pathogen inoculum. The strain of *P. ultimum* var. *sporangiiferum* used in this study was originally isolated from an infected root of a wheat seedling obtained from a field near Pullman, Washington. The isolate was maintained on potato-carrot agar (38) and transferred to fresh medium every 6 mo.

Estimation and adjustment of inoculum density of *Pythium* spp. in soil and reinfestation of pasteurized soil with *P. u. sporangiiferum*. The inoculum density (ID) of *Pythium* spp. in soil was determined by the method of Ingram and Cook (18). Briefly, 4 g of a composite soil sample was suspended in 100 ml of sterile water with 0.1% agar in a glass bottle, and 1.0 ml of this soil suspension was then plated onto a selective medium (26) in each of three petri dishes. Rifampicin (50 μg/ml) was substituted for rose bengal in this medium (9). After incubation at room temperature for 24 h, soil particles retained on the agar were washed off under running tap water, and colonies of *Pythium* spp. were verified at 15×. The colonies were counted after 72 h, and the ID was determined with three replicates (a total of nine dishes).

To adjust the ID of *Pythium* spp. in soil, each naturally infested field soil sample was diluted with a predetermined amount of the same soil treated with aerated steam at 60 C for 20 min to eliminate *Pythium* inoculum (2). Different amounts of pasteurized soil were used to create the desired range of IDs. The natural and corresponding pasteurized soils were mixed thoroughly in a twin-shell blender for at least 20 min, and the final ID of *Pythium* spp. in each blend was then confirmed by the dilution-plate method described above.

Larkin silt loam was pasteurized and reinfested with inoculum of *P. u. sporangiiferum* (hereafter referred to as pasteurized-reinfested soil). The inoculum was produced by growing the pathogen in potato-carrot broth for 3–4 wk at 15 C (38). Then, 200 mycelial mats were harvested on cheese cloth; rinsed twice with distilled water; comminuted in a blender at low speed for 2 min; and mixed as a water suspension of mycelium, oospores, and sporangia with 3 kg of pasteurized soil, bringing the water content to 20% (w/w). After incubation at 15 C for 1 wk, the soil was air dried (to reduce the inoculum to mainly or almost exclusively oospores), and the ID was determined. Samples of this soil were then diluted with different amounts of the non-infested pasteurized soil to create the desired IDs of *P. u. sporangiiferum* as described above. The actual populations were confirmed by dilution-plate counting.

Adjustment of soil matric potential. The hanging column method with a 350-ml porous plate funnel (medium porosity) was used to adjust and maintain soil matric potential (ψ_m) in most experiments conducted at −10 J/kg (17). Since only Larkin silt loam was used in experiments that required soils drier than −10 J/kg, a moisture release curve was first constructed for this soil, and the soil container method was used to adjust ψ_m as follows. Samples of air-dried soil (400 g) contained in 1.7- or 1.9-quart plastic bowls (Rubbermaid Inc., Wooster, OH) were wetted to a water content equivalent to −10 J/kg (30.4% by weight). The bowls were covered with tight-fitting lids and incubated at 15 C for 24 h. After equilibration, the lids were removed, and the soils were allowed to air dry in a growth chamber with circulating air for different periods of time (or to adjust several soils each to a certain ψ_m, soils in bowls were air dried for the same period of time). The bowls were resealed after the soils were dried for different periods of time at 15 C until the driest soil (lowest ψ_m) was ready for use. Some bowls were kept sealed from the start to maintain ψ_m at the original −10 J/kg. Before the seeds were planted, triplicate soil samples (each about 15 g) were taken from each container at the planting depth (1 cm) and oven dried at 105 C for 24 h to determine water content. The mean water content was then converted to ψ_m according to the water release curve. Since final values of ψ_m among soils dried for the same amount of time were slightly different, ψ_m is expressed by the average value and standard deviation.

Measurement of the incidence of embryo infection by *Pythium* spp. in soil. With both the hanging column and soil container methods, wheat seeds were planted in the soils after equilibration for 24 h following adjustment of ψ_m. Twenty seeds were planted in each of two replicate hanging columns, which then were covered with a plastic bag to prevent further drying. Forty seeds were planted in each soil container, which then was covered with its

TABLE 1. Soil chemical and physical properties and inoculum densities of *Pythium* spp. for 12 wheat-field soils and the correlation with incidence of embryo infection (EI)

Site of collection	Soil type	Incidence of EI (% of seeds)	Inoculum density[r]	Soil pH	CEC[s] (meq/100 g of soil)	Exchangeable cation content (meq/100 g of soil)				Clay content (%)	Organic carbon content (%)	Organic nitrogen content (%)	Nutrient diffusivity[t] ($\times 10^{-10}$ m^2/s)	Gas diffusivity[t] ($\times 10^{-10}$ m^2/s)
						K	Na	Ca	Mg					
Potlatch, ID	Silt loam	91.7	163	4.4	21.1	0.55	0.91	8.72	1.13	15.2	1.8	0.34	1.89	5.5
Fairfield, WA	Silt loam[u]	85.0	276	4.6	20.6	0.62	0.80	12.60	1.93	20.6	ND[v]	ND	5.14	1.6
Tekoa, WA	Silt loam	81.3	978	5.5	28.2	2.06	0.84	17.47	1.86	19.8	2.6	0.34	3.32	3.1
Joel, ID	Silt loam[w]	76.3	342	4.5	23.7	0.55	0.86	10.40	2.35	17.7	1.5	0.34	2.59	4.1
Potlatch, ID	Silt loam	76.3	606	5.1	19.2	0.48	0.88	6.45	1.00	11.1	1.3	0.23	1.89	5.3
Farmington, WA	Silt loam	67.1	653	5.4	26.0	1.87	0.81	14.07	2.32	19.1	2.0	0.25	4.21	2.3
Colfax, WA	Silt loam	61.3	1,281	6.1	26.9	2.23	0.92	14.32	2.18	16.1	2.4	0.30	3.20	3.2
Palouse, WA	Silt loam[x]	56.7	215	4.9	24.2	1.07	0.90	12.33	1.81	22.1	2.0	0.38	1.75	4.9
Diamond, WA	Silt loam	43.8	275	7.2	27.2	1.74	0.79	16.66	2.26	12.8	1.7	0.26	4.80	1.8
Prosser, WA	Loamy sand	42.5	84	5.5	13.0	0.78	0.97	5.71	1.67	6.0	ND	ND	0.35	8.4
Endicott, WA	Silt loam[y]	26.3	228	7.6	24.1	1.71	0.90	16.58	1.42	7.6	1.6	0.26	4.67	1.9
Kamiak Butte, WA	Silt loam	16.3	45	5.1	27.3	0.21	0.93	14.42	4.41	14.4	0.6	0.19	3.67	2.8
Correlation coefficient (R^2)[z]			0.01	0.64*	0.33	0.23	<0.01	0.27	0.03	0.22	0.02	0.17	0.14	0.19

[r] Propagules per gram of soil.
[s] Cation exchange capacity.
[t] At −10 J/kg.
[u] Larkin silt loam (fine-silty, mixed, mesic, ultic agrixerolls).
[v] Not determined.
[w] Lovell silt loam (fine-silty, mixed, frigid, ultic haploxeralfs).
[x] Thatura silt loam (fine-silty, mixed, mesic, xeric argialbolls).
[y] Pedigo silt loam (coarse-silty, mixed, mesic, cumlic haploxerolls).
[z] The correlation coefficient was calculated in relation to the incidence of EI transformed by the arcsine transformation determined with three replicates among 10 soils listed, excluding the soils collected from Prosser and Kamiak Butte containing less than 100 propagules per gram of soil. An asterisk indicates a significant negative linear correlation at $P = 0.05$.

lid. After incubation at 15 C for 48 h, the seeds were removed, washed with a strong jet of water, wrapped in cheese cloth, and washed under running tap water overnight. The washed seeds were plated, embryo end down, onto water agar containing 50 μg of rifampicin per milliliter. Hyphae of *Pythium* spp. growing from the embryos were examined under a dissecting microscope (15×) for 2–3 days after plating. The incidence of embryo infection (EI; percentage of seeds) was determined with 40 seeds per determination and two to four replicates, depending on the experiment.

Determination of ID-EI relationship in various soils. The ID-EI relationship was studied in three soils (Larkin silt loam, Lovell silt loam, and Thatuna silt loam), each adjusted to −10 J/kg by the hanging column method, and in the pasteurized-reinfested soil (Larkin silt loam) adjusted to −11 ± 1, −45 ± 5, and −88 ± 22 J/kg by the soil container method. The incidence of EI was determined for at least six incrementally different IDs (as high as the original ID) with two or three replicates for each ID. The ID-EI relationship was then analyzed by an adaptation of the Lineweaver-Burk double reciprocal plot technique (34) for studying ID-disease incidence relationships. In our study, the original Michaelis-Menten equation for enzyme kinetics was modified for the ID-EI relationship:

$$EI\,(\%) = \frac{EI_{max}}{1 + \frac{ID_{50}}{ID}} + C,$$

where EI_{max} is the maximum EI (%), ID is the estimated inoculum density (propagules per gram of soil), ID_{50} is the ID at one-half of EI_{max}, and C is a constant coefficient. The most probable values for three variable determinants (EI_{max}, ID_{50}, and C) were found by the Marquardt nonlinear least squares method (32).

Measurement of EI in soil adjusted at different ψ_m. A natural field soil (Larkin silt loam), either air dried for storage or not air dried before use (taken freshly from the field), and the same soil pasteurized and reinfested with *P. u. sporangiiferum* at 20, 50, and 150 propagules per gram were adjusted to eight different ψ_m of −10 to approximately −240 J/kg by the soil container method. The incidence of EI then was determined in each soil as described above. The experiment was conducted twice, and the data were analyzed by linear regression.

Relationships between EI and chemical and physical properties of the soils. The incidence of EI was determined in each of 12 natural field soils adjusted at −10 J/kg with three replicates. An arcsine transformation was applied to the incidence of EI, and the transformed data were analyzed in relation to each of the soil properties by linear regression.

Several soil properties were determined for each of the 12 soils at least twice for each bulked sample to reveal the possible basis for different EI_{max} values for different soils. Soil pH was measured by suspending a 5-g soil sample in 5 ml of 10 mM $CaCl_2$ and determining pH with a single glass electrode. Cation exchange capacity was determined by the method of Rhoades (33), and concentrations of exchangeable cations (K, Na, Ca, and Mg) were measured by atomic absorption spectrometry. Soil particle distribution was determined by a sedimentation analysis made by the pipette method, and each soil was classified (14). Organic matter content was measured by the carbon-hydrogen-nitrogen Determinator (Leco Corporation, St. Joseph, MI). Nutrient and gas diffusivities at −10 J/kg were calculated (8,29).

Measurement of EI in soil adjusted to different pH values. The influence of soil pH on EI was studied in an acid soil adjusted to higher pH values and an alkaline soil adjusted to lower pH values. The Larkin silt loam (originally pH 4.6) was amended with different amounts of $Ca(OH)_2$ (0, 1.5, 3.0, 4.5, 6.0, or 9.0 g/2.5 kg of soil) to adjust soil pH to different values as high as 7.5. Each soil-lime combination was mixed thoroughly in a twin-shell blender and moistened to approximately 20% water content (approximately −50 J/kg) in a polyethylene bag. After incubation (equilibration) at 15 C for 7 days, the soils were air dried, and pH was measured for each soil. The Pedigo silt loam (originally pH 7.6) was moistened with different amounts of 0.18 N H_2SO_4 (0, 350, 1,050, 1,400, 1,750, or 2,100 ml/2.5 kg of soil) to adjust soil pH to different values as low as 4.3. These amended samples were each incubated and then air dried as described above for the soil samples amended with $Ca(OH)_2$. For larger amounts of H_2SO_4, the soil was moistened once with 500 ml, incubated, and air dried. The procedure was then repeated until the desired soil pH was achieved. The other soils adjusted to higher pH values were air dried and remoistened with water only after all H_2SO_4 had been added. As a control to determine the effect of sulfate ions on EI, soil also was amended with $CaSO_4$ in quantities that provided the same amount of sulfate ions added by H_2SO_4. The incidence of EI was determined in each soil adjusted at −10 J/kg by the hanging column method, and the experiment was replicated three times.

Measurement of EI in soil treated with fungicidal and bactericidal chemicals. The Larkin silt loam was sprayed uniformly with a solution of benomyl (E. I. DuPont De Nemours & Co., Wilmington, DE), chloramphenicol chloromycetin (United States Biochemical Corp., Cleveland, OH), and iprodione (Rhone-Poulenc Inc., Monmouth Junction, NJ) to produce final concentrations of 5.0, 7.2, and 5.0 μg a.i./g of soil, respectively. Soils were treated with a single chemical or with combinations of chemicals. The incidence of EI was determined in each treated soil adjusted at −10 J/kg with four replicates. Soil matric potential was adjusted by the soil container method in this experiment; soil in containers was wetted to the water content needed for a ψ_m of −10 J/kg and equilibrated for 24 h before seeds were planted. This experiment was also conducted with the same soil adjusted to pH 7.4 by the method described above. The containers with soil treated with the different chemicals were arranged in a completely randomized design. Data were analyzed by analysis of variance (ANOVA), and means were separated by Fisher's least significant difference.

Effect of seed age on the incidence of EI. Wheat seeds of three cultivars (Hill 81, Daws, and Nugaines) harvested in two different crop years (current seeds = new, and 4-yr-old seeds = aged) were surface disinfested as described previously and used to determine

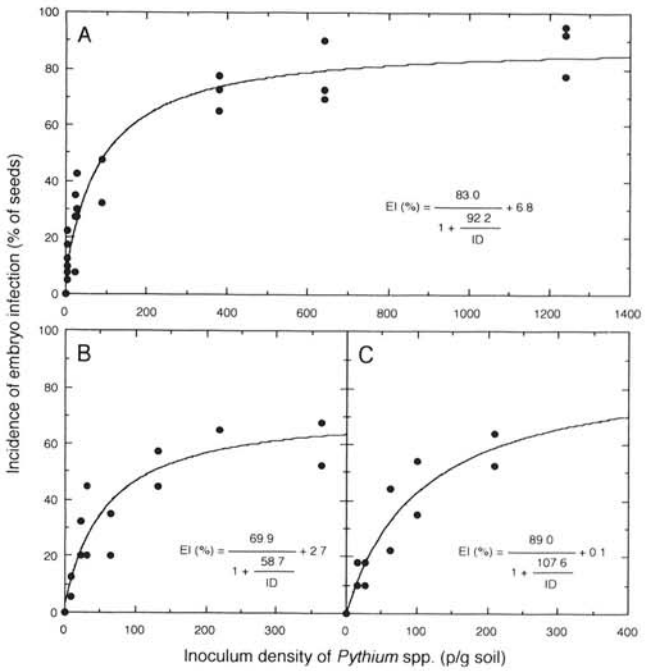

Fig. 1. Inoculum density (ID) and embryo infection (EI) relationships fit to the modified Michaelis-Menten equation in **A,** Larkin silt loam, **B,** Lovell silt loam, and **C,** Thatuna silt loam naturally infested with *Pythium* spp. To produce different IDs, each soil was diluted with the same respective soil pasteurized to eliminate *Pythium* inoculum. p/g = Propagules per gram of soil.

the incidence of EI in three field soils held at −10 J/kg. In a second experiment, the new and aged seeds of the three cultivars were soaked in sterilized water at 5 C for 6 h, air dried overnight in a vented hood, and planted in the Larkin silt loam held at −10 J/kg to measure the incidence of EI. Nonsoaked seeds were included as controls.

In both experiments, treatments were replicated two or three times, and data were analyzed by three-way ANOVA.

RESULTS

ID-EI relationships in three soils naturally infested with *Pythium* spp. The incidence of EI in soil naturally infested with *Pythium* spp. was related directly to ID at 0–200 propagules per gram but not when ID exceeded 200 propagules per gram in Larkin, Lovell, and Thatuna silt loams (Fig. 1). In Larkin and Lovell silt loams, the average incidences of EI at the original ID (no dilution with pasteurized soil) were 88.3 and 60.0%, respectively. These values were similar to the estimated EI_{max} of 83.0 and 69.9%, respectively, for these two soils (Fig. 1A and B). In Thatuna silt loam, the average incidence of EI at the original ID (58.3%) was considerably lower than the estimated EI_{max} of 89.0% (Fig. 1C). Since this soil without dilution contained only 211 propagules per gram, the ID-EI relationship could not be estimated accurately at IDs higher than 211 propagules per gram of soil.

Effect of ψ_m on EI and the ID-EI relationship in the pasteurized-reinfested soil. The incidence of EI in the pasteurized-reinfested soil was greatest at −10 J/kg (approximately 70% at 20 propagules per gram and approximately 100% at both 50 and 150 propagules per gram) and approximately half of these maxima at −120 J/kg (Fig. 2A, B, and C). Estimated variance (95% confidence intervals) for three regressed lines (Fig. 2D) revealed that the ψ_m-EI relationship at 50 propagules per gram of soil was not significantly different from that at 150 propagules per gram of soil. However, the ψ_m-EI relationship at 20 propagules per gram of soil was significantly different from that at 50 and 150 propagules per gram of soil; i.e., the incidence of EI was consistently lower at 20 than at 50 or 150 propagules per gram of soil at ψ_m between approximately −10 and approximately −85 J/kg.

The incidence of EI in the pasteurized-reinfested soil held at −11 ± 1 J/kg rose markedly in response to increasing ID at 0–50 propagules per gram and reached approximately 90% at only 38 propagules per gram (Fig. 3A). Only 8.2 propagules per gram of soil were required to achieve 50% EI_{max}. The estimated EI_{max} was 100%, and the greatest incidence of EI at the highest ID (360 propagules per gram of soil) also was approximately 100%. Moreover, EI_{max} in this soil was not affected when the soil was dried to −45 ± 5 J/kg (Fig. 3B) and was only slightly lower when ψ_m was adjusted at −88 ± 22 J/kg (Fig. 3C). However, the ID_{50} in this pasteurized-reinfested soil increased to 21.1 and 36.0 propagules per gram when the soil was at −45 ± 5 and −88 ± 22 J/kg, respectively (Fig. 3B and C).

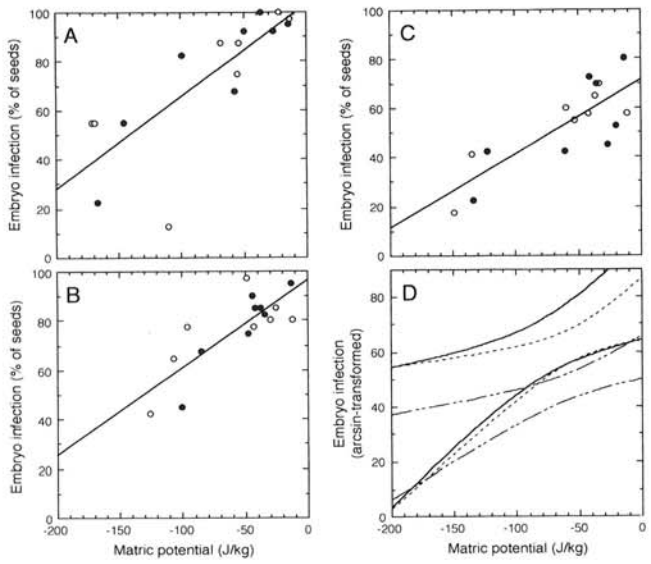

Fig. 2. Incidence of embryo infection (EI) in soil pasteurized and reinfested with *Pythium ultimum* var. *sporangiiferum* and adjusted to different soil matric potentials. Since the results from two experiments were similar (○ = data for the second experiment), the two sets of data were combined in the figure and analyzed by linear regression. There were positive linear relationships between the values of soil matric potential (x) and the incidence of EI transformed by the arcsine transformation (y): **A,** 150 propagules per gram of soil ($y = 0.27x + 82.62$; $r^2 = 0.65$); **B,** 50 propagules per gram of soil ($y = 0.23x + 75.50$; $r^2 = 0.59$); and **C,** 20 propagules per gram of soil ($y = 0.18x + 57.79$; $r^2 = 0.64$). The regressed lines were superimposed on the figures in nontransformed percentage scale. **D,** Estimated variance (95% confidence intervals) for three regressed lines: 150 (—), 50 (- - -), and 20 (···) propagules per gram of soil.

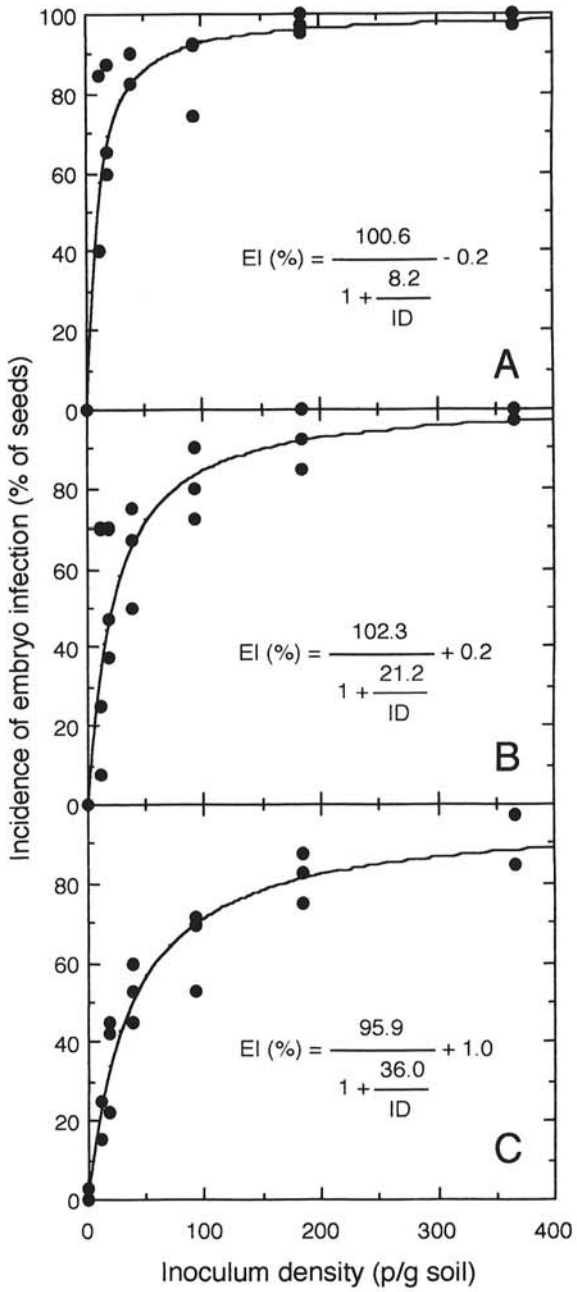

Fig. 3. Inoculum density (ID) and embryo infection (EI) relationships fit to the modified Michaelis-Menten equation in Larkin silt loam pasteurized and reinfested with *Pythium ultimum* var. *sporangiiferum* at three soil matric potentials: **A,** −11 ± 1 J/kg; **B,** −45 ± 5 J/kg; and **C,** −88 ± 22 J/kg.

Influence of air drying of soil on the ψ_m-EI relationship. Different ψ_m-EI relationships were obtained in the same natural Larkin silt loam when the soil was stored moist until use and when the soil was air dried and then rewetted before use. The ID of *Pythium* spp. was estimated to be 700 propagules per gram in the soil stored moist and only 217 propagules per gram in the same soil once air dried. At −10 J/kg, the incidence of EI was approximately 85% whether or not the soil had been air dried before adjustment of ψ_m. However, at lower ψ_m, the incidence of EI was less in the soil stored moist (despite a higher ID) than in the same soil air dried and then rewetted (Fig. 4A and B). In other words, there was a greater incremental drop in the incidence of EI in response to decreases in ψ_m in the soil stored moist than in the same soil air dried and then rewetted. Once soil was air dried, ψ_m at approximately −120 J/kg was required to limit EI to half of EI_{max}, whereas in the soil kept moist, adjustment of ψ_m to only approximately −60 J/kg had the same effect. The incidence of EI was negligible in soils drier than −200 J/kg, regardless of whether the soil was air dried or stored moist.

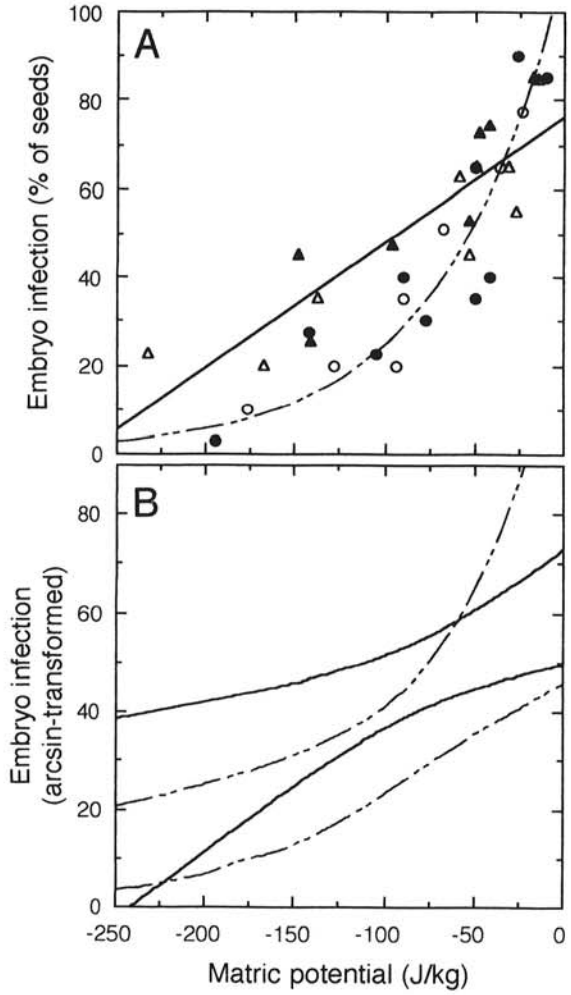

Fig. 4. A, Incidence of embryo infection (EI) in Larkin silt loam collected near Fairfield, Washington, and adjusted to different soil matric potentials. The soil had been either kept moist (●) or air dried and rewetted (▲) before adjusting matric potential. Since the results from two experiments were similar (○ and △ = data for the second experiment), the two sets of data were combined in the figure and analyzed by linear regression. There were positive exponential and positive linear relationships between the values of soil matric potential (x) and the incidence of EI transformed by the arcsine transformation (y), respectively, in soil kept moist (log $y = 0.0038x + 1.87$; $r^2 = 0.86$) and in soil air dried and rewetted ($y = 0.17x + 61.31$; $r^2 = 0.75$). The regressed lines were superimposed on the figure in nontransformed percentage scale. **B,** Estimated variance (95% confidence intervals) for two regressed lines: soil kept moist (—) and soil air dried and rewetted (···).

Relationship of EI to soil properties. No significant relationship was found between the incidence of EI and any of the chemical or physical properties determined for the 12 soils, yet the incidence of EI was 16.3–91.7% in the 12 soils held at −10 J/kg. However, when the soils collected from near Prosser and Kamiak Butte, Washington, were omitted from the analysis (these two soils had *Pythium* populations of less than 100 propagules per gram), a significant negative linear correlation was found between the incidence of EI and soil pH (Table 1).

Effect of soil pH on EI. In the acid soil (Larkin silt loam) amended with $Ca(OH)_2$, the incidence of EI was significantly less when soil pH was adjusted to 7.6 than at other acidities. Conversely, in the alkaline soil (Pedigo silt loam) treated with H_2SO_4, the incidence of EI was significantly greater when the soil was acidified to pH 5.1–6.7 than at other acidities (Fig. 5). Further acidification of this soil to pH 4.3 resulted in the lowest EI. After equilibrium for adjustment of soil pH, IDs of *Pythium* spp. were 178, 189, 267, 189, 278, and 178 propagules per gram in Larkin silt loam at pH 4.8, 5.3, 5.8, 6.4, 6.9, and 7.6, respectively,

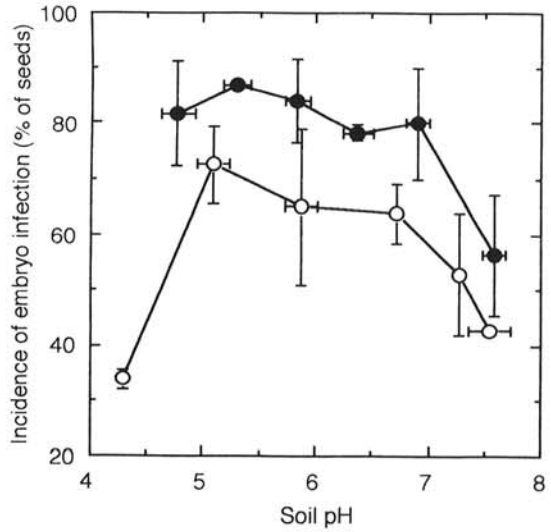

Fig. 5. Incidence of embryo infection (EI) in Larkin silt loam (●) and Pedigo silt loam (○) adjusted to different pH values. The incidence of EI was determined with three replicates over time in each soil maintained at −10 J/kg. Soil pH of Larkin silt loam (original pH = 4.6) was raised with calcium hydroxide, and the Pedigo silt loam (original pH = 7.6) was acidified with 0.18 N sulfuric acid. Vertical and horizontal bars represent standard deviations for the incidence of EI and soil pH, respectively.

TABLE 2. Influence of soil pH and soil chemical treatments on embryo infection by *Pythium* spp.

Soil treatment[x]	Incidence of embryo infection[y,z] (% of seeds) pH 5.3	pH 7.4
Untreated	69.4 abc	55.6 b
Chl	75.0 a	78.6 a
Ben	67.5 abc	58.1 b
Ipr	60.0 abc	54.4 b
Chl + Ben	63.1 abc	75.5 a
Ben + Ipr	55.0 c	54.5 b
Ipr + Chl	71.9 ab	78.8 a
Chl + Ben + Ipr	58.8 bc	76.9 a

[x]The final concentration of each chemical compound in soil was 7.2, 5.0, and 5.0 μg a.i./g of soil for chloramphenicol (Chl), benomyl (Ben), and iprodione (Ipr), respectively.
[y]Seeds were planted in Larkin silt loam held at −10 J/kg, and the incidence of embryo infection by *Pythium* spp. was determined with four replicates.
[z]Incidence data transformed by the arcsine transformation were analyzed by the analysis of variance followed by the Fisher's LSD test. Values in each column followed by the same letter are not significantly different at $P = 0.05$.

and 151, 228, 206, 493, 398, and 310 propagules per gram in Pedigo silt loam adjusted to pH 4.3, 5.1, 5.9, 6.7, 7.3, and 7.6, respectively. The addition of $CaSO_4$ to this soil to provide the same relative amounts of sulfate ions as was supplied as H_2SO_4 changed neither soil pH (constant at 7.4) nor the incidence of EI (32.5–43.0%). While the ID of *Pythium* spp. fluctuated to some extent in both soils during soil pH equilibration, all treated soils contained more than 150 propagules per gram, and the higher IDs were not always related to a higher incidence of EI.

Effect of soil chemical treatments on EI. In Larkin silt loam at pH 5.3 (near optimal for EI), chloramphenicol applied in combination with other fungicides did not always produce a higher incidence of EI. However, all chloramphenicol treatments, including all chloramphenicol-fungicide combinations (but not the fungicides alone), resulted in higher incidence of EI in the same soil adjusted to pH 7.4 with $Ca(OH)_2$ (Table 2).

Effect of seed age on EI. Average incidence of EI was approximately 13% greater (significant at $P = 0.05$) with aged than with new seeds (Table 3); there were no significant interactions within any of the seed age-cultivar-soil combinations by the three-way ANOVA. The incidence of EI was consistently and significantly lower with presoaked than with unsoaked seeds, regardless of cultivar and age of seed (Table 4).

DISCUSSION

This study demonstrates that the incidence of EI by *Pythium* spp. during wheat seed germination in some common silt loams of eastern Washington and adjacent northern Idaho soils is a function of the pathogen population at lower IDs but approaches EI_{max} at higher IDs because of soil properties. Most soils in this area contain *Pythium* spp. at levels greater than 200 propagules per gram (11). Among the 12 soils studied, nine contained more than 200 propagules per gram, yet EI_{max} values in these soils were 26.3–85.0% and were unrelated to ID. The highest percentage of EI was observed in a soil collected near Potlatch, Idaho, with only 163 *Pythium* propagules per gram. These observations suggest that ID is not the limiting factor for EI of germinating wheat seeds in most soils of this region.

In our tests for ID-EI relationships, we used natural soils each diluted with the same respective soils treated by pasteurization to eliminate inoculum of *Pythium* spp. At lower IDs (lower proportion of natural to pasteurized soil), the sample composed of mostly pasteurized soil may have contained fewer antagonists, and hence the incidence of EI was proportional to ID. At higher IDs, however, the samples composed of mostly natural soil may have limited the incidence of EI because of higher populations of antagonists. We used the modified Michaelis-Menten equation to describe the ID-EI relationship. The same mathematical principle (the Lineweaver-Burk double reciprocal plot technique) also has been used to analyze the ID-disease relationship in the interaction between pathogenic and nonpathogenic isolates of *Fusarium oxysporum* (34). The Michaelis-Menten equation describes enzyme kinetics and is based on a biochemical equilibrium reaction, whereas there is no equivalent equilibrium interaction between *Pythium* spp. and infection sites or *Pythium* spp.-infection site complexes during infection of wheat embryos. Thus, the slope values (ID_{50}) may have no particular epidemiological significance. Nevertheless, the modified Michaelis-Menten equation is useful for estimating EI_{max}, especially in the blends dominated by natural soil. In addition, EI_{max} cannot be obtained easily by other linearization approaches in which the regressed lines rise endlessly as ID becomes greater. An EI_{max} indicates a "*Pythium* carrying capacity" for each wheat seed-soil-pathogen system to support infection of wheat embryos. Soil with a high *Pythium* carrying capacity (high EI_{max}) can be considered to be conducive to EI, whereas soil with a low *Pythium* carrying capacity (low EI_{max}) can be considered suppressive.

Of the many chemical and physical properties of soil measured, only soil pH was negatively related to EI_{max}. This relationship was verified experimentally. Field soils in eastern Washington and northern Idaho have become increasingly acidic because of the long-term use of ammonium-based nitrogen fertilizers. In addition, the 45–55 cm of annual precipitation in the easternmost region of Washington and adjacent northern Idaho accelerates acidification of soils. Coincidentally, the soils in this area have the highest clay contents for the region. The relatively recent (within the past 20–30 yr) increase in acidification of the soils may account for the *Pythium* damage to wheat that has become so important in this region (11,12). Soil pH could influence the *Pythium* carrying capacity through a direct effect on activity of *Pythium* spp. (15,24) or indirectly through increasing soil suppressiveness to *Pythium* spp. (23,31). The use of calcium to raise soil pH also may have an indirect effect on EI by affecting resistance of the host (4,20,21).

P. u. sporangiiferum was a very efficient colonist of wheat embryos in the pasteurized-reinfested soil compared with the mixture of *Pythium* spp. in natural soils. This is probably because few antagonists were present in the pasteurized soil, including weakly virulent *Pythium* spp., that could compete with effective

TABLE 3. Influence of seed age on the incidence of embryo infection (EI) by *Pythium* spp.

Wheat seeds		Incidence of EI in three silt loams[x] (% of seeds)			Average across three soils[y] (%)
Cultivar	Age[w]	Larkin	Thatura	Pedigo	
Daws	New	80.8	81.7	64.2	75.6 bc
	Old	95.0*[z]	94.2	58.3	82.5 c
Hill 81	New	84.2	85.8	39.2	69.7 b
	Old	96.7*	90.0	54.7	80.5 c
Nugaines	New	69.2	68.3	29.1	55.5 a
	Old	89.2*	86.7*	55.0*	77.0 bc
Average across three cultivars[y]	New	78.1 y	78.6 y	44.2 w	66.9
	Old	93.6 z	90.3 z	56.0 x	80.0

[w] New = harvested in the current year, and old = harvested 4 yr earlier.
[x] Seeds were planted in each soil held at −10 J/kg, and the incidence of EI was determined with three replicates for each seed age-cultivar-soil combination. Incidence data transformed by the arcsine transformation were analyzed by the three-way (age of seed, cultivar, and soil) analysis of variance followed by the Fisher's LSD test.
[y] Values followed by the same letter are not significantly different at $P = 0.05$. Overall average incidence was significantly different ($P = 0.05$) between new and aged seeds with no interaction for any combinations.
[z] * = Significantly different ($P = 0.05$) from value for new seeds of the same cultivar and in the same soil.

TABLE 4. Influence of soaking seeds on embryo infection (EI) by *Pythium* spp.

Wheat seeds		Incidence of EI[v] (% of seeds)		
Cultivar	Age[u]	Not soaked	Soaked[w]	Average
Daws	New	96.3 cd[x]	87.5 abc	91.9 xy[y]
	Old	100.0 d	96.3 cd	98.2 y
Hill 81	New	95.0 bcd	75.0 a	85.0 x
	Old	96.3 cd	86.3 abc	91.3 x
Nugaines	New	88.8 abc	82.3 ab	85.6 x
	Old	96.3 cd	90.0 abc	93.2 xy
Overall average[z]		95.5	86.3	

[u] New = harvested in the current year, and old = harvested 4 yr earlier.
[v] Seeds were planted in Larkin silt loam held at −10 J/kg, and the incidence of EI was determined with two replicates for each cultivar-seed age-soaking treatment combination. Incidence data transformed by the arcsine transformation were analyzed by the three-way (cultivar, age of seed, and soaking) analysis of variance followed by the Fisher's LSD test.
[w] Seeds were soaked in sterilized distilled water at 5 C for 6 h and air dried overnight in a vented hood before planting.
[x] Individual values followed by the same letter (a, b, c) are not significantly different at $P = 0.05$.
[y] Average values followed by the same letter (x, y) are not significantly different at $P = 0.05$.
[z] Overall average incidence was significantly different ($P = 0.05$) between soaked and unsoaked seeds with no interaction for any combinations.

embryo colonists. At least 10 *Pythium* spp. are pathogenic to wheat in the Pacific Northwest (9), and the same EI by *P. irregulare, P. torulosum,* or *P. heterothallicum* required greater ID than that by *P. ultimum* (18). EI_{max} may be directly related to the actual portion of *P. ultimum* in the total *Pythium* population in soil, which includes weakly virulent or nonpathogenic *Pythium* spp.

In the pasteurized-reinfested soil, the ψ_m-EI relationship was similar at 50 and 150 propagules per gram, but the incidence of EI was consistently lower at 20 than at 50 or 150 propagules per gram at approximately −10 to approximately −85 J/kg. Moreover, at −11 J/kg, the incidence of EI approached EI_{max} (100%) only at approximately 50 propagules per gram, whereas at −45 and −88 J/kg, EI_{max} was not affected but the IDs required to achieve 50% EI were progressively higher (21.2 and 36.0 propagules per gram, respectively). Therefore, *Pythium* infection, but not the *Pythium* carrying capacity, may be limited by ψ_m. The incidence of EI in the pasteurized-reinfested soil may have been near the *Pythium* carrying capacity at ≥50 propagules per gram, and the sensitivity of EI to ψ_m may be best demonstrated at IDs below the *Pythium* carrying capacity (<50 propagules per gram). In fact, ID and ψ_m may affect the incidence of EI in a similar way. Nutrient diffusivity in soil is directly related to the cube of soil water content (29); hence, nutrients diffuse markedly faster and farther in wet soil than in dry soil. Thus, the chance for a *Pythium* propagule to infect an embryo increases with either increasing ID or increasing ψ_m.

The incidence of EI was related exponentially to ψ_m in a natural soil kept moist and linearly to ψ_m in the same soil air dried and then remoistened before use. In other words, there was a greater incremental drop in the incidence of EI in response to a decrease in ψ_m in the soil kept moist than in soil air dried and then remoistened. The incidence of EI in the pasteurized-reinfested soil also was linearly rather than exponentially related to ψ_m. Pasteurization and air drying both may increase nutrient availability for *Pythium* spp. Both processes kill competitors, and the dead microbial biomass could provide an additional nutrient source. Suppressiveness of soils to *Pythium* spp. may be caused by competition from soil microorganisms (5,6), which can be overcome by increasing nutrient availability (1,20,22).

Pythium carrying capacity also can be influenced by the seed quality. Seeds leak solutes during the initial stage of imbibition, since the ruptured cell membranes of dried seed are reassembled more slowly than water is imbibed into the seed (37). Seeds also release volatile substances (ethanol or acetaldehyde) that stimulate germination of sporangia of *P. ultimum* (27,30). In general, aged or damaged seeds release more exudates than new or intact seeds (35,36) and therefore provide more nutrients (or stimulants) for *Pythium* spp. In our study, EI_{max} in one experiment (but not another) was significantly greater with aged seeds than with new seeds, regardless of the cultivar or soil used. In another study (17), percent emergence and height of wheat seedlings were significantly lower with aged seeds than with new seeds in *Pythium*-infested soil but not in pasteurized soil. In our experiments, EI_{max} was significantly lower when seeds were soaked in cold (5 C) water for 6 h before planting than when seeds were not soaked. Considerable amounts of seed exudates can be lost during soaking. Exudation during soaking can also be greater in cold than in warm (25–31 C) water with many kinds of seeds, perhaps because cell membranes are reassembled slowly at low temperatures (37).

EI_{max} or *Pythium* carrying capacity may be determined in the spermosphere of wheat mainly by two parameters: the size of the nutrient supply from the seed (greater in wet than in dry soil, owing to the greater diffusivity, and generally greater with aged than with new seed) and competition from the associated microorganisms (greater in alkaline than in acid soil and in soil kept moist rather than air dried before planting). Although the *Pythium* carrying capacity is a defined property for each wheat seed-soil system, the actual incidence of EI is a fraction of the capacity and can be controlled either by the initial ID of *Pythium* spp. or by nutrient diffusivity. Furthermore, the *Pythium* carrying capacity might be lowered in response to one or more practices or treatments that reduce *Pythium* damage to crops, including use of new high-quality seed, use of osmoprimed seeds to reduce solute leakage upon sowing (7,28), raising soil pH (e.g., liming), preplant irrigation, and addition of an antagonistic microorganism on the seed before planting. When a soil is expected to have a high *Pythium* carrying capacity, seeding when ψ_m is between −50 and −100 J/kg is recommended to reduce the incidence of EI.

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