Identification of Resistance to *Pythium* Seedling Diseases in Alfalfa Using a Culture Plate Method

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ABSTRACT

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Pathogenicity and virulence of three *Pythium* spp. to alfalfa seedlings and resistance of alfalfa germ plasm to *Pythium* seedling diseases were characterized using a culture plate method. Twenty-five seeds were placed on the surface of a 3-day-old colony of *Pythium* growing on water agar and incubated at 12, 18, or 24 C for 5 days. Disease severity was rated using a 5-class scale (1 = healthy seedling, 5 = dead seed). Pathogenic cultures of *Pythium ultimum, P. paroecandrum,* and a *P. sylvaticum*-like isolate, all from alfalfa fields in Minnesota, varied in virulence to the Beltsville International Composite-7 alfalfa population. Moderately virulent isolates induced greater disease at 12 and 18 C than at 24 C, but highly virulent isolates were uniformly virulent over the three temperatures. Twelve alfalfa entries representing diverse germ plasm backgrounds varied significantly in resistance to *P. ultimum* and *P. paroecandrum*. Alfalfa seed weight, seedling growth rate, rate of germination, and fall dormancy were not correlated with this resistance. The culture plate method is useful for evaluating alfalfa germ plasm for resistance to *Pythium* seedling diseases.

Additional keywords: damping-off, seed rot

Members of the genus *Pythium* cause seed rot and pre- and postemergence damping-off of alfalfa (*Medicago sativa* L.). Several *Pythium* spp. can infect the embryo, primary and secondary roots and hypocotyls of seedlings, and secondary roots of older plants (8). Most alfalfa seedlings become resistant to damping-off by about 5 days after seeding (9,11), but infection of feeder roots can occur at any stage of plant development (14). Species pathogenic to alfalfa seedlings include: *P. acanthicum* (17), *P. debaryanum* (8,9,11,12), *P. irregulare* (11,12,15, 17,26), *P. paroecandrum* Drechs. (11,12, 15,26,28), *P. splendens* (11,12), *P. sylvaticum* W.A. Campbell & J.W. Hendrix (28), and *P. ultimum* Trow (11–13, 15,17,26).

Soil temperatures affect development of pre- and postemergence damping-off caused by *Pythium* spp. (18,27). Each *Pythium* species has an optimum soil temperature for maximum activity. Preemergence damping-off caused by *P. ultimum* occurs over a wide range of temperatures (16–31 C), whereas postemergence damping-off caused by *P. irregulare* and *P. paroecandrum* is most severe at low temperatures (16 and 21 C, respectively) (14). *P. ultimum* and *P. debaryanum* uniformly cause seed rot and kill all alfalfa seedlings before emergence at 16–28 C (12). *P. paroecandrum* is less virulent than *P. ultimum* and *P. debaryanum* and causes less reduction in alfalfa stand at 20–24 C than at 16 C (12). *P. debaryanum* causes pre- and postemergence damping-off of alfalfa seedlings at 16 and 24–28 C, respectively (9).

Fungicide seed treatments, cultural practices, and biocontrol agents may provide some reduction of Pythium seed rot and damping-off. There is evidence for cultivar differences in resistance to *Pythium*-incited diseases in alfalfa, annual *Medicago* spp., and subterranean clover. Alfalfa cultivars differed in shoot growth when their rootlets were infected by *P. irregulare* (15). Annual *Medicago* spp. varied in resistance to root rot caused by *Pythium* spp. (7). Subterranean clover cultivars differed in resistance to seedling damping-off and root rot caused by *P. irregulare* (3). Plant resistance, therefore, is a potential control for medic root diseases.

There is evidence that susceptibility of alfalfa seedlings to damping-off may be related to host characteristics such as seed weight, rate of germination, and seedling vigor. Hawthorne (17) demonstrated a positive relationship between alfalfa seed weight and resistance of alfalfa seedlings to damping-off caused by *Pythium* spp. Hancock (13) suggested that vulnerability of alfalfa seedlings to damping-off may be related to seed germination rate.

Our objectives were to: 1) develop a laboratory method to determine pathogenicity and virulence of selected *Pythium* species on alfalfa seedlings; 2) determine if *Pythium* spp. interact with temperature in disease development; 3) evaluate alfalfa cultivars for resistance to Pythium seed rot and damping-off; and 4) determine if host characteristics associated with seed vigor affect disease.

MATERIALS AND METHODS

***Pythium* isolation.** Soil samples were collected in alfalfa fields at: the Southern Experiment Station, Waseca, MN, on 25 June 1991; the Southwest Experiment Station, Lamberton, MN, on 26 June 1991; the North Central Experiment Station, Grand Rapids, MN, on 25 July 1991; the USDA/ARS and University of Minnesota Phytophthora root rot evaluation nursery at St. Paul, MN, on 8 August 1991; and the University of Wisconsin Aphanomyces root rot evaluation nursery at Marshfield, WI, during July 1991. Ten soil cores were taken to a depth of 10 cm with a 2.5-cm-diameter soil tube and pooled to form a composite sample. One to four composite soil samples were collected at each location.

Pythium spp. were isolated from these soils by baiting with alfalfa seedlings, using a modification of the rolled towel technique (23). Alfalfa entries used for baiting included the cultivar Baker (20), the BIC-7 (Beltsville International Composite-7) population (6), and the experimental population MNGRN-14 (30). The BIC-7 population is a broad-based germ plasm pool and a source of resistance to many diseases and insect and nematode pests of alfalfa. MNGRN-14 is an experimental population selected in Grand Rapids for resistance to the root-lesion nematode, *Pratylenchus penetrans*.

Seeds of each alfalfa entry were surface-treated by soaking in 95% ethanol for 3 min, transferred to 10% NaOCl (sodium hypochlorite) for 10 min, and rinsed with sterile distilled water. Approximately 20 seeds were placed between moist, folded germination papers inside a plastic pouch and incubated for 2 days at 24 C. A 2- to 3-mm layer of soil was placed adjacent

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to the hypocotyl and root-hair zone of 2-day-old seedlings. Germination papers containing the seedlings and soil were rolled, placed inside plastic pouches, and incubated for 3 days in growth chambers set at 12, 18, and 24 C with a 14 h day (light intensity approximately 95 μmol m^{-2} s). Seedlings were washed under flowing tap water for 2–3 h, blotted dry in sterile paper towels, placed on 1.5% water agar (WA) in petri dishes, and incubated for 2–4 days at the temperature used for baiting (Table 1). Fungi that grew from alfalfa seedling tissue were hyphal-tipped to cornmeal agar (CMA) and incubated at the corresponding baiting temperature.

Two additional *Pythium* isolates were included in the pathogenicity tests for comparison to the alfalfa isolates. One *Pythium* isolate (90-6-7) had been recovered at approximately 22–24 C from a diseased sugar beet plant (C. E. Windels, University of Minnesota, Northwest Experiment Station, Crookston, MN). A second isolate had been recovered at approximately 22–24 C from an alfalfa plant with severe tap root rot in a 2-yr-old field in Marshfield, WI (S. L. Nygaard, W-L Research, Inc., Evansville, WI). All of the *Pythium* isolates were maintained on CMA at 4 C until pathogenicity tests were made and, for long-term storage, were maintained on CMA blocks in sterile water at room temperature (22).

Four selected isolates (W3, L3, L4, and GR1) that differed in morphological characteristics and virulence to alfalfa seedlings were identified using the keys of Dick (10) and Van der Plaats-Niterink (31). Species identification was confirmed by M. E. Stanghellini, University of Arizona, Tucson, and by C. E. Windels.

Culture plate method for seedling disease evaluation. We modified a method similar to one used to select alfalfa germ plasm for resistance to seedling damping-off caused by *Rhizoctonia solani* (4,24). A 3-mm-diameter disk of *Pythium* inoculum (mycelia and sporangia) was removed from the edge of a 2- to 3-day-old colony growing on CMA, placed in the center of a 9-cm-diameter petri plate containing 1.5% WA, and incubated at 24 C for 3 days. Twenty-five surface-treated alfalfa seeds were spaced equidistantly in a radiate pattern on the inoculated agar surface with a vacuum template. Plates were incubated in growth chambers at 12, 18, and 24 C for 5 days with a 14 h day (light intensity approximately 95 μmol m^{-2} s). Uninoculated plates of WA containing 25 surface-treated seeds were checks for determining expected percentage of seed germination, hard seed, and dead seed at each temperature.

Disease severity was rated, using a 5-class scale: 1 = healthy seedling, primary root free of necrosis or only slight discoloration; 2 = infected seedling, primary root tip necrotic but firm; 3 = infected seedling, primary root tip soft and rotted; 4 = dead seedling, germinated seed with rotted radicle; and 5 = dead seed, nongerminated rotted seed (Fig. 1). Disease ratings were expressed as an average severity index (ASI) = (numerical value of class × the number of individuals in a class)/the expected number germinated seeds. the percentage of resistant plants was calculated as: (the total of classes 1 and 2 divided by the expected number of germinated seeds) × 100.

Pathogenicity tests. Twelve isolates of *Pythium* (including W3, L3, L4, and GR1) obtained from Minnesota and Wisconsin alfalfa field soils plus the isolates provided by Windels and Nygaard (Table 1) were evaluated for pathogenicity to the BIC-7 population at 12, 18, and 24 C using the culture plate method. The experimental design was a split-plot with temperature as the whole plot factor, time as the whole plot block, and isolates as the subplot factor. There were three replications per treatment, and the experiment was repeated once. The experiment was treated as a fixed-variable model. An uninoculated plate of WA containing 25 surface-treated seeds of the BIC-7 population was used as a check within each replication. There was no heterogeneity of variance between the two experiments; therefore, both experiments were analyzed as one with six replications.

Germ plasm × isolate × temperature experiment. Resistance of 12 alfalfa entries to each of three *Pythium* isolates was evaluated at 12, 18, and 24 C using the culture plate method. The three *Pythium* isolates included: W3, a sporangial isolate of *P. ultimum* (Waseca, MN); L3, an isolate of *P. paroecandrum* (Lamberton, MN); and GR1, an isolate of *P. ultimum*, (Grand Rapids, MN). Eleven alfalfa cultivars (Alouette, Apollo Supreme, Belmont, Centurion, Cimarron VR, Florida 77, Indian, Legend, Pierce, RamRod, and WL-322-HQ) and one experimental population (Aph-12G) were chosen to represent the range of resistance to *Pythium*-incited diseases observed by Altier (1) when she evaluated 253 North American alfalfa cultivars. The experimental design was a split-plot with temperature as the whole plot factor; subplots were *Pythium* isolate-alfalfa entry treatment combinations with three replications. An uninoculated

Table 1. Description of *Pythium* isolates recovered from alfalfa field soils baited in Minnesota and Wisconsin

Pythium isolate[a]	Alfalfa entry baited	Location	Isolation temperature (C)
BIC-L3-24	BIC-7	Lamberton, MN	24
GR1	BIC-7	Grand Rapids, MN	24
BIC-SP1-24	BIC-7	St. Paul, MN	24
BIC-M-24	BIC-7	Marshfield, WI	24
W3	BIC-7	Waseca, MN	18
BIC-L4-18	BIC-7	Lamberton, MN	18
MN-W1-12	MNGRN-14	Waseca, MN	12
MN-W2-12	MNGRN-14	Waseca, MN	12
BIC-W2-12	BIC-7	Waseca, MN	12
L3	MNGRN-14	Lamberton, MN	12
B-L4-12	Baker	Lamberton, MN	12
L4	BIC-7	Lamberton, MN	12

[a]GR1 and W3 = *P. ultimum*, L3 = *P. paroecandrum*, L4 = *P. sylvaticum*-like. BIC-7 = Beltsville International Composite-7 population.

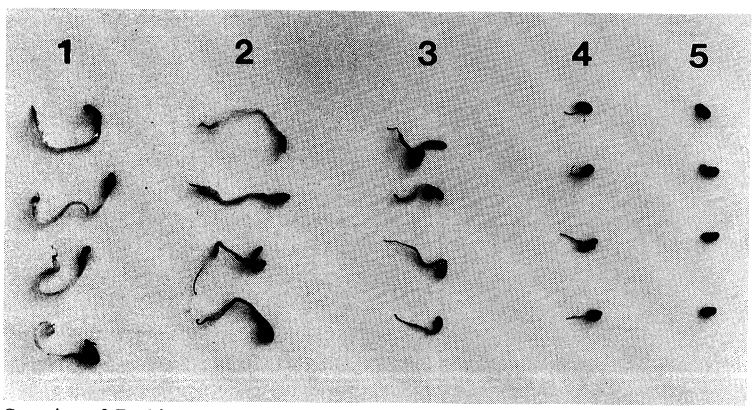

Fig. 1. Severity of Pythium seed rot and damping-off on alfalfa, using a 5-class rating scale of individual seedlings: 1 = healthy; 2 = primary root tip necrotic but firm; 3 = primary root tip rotted and soft; 4 = dead seedling; and 5 = dead seed.

plate of WA containing 25 surface-treated seeds of each entry was used as a check within each replication.

Host characteristics associated with seed vigor. Seed weight, rate of germination, and seedling growth were determined for each of the 12 alfalfa entries evaluated in the cultivar × isolate × temperature experiment. Seed weight was expressed as weight (grams) per 100 seeds. The alfalfa entries were grown in 1.5% WA plates (25 seeds per plate) for 7 days at 18 C with a 14 h day (light intensity approximately 95 μmol m^{-2} s). Rate of germination was determined by daily counting as described by Maguire (21). On each of the first 5 days, the number of seeds that germinated normally was recorded and divided by the number of days seeds had been incubated. The values obtained at each count were summed, and the speed of germination was expressed as germination rate. Seedling growth rate was determined after 7 days, as previously described (2). Abnormal seedlings and dead seeds were discarded, and normal seedlings were severed from cotyledons and dried at 80 C for 24 h. Seedling growth rate was expressed as total dry weight per entry divided by the number of normal seedlings (expressed as dry weight [grams] per 1,000 seedlings). The experimental design was a randomized complete block with three replications.

Fall dormancy, measured by fall plant height, was used as another estimate of growth rate (5). Generally, nondormant entries have faster growth rates than dormant entries. Fall dormancy values for 10 of the alfalfa entries were provided by D. K. Barnes (USDA/ARS, St. Paul, MN, *unpublished data*).

Statistical analyses. Data for each experiment were subjected to analysis of variance and treatment means were separated with Fischer's protected LSD ($P < 0.05$) by SAS (25). Coefficients of linear correlation (r) were calculated between fall dormancy, seed weight, rate of germination, seedling growth rate, and ASI for 12 alfalfa entries.

RESULTS AND DISCUSSION

***Pythium* isolation.** The alfalfa entries used as bait, isolation temperatures, and geographic origins of 12 isolates of *Pythium* spp. are described in Table 1. Soil samples were collected about 1 mo earlier at the southern locations (Waseca and Lamberton, MN) than at Grand Rapids and St. Paul, MN, and Marshfield, WI. Recovery of *Pythium* isolates at 12 and 18 C were predominantly from soil samples collected during late June at the southern locations. *Pythium* isolates were recovered at 24 C, but not at lower temperatures, from soil samples collected during late July and August at Grand Rapids, St. Paul, and Marshfield. *Pythium* isolates were recovered from Lamberton soils at all three temperatures.

Isolates GR1, W3, L3, and L4 were identified to species because they differed in morphological characteristics and in virulence to alfalfa seedlings. Isolates GR1 and L3 were identified by the first author as *P. ultimum* and *P. paroecandrum*, respectively. Isolates W3 and L4 were identified as a sporangial isolate of *P. ultimum* and as a *P. sylvaticum*-like isolate, respectively, by M. E. Stanghellini. The three identified species have been previously reported as highly pathogenic to alfalfa seedlings (11–13, 15,17,19,26,28).

Differences in soil type and soil temperature at the five locations may account for differential prevalence of *Pythium* species, because different species have optimum soil-temperature ranges for maximum activity (18). Seasonal differences in soil temperatures account for cyclical patterns of root colonization of alfalfa by different species of *Pythium* (14,16), with *P. irregulare* and *P. violae* predominantly isolated during winter and early spring and *P. ultimum* during the summer months.

Pathogenicity tests. All *Pythium* isolates were pathogenic to alfalfa seedlings (Table 2). There was a wide range in virulence among isolates of *Pythium* spp., as determined by disease severity (ASI) and percentage of resistant plants in the BIC-7 alfalfa population (Table 2). Five isolates (L4, BIC-L4-18, B-L4-12, BIC-W2-12, and MN-W2-12) were of low virulence and primarily caused postemergence damping-off. *P. ultimum* (isolate W3) was of intermediate virulence and caused seed rot and pre- and postemergence damping-off. *P. paroecandrum* (isolate L3) was highly virulent and induced seed rot and pre- and postemergence damping-off symptoms. The remaining seven isolates (including *P. ultimum* [GR1]) were highly virulent and induced primarily seed rot and preemergence damping-off symptoms.

These results do not support previous reports (12,15,26) that *P. paroecandrum* is less virulent than *P. ultimum*, possibly due to differences in isolates. The *P. sylvaticum*-like isolate (L4) was less virulent than *P. paroecandrum* (L3), which agrees with a previous report by Hwang (19). The ranking of isolates for virulence by percent resistant plants was similar to ranking by ASI (Table 2).

Disease severity increased and percent resistant plants decreased with decreasing temperatures (Table 2). These results parallel other findings (9,15,29) that decreasing temperatures increased severity of *Pythium*-incited seedling diseases.

There was a significant interaction between isolates and temperature ($P < 0.01$). Isolates with low or intermediate virulence induced greater disease and greater reductions in percentage of resistant plants at 12 and 18 C than at 24 C. These results parallel those of Hancock (15) who also observed greater disease severity at low temperatures. In contrast, highly virulent isolates were more uniformly virulent over the range of temperatures compared to the less virulent isolates.

Temperature did not affect virulence of *P. ultimum* isolate GR1, which agrees with previous reports that *P. ultimum* is uniformly virulent over a range of temperatures (13,26). In contrast, *P. ultimum* isolate W3 was more virulent at 12 C than at 24 C ($P < 0.05$). Virulence of the *P. sylvaticum*-like isolate L4 also

Table 2. Pathogenicity of 14 *Pythium* isolates to Beltsville International Composite-7 (BIC-7) alfalfa population at three temperatures, as determined by disease severity and percent resistant plants

	Disease severity[a]			Percent resistant plants[b]		
***Pythium* isolate**	**12 C**	**18 C**	**24 C**	**12 C**	**18 C**	**24 C**
P. sylvaticum-like (L4)	3.8	3.2	2.8	1.5	13.7	34.7
BIC-L4-18	3.4	3.3	2.8	3.6	13.0	32.4
B-L4-12	3.7	3.2	2.7	2.2	4.9	38.7
BIC-W2-12	3.6	3.0	2.8	2.3	12.4	30.5
MN-W2-12	3.7	3.2	2.9	2.2	13.2	30.5
P. ultimum (W3)	4.1	3.9	3.2	0.0	2.3	7.9
P. paroecandrum (L3)	4.1	4.1	3.9	0.0	0.0	0.0
Nygaard	4.1	4.1	4.0	0.0	0.0	1.6
Windels	4.1	4.1	4.1	0.0	0.0	0.0
BIC-M-24	4.1	4.1	4.1	0.0	0.0	0.0
P. ultimum (GR1)	4.1	4.1	4.1	0.0	0.0	0.0
MN-W1-12	4.2	4.1	4.1	0.0	0.0	0.0
BIC-L3-24	4.2	4.1	4.2	0.0	0.0	0.0
BIC-SP1-24	4.2	4.1	4.2	0.0	0.0	0.0
$LSD_{(0.05)}$[c]		0.2			6.0	

[a] Average severity index (ASI) based on a 5-class scoring system of individual seedlings: 1 = healthy; 2 = primary root tip necrotic and firm; 3 = primary root tip rotted and soft; 4 = dead seedling; and 5 = dead seed.

[b] Percent resistant plants = percentage of seedlings in classes 1 and 2.

[c] $LSD_{(0.05)}$ applies among *Pythium* isolates within each temperature (vertically in columns) and among temperatures within each *Pythium* isolate (across rows).

was affected by temperature ($P < 0.05$); preemergence damping-off at 12 C and postemergence damping-off occurred at 18 and 24 C. At 12 and 18 C *P. paroecandrum* isolate L3 caused severe seed rot and preemergence damping-off (classes 5 and 4 in the disease rating scale, respectively), whereas at 24 C some seedlings developed postemergence damping-off (class 3). These results agree with previous reports that virulence of *P. paroecandrum* is temperature-dependent (12,15).

Germ plasm × isolate × temperature experiment. The effects of *Pythium* isolate and temperature on disease development in alfalfa germ plasm to *Pythium* spp. (1) are presented in Table 3. Isolates varied significantly in virulence. *P. ultimum* isolate W3 was least virulent, whereas *P. ultimum* isolate GR1 was moderately highly virulent. *P. paroecandrum* isolate L3 was the most virulent. In this experiment, levels of virulence expressed by *P. ultimum* isolate W3 and *P. paroecandrum* isolate L3 were decreased and increased, respectively, compared to the previous pathogenicity test. Differences in virulence of isolates for the two tests may have occurred because different alfalfa populations were evaluated in these tests.

Temperature influenced the expression of disease severity and the percentage of resistant plants. Disease severity increased and percentage of resistant plants decreased with decreasing temperatures (Table 3). These results strongly agree with results of effects of temperature on virulence of 14 *Pythium* isolates to the BIC-7 population.

There was a significant interaction ($P < 0.01$) between *Pythium* isolates and temperature for disease severity (Table 3). The effect of temperature on disease development was isolate-dependent. At lower temperatures, the isolate of *P. ultimum* (W3) with low virulence induced greater disease, and lower percentages of resistant plants resulted. *P. ultimum* isolate GR1 was highly virulent over the range of temperatures. *P. paroecandrum* isolate L3, was the most virulent and caused severe seed rot at 12 C; however, at 24 C, radicles of some alfalfa seedlings emerged before rotting occurred. These results suggest that the differential expression of virulence in the species *P. paroecandrum* may depend on temperature, as previously reported (12, 15,26).

Our experiments have shown that temperature can affect development of seed rot and damping-off caused by different isolates of *Pythium* spp. When disease development was influenced by a temperature × *Pythium* isolate interaction, disease severity was intermediate at 18 C, compared to 12 and 24 C. A temperature of 18 C is also the average soil temperature during the spring planting period for alfalfa in Minnesota. Therefore, we recommend 18 C as the standard temperature for evaluating alfalfa germ plasm for resistance to *P. ultimum* and *P. paroecandrum*.

There was a significant interaction ($P < 0.01$) between *Pythium* isolates and alfalfa entries for disease severity (Table 4). The 12 alfalfa germ plasms varied greatly in levels of resistance to seedling diseases caused by *Pythium* spp. The least virulent isolate, *P. ultimum* W3, was most useful for characterizing host-plant resistance, because the ASI range was much greater than for *P. ultimum* GR1 or *P. paroecandrum* L3. A greater ASI range allows for better separation of the host-resistance response among alfalfa germ plasms. Five entries (Florida 77, Indian, RamRod, Cimarron VR, and Apollo Supreme) were moderately resistant, and one entry (Belmont) was highly susceptible. The six remaining entries (WL-322-HQ, Alouette, Aph-12G, Centurion, Legend, and Pierce) had intermediate disease severity reactions.

Altier (1) evaluated 253 North American alfalfa cultivars for resistance to *Pythium* spp. and observed a wide range of reactions among cultivars. Other workers (3,7,15) also have reported variability in resistance to *Pythium* spp. among different cultivars of subterranean clover, annual medics, and alfalfa. These findings suggest that plant resistance is a potential means of managing alfalfa seedling diseases caused by *Pythium* spp.

Host characteristics associated with seed vigor. Seed weight, seedling growth rate, rate of germination, and fall dormancy varied for the 12 alfalfa germ plasms evaluated in the germ plasm × *Pythium* isolate × temperature experiment ($P < 0.05$) (Table 5). Coefficients of linear correlation (r) were used to study the relationship between resistance to *Pythium* seedling diseases and host characteristics associated with seed vigor.

Within the range represented by the 12 alfalfa entries, the four seed vigor characteristics measured were not cor-

Table 3. Effect of temperature on resistance of alfalfa to seedling diseases caused by three *Pythium* isolates, as determined by disease severity and percent resistant plants[a]

	Disease severity[b]			Percent resistant plants[c]		
***Pythium* isolate**	**12 C**	**18 C**	**24 C**	**12 C**	**18 C**	**24 C**
P. ultimum (W3)	3.7	2.9	2.0	17.6	45.2	75.2
P. ultimum (GR1)	4.3	4.3	4.3	0.0	0.0	0.7
P. paroecandrum (L3)	4.9	4.7	4.6	0.0	0.0	1.1
$LSD_{(0.05)}$[d]		0.1			2.9	
$LSD_{(0.05)}$[e]		0.4			2.9	

[a] Averaged over 12 alfalfa germ plasms.
[b] Average severity index (ASI) based on a 5-class scoring system of individual seedlings: 1 = healthy; 2 = primary root tip necrotic and firm; 3 = primary root tip rotted and soft; 4 = dead seedling; and 5 = dead seed.
[c] Percent resistant plants = percentage of seedlings in classes 1 and 2.
[d] $LSD_{(0.05)}$ applies among *Pythium* isolates within each temperature (vertically in columns).
[e] $LSD_{(0.05)}$ applies among temperatures within each *Pythium* isolate (across rows).

Table 4. Comparison of 12 alfalfa entries for resistance to seedling diseases caused by three *Pythium* isolates (W3, L3, and GR1)[a]

	Disease severity[b]			Percent resistant plants[c]		
Alfalfa entry	**W3**	**GR1**	**L3**	**W3**	**GR1**	**L3**
Florida 77	2.2	4.1	4.6	66.5	0.0	0.9
Indian	2.3	4.0	4.4	61.7	0.9	2.3
RamRod	2.3	4.0	4.4	62.2	1.2	0.6
Cimarron VR	2.3	4.2	4.7	64.2	0.0	0.0
Apollo Supreme	2.3	4.2	4.7	59.3	0.5	0.5
WL-322-HQ	2.7	4.3	4.8	48.0	0.0	0.0
Alouette	2.8	4.2	4.8	46.3	0.0	0.0
Aph-12G	3.2	4.2	4.7	32.3	0.0	0.0
Centurion	3.4	4.4	4.8	32.5	0.0	0.0
Legend	3.3	4.4	4.9	31.8	0.0	0.0
Pierce	3.5	4.5	4.9	25.2	0.0	0.0
Belmont	3.9	5.0	5.0	21.5	0.0	0.0
$LSD_{(0.05)}$[d]		0.2			5.9	

[a] W3 and GR1 = *P. ultimum*; L3 = *P. paroecandrum*; data averaged over three incubation temperatures (12, 18, and 24 C).
[b] Average severity index (ASI) based on a 5-class scoring system of individual seedlings: 1 = healthy; 2 = primary root tip necrotic and firm; 3 = primary root tip rotted and soft; 4 = dead seedling; and 5 = dead seed.
[c] Percent resistant plants = percentage of seedlings in classes 1 and 2.
[d] $LSD_{(0.05)}$ applies among entries within each *Pythium* isolate (vertically in columns) and among *Pythium* isolates within temperatures (across rows).

Table 5. Seed weight, seedling growth rate, rate of germination, and fall dormancy of 12 alfalfa germ plasms

Alfalfa entry[a]	Seed weight[b]	Seedling growth rate[c]	Germination rate[d]	Fall dormancy[e]
Florida 77	0.247[f]	1.108[g]	21.39[g]	6.16
Indian	0.195	0.888	20.47	7.18
RamRod	0.245	1.016	12.08	3.80
Cimarron VR	0.197	0.805	17.70	3.08
Apollo Supreme	0.247	0.884	13.88	3.48
WL-322-HQ	0.249	0.844	15.67	3.26
Alouette	0.208	0.885	13.87	NA[h]
Aph-12G	0.208	0.816	13.51	NA
Centurion	0.210	0.897	15.55	3.79
Legend	0.271	0.855	16.69	3.99
Pierce	0.222	0.816	20.38	6.29
Belmont	0.282	0.905	13.52	4.38
$LSD_{(0.05)}$	0.008	0.155	2.87	0.75

[a] Alfalfa entries are ranked from most resistant to *Pythium* spp. (top) to most susceptible (bottom).
[b] Grams per 100 seeds.
[c] Seedling growth rate determined after a 7-day incubation period in water agar plates: Normal seedlings were cut from cotyledons and dried at 80 C for 24 h; total dry weight was corrected by the number of normal seedlings and expressed as grams per 1,000 seedlings.
[d] Germination rate was calculated after a 5-day incubation period in water agar plates: In each one of the 5 days, the number of seeds that germinated normally was recorded and divided by the day number; the values at each counting were summed to obtain the germination rate.
[e] Fall dormancy expressed as plant height (in centimeters) during October, 25–30 days after clipping.
[f] Data is an average of three replications with 100 seeds per replication.
[g] Data is an average of three replications with 25 seedlings per replication.
[h] NA = not available.

related with disease severity (ASI) caused by three *Pythium* isolates (data not shown). Rate of germination was positively correlated with fall dormancy ($r = 0.75$; $P < 0.05$). This was expected because nondormant entries generally have faster growth rates than dormant entries. Cultivars differed for seed weight, seedling growth rate, rate of germination, and fall dormancy characteristics within both the most resistant and most susceptible groups of alfalfa entries (Table 5). For example, the five most resistant cultivars (mean ASI range: 3.6–3.7; mean percent resistant plants: 20.1–22.5%) represented a very wide range of measurements for each of the four host characteristics associated with seed and seedling vigor.

The lack of correlation between seed vigor characteristics of the host and disease severity strongly suggests genetic control of seedling resistance to *Pythium* spp. In contrast, Hancock (13) compared two alfalfa cultivars and suggested that germination rate may affect vulnerability to preemergence damping-off caused by *P. ultimum, Rhizoctonia solani,* and *Fusarium* spp. Hawthorne (17) used different seed lots of only one alfalfa cultivar and demonstrated a positive relationship between alfalfa seed weight and resistance of alfalfa seedlings to damping-off caused by *Pythium* spp. Altier (1) observed that the level of resistance to Pythium seed rot and damping-off was not associated with fall dormancy in an evaluation of 253 North American alfalfa cultivars; the results presented evidence of an independent mechanism controlling host-plant resistance to alfalfa seedling diseases caused by *Pythium* spp.

The results of our studies indicate that the culture plate method is useful for studying pathogenicity and virulence of *Pythium* spp. and for characterizing alfalfa germ plasm for seedling resistance to *Pythium*. The method optimized efficiency because it did not require much time and space, gave reliable infection without escapes, and provided reproducible results between experiments. The existence of genetic variability for resistance to *P. ultimum* and *P. paroecandrum* in the 12 alfalfa germ plasms evaluated suggests that host-plant resistance is a potential means of managing *Pythium* seedling diseases of alfalfa.

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