Phytophthora Root Rot of Alfalfa in Wyoming

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ABSTRACT

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Phytophthora root rot was found for the first time in all major alfalfa-producing areas in Wyoming. *Phytophthora megasperma* f. sp. *medicaginis* was isolated from 3-mo- to 3-yr-old alfalfa plants that exhibited typical symptoms of Phytophthora root rot (PRR). An isolate of *P. megasperma* obtained from alfalfa caused damping-off of alfalfa and sainfoin seedlings and root rot of mature alfalfa plants in greenhouse tests. Alfalfa cultivars with resistance to PRR had better stands, less disease, and higher forage yields than susceptible cultivars when grown in a field naturally infested with *P. megasperma*. A stand reduction of 75% in the PRR-susceptible cultivar Skyline 400 was attributed to the disease during the first year of production.

Phytophthora root rot (PRR) of alfalfa, caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin, causes a rot of the taproots and lateral roots of mature plants (3), as well as seedling blight (9). Widespread distribution of this disease has been reported in several regions in the United States, Canada, and Australia (5); however, reports of this disease in the Rocky Mountain states are lacking.

Although several cultural practices including crop rotation have been suggested to reduce losses from *P. megasperma*, effective control has been obtained primarily through the use of resistant cultivars (5). Although PRR-resistant cultivars are available, certain areas are still predominantly planted with susceptible cultivars.

The alfalfa strain of *P. megasperma* appears to be specific for alfalfa in nature. However, its pathogenicity on other legume crops including sainfoin (*Onobrychis viciifolia* Scop.) has been documented in greenhouse tests (6).

Grower complaints concerning the short life (less than 3 yr) of irrigated alfalfa stands have been common in Wyoming. Much of this loss has been blamed on winterkill or unknown factors. In July 1980, several 2- and 3-yr-old alfalfa fields in the Big Horn River Basin of north central Wyoming were noted to have thin stands. Typical symptoms of PRR were observed on the unhealthy plants.

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The purpose of this paper is to report for the first time the occurrence and distribution of Phytophthora root rot in Wyoming and the reaction of 20 alfalfa cultivars during the first year of production in a field naturally infested with *P. megasperma*.

MATERIALS AND METHODS

Initial isolations. Alfalfa plants with symptoms of PRR were removed from a 3-yr-old stand near Worland, WY, and brought to the laboratory for isolation. Root pieces were removed, disinfested in a 0.05% solution of NaOCl for 3 min, rinsed in sterile distilled water (SDW), blotted on sterile filter paper, and partially submerged in a *Phytophthora*-selective (PS) medium (2). After 4–6 days, colonies with *Phytophthora*-like mycelium were transferred to PS medium and V-8 juice agar medium and observed for fungal growth and fruiting structures. Agar disks (5-mm-diam.) were taken from the suspect colonies and placed in small petri dishes (60 × 15 mm) that were partially filled with SDW and contained five 5-day-old alfalfa seedlings. Seedlings were observed after 5 days for the presence of sporangia and oospores at which time the average dimensions of 25 of each fungal structure were determined. Isolation of *P. megasperma* also was attempted from soil adjacent to roots of diseased plants by using an alfalfa seedling bait-out technique (8).

Pathogenicity studies. Studies were conducted with alfalfa and sainfoin seedlings and with mature plants of alfalfa. Sainfoin was tested because it is closely related to alfalfa and is grown as a forage crop in Wyoming.

The pathogenicity of the *P. megasperma* isolate was determined with seedlings by measuring damping-off incidence. Alfalfa cultivars Agate and Saranac (USDA-recommended PRR-resistant and susceptible checks, respectively) (1) and the sainfoin cultivar Remont were used in the test. Clay pots (15-cm-diam.) were filled with 1:1 (v/v) previously autoclaved sand and Metro Mix 200 (W. R. Grace & Co., Cambridge, MA 02140). Three furrows (10 × 1 cm) were made in the growing medium of each pot. Each furrow received either 10 ml of inoculum (inoculated treatment) or 10 ml of SDW (uninoculated treatment) after which seeds were planted (10 seeds per furrow, 30 seeds per pot) and the furrows closed. Inoculum was prepared as described previously (7). Mycelial mats from four 2-wk-old cultures were washed and macerated in SDW. Cultures were grown in 32-oz medicine bottles containing 160 ml of a liquid V-8 juice medium. Five pots received *P. megasperma* inoculum and five received SDW for each cultivar of each species (30 pots total). Clay saucers were placed under pots to provide high soil moisture. The number of live seedlings and resultant percent kill were determined after 2 wk.

The mature plant study consisted of inoculating healthy 12-mo-old alfalfa plants (Agate and Saranac). Before establishment, seeds were treated with *Rhizobium meliloti*. Plants were grown in 15-cm-diameter clay pots (three plants per pot) containing a pasteurized mixture of soil-sand-peat (2:1:1, v/v/v). Holes 10 cm deep (three per pot) were made in the growing medium and extended downward to the root zone to facilitate movement of the inoculum. Fifty milliliters of inoculum prepared as described in the seedling study were poured on the soil surface of each pot. Holes were pinched shut and pots thoroughly watered. Saucers were placed under each pot. The test consisted of 10 pots of each cultivar, five inoculated and five uninoculated. Tops of plants were clipped off at inoculation and thereafter at 10% bloom. After 3 mo, plants were removed and rated for PRR. Isolations were made from several diseased roots to verify the presence of *P. megasperma*. All tests were conducted in a greenhouse with day/night temperatures maintained at 27 and 13 ± 2 C, respectively. Fluorescent lamps were provided to extend the day length to 12 hr.

Cultivar trial. An alfalfa cultivar yield trial was established on 29 April 1981, near Riverton, WY. The field had been planted in barley for the past 3 yr and was flood-irrigated. The test consisted of 20 cultivars planted at a seedling rate of 11.2 kg pure-live seed per hectare in a randomized complete block design with 4 replicates. Each plot was 0.6 (five rows spaced 12.5 cm apart) × 4.6 m. To evaluate the reaction of cultivars to PRR, visual stand counts, disease ratings, and forage yields were taken. A visual estimate of the percentage of stand remaining was made on 21 July 1981. One week later, plots were rated for disease on a scale of 1–5 (1 = none, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe disease), and included the percent dead or dying plants as well as discoloration and stunting. Plots were harvested on 6 October 1981. A composite soil sample, representative of the test site area, was collected to determine its physical composition.

Phytophthora survey. To determine how extensive PRR was in the state, a survey was made in three major irrigated alfalfa areas during the 1980 and 1981 growing seasons. The three areas surveyed were the Big Horn Basin (Hot Springs, Washakie, and Big Horn counties), Wind River Basin (Fremont County), and Eastern North Platte Valley (Goshen and Platte counties). Plants were removed from fields showing signs of stand depletion. Plants with root rot were taken to the laboratory for isolation as described previously. In addition, alfalfa samples mailed to the University of Wyoming Plant Disease Clinic were considered part of the survey.

RESULTS

Initial isolations. After 4–6 days, mycelium characteristic of *Phytophthora* spp. could be seen growing in the PS medium from several root pieces. Ten-day-old subcultures of suspect colonies contained mycelium and aerially produced sporangia on PS medium and mycelium and oospores on V-8 juice medium, all characteristic of *P. megasperma* (3). Alfalfa seedlings infected with *Phytophthora* spp. that had a water-soaked appearance were placed on glass slides and viewed under a compound microscope. Oospores (mean diam. 26 μm) were produced readily within the hypocotyl and root. Numerous sporangia were observed growing from the surface of seedlings. Sporangia (mean length and width 64 × 39 μm) were usually formed terminally on a simple filament and had distinct papillae. Clusters of intercalary hyphal swellings also were present in the PS medium. Sporangia and oospores were present on alfalfa seedlings used to bait out *P. megasperma* from soil collected beneath diseased plants. Sporangia growing from infected seedlings were usually nonpapillate.

Pathogenicity studies. After 2 wk, seedling stands were greatly reduced in the alfalfa cultivars Agate and Saranac

Table 1. Pathogenicity of a Wyoming isolate of *Phytophthora megasperma* on alfalfa and sainfoin seedlings

Legume and cultivar tested	Reaction to PRR[v]	Plant reaction 2 wk after treatment[w]		
		No. of healthy seedlings[x]		Percent loss[y] (percent of control)
		Inoculated	Uninoculated	
Alfalfa[z]				
Agate	R	17 a	95 b	82.1 a
Saranac	S	2 a	128 b	98.4 b
Sainfoin				
Remont	U	3 a	85 b	96.5 b

[v] R = resistant, S = susceptible, U = unknown.
[w] Values in columns followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range tests.
[x] Values are the total of five replicates.
[y] Loss was caused by preemergence and postemergence damping-off.
[z] Cultivars Agate and Saranac are the USDA-recommended standard resistant and susceptible checks, respectively, for Phytophthora root rot.

and in the sainfoin cultivar Remont when compared with their respective uninoculated controls (Table 1). Saranac and Remont had significantly higher percent seedling loss than Agate (Table 1).

Three months after inoculation, a higher but not significant number of 1-yr-old Saranac plants had PRR than did Agate plants (Table 2). Mean root disease ratings of inoculated plants for Saranac and Agate were not significantly different.

Cultivar trial. By early July, certain cultivars showed severe stand losses. Taproots were rotted off 2–4 cm below the crown and displayed symptoms typical of PRR. *P. megasperma* was readily isolated from diseased roots randomly collected within the test site. Cultivars with known resistance to PRR had better stands, less disease, and higher forage yields than susceptible cultivars or cultivars whose reaction was unknown (Table 3). A 75% reduction in forage yield occurred in the PRR-susceptible cultivar Skyline 400 as compared with the PRR-resistant cultivar WL-312. Soil from the test site contained 30.2% sand, 34.6% silt, 35.2% clay, and 0.7% organic matter.

Phytophthora survey. Phytophthora root rot was found in all three of the major irrigated alfalfa areas surveyed (Fig. 1). It was not found in Hot Springs County in the Big Horn Basin or in Platte County in the Eastern North Platte Valley. From samples submitted to the Plant Disease Clinic, it was also found in Carbon and Albany counties. It was not found in areas where alfalfa is grown under dryland conditions (mean annual precipitation of 38 cm or less). Established stands (2-yr or older) that had PRR often showed symptoms of decline. Stand losses were observed in two fields seeded only 3–4 mo before our observations. *P. megasperma* was readily recovered from roots of 3-mo- to 3-yr-old plants showing typical PRR symptoms as well as from adjacent soil. Many of the alfalfa fields in the Big Horn Basin where PRR was present also had plants infected with the alfalfa stem nematode (*Ditylenchus dipsaci* (Kühn) Filip.). This pest undoubtedly is also involved in losses in this area.

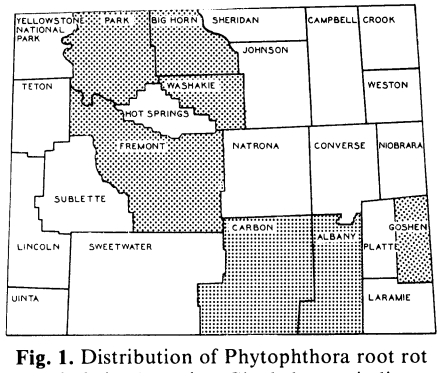

Fig. 1. Distribution of Phytophthora root rot of alfalfa in Wyoming. Shaded areas indicate counties where one or more fields were found to be infested with *Phytophthora megasperma*. Unshaded areas indicate counties either not surveyed or where the disease was not found.

DISCUSSION

This is the first report of PRR of alfalfa from Wyoming. The disease appeared to be more prominent in fields where the soil clay content was high.

Our pathogenicity studies conducted in the greenhouse agree with a previous report (6) that sainfoin is an excellent host for *P. megasperma*. We have not observed PRR on this crop in the field; however, because stand decline is a common problem with sainfoin in most northwestern states (4), further studies should be conducted to determine its possible involvement.

Results of the field trial indicated that spring-seeded stands of PRR-susceptible alfalfa cultivars may be severely reduced by *P. megasperma* during the first year. Because severe PRR developed in a field that had been in barley for 3 yr since the previous alfalfa crop, a short-term rotation (2–3 yr out of alfalfa) may offer little help in controlling PRR in Wyoming. Several cultivars showed excellent resistance to PRR. Evaluation of these cultivars will be continued over several years to determine their long-term survival in *P. megasperma*-infested fields and general adaptability to Wyoming growing conditions. Most of the 165,992 ha of irrigated alfalfa grown in Wyoming are believed to be planted with PRR-susceptible cultivars. A survey in the Wind River Basin, where the cultivar test was conducted and where 22,663 ha of

Table 2. Pathogenicity of a Wyoming isolate of *Phytophthora megasperma* from alfalfa on 1-yr-old alfalfa plants

Alfalfa[w] cultivar tested	Known reaction to PRR[x]	Plant reaction 3 mo after inoculation[y]			
		Root disease[z] rating		Percent plants with Phytophthora root rot	
		Uninoculated	Inoculated	Uninoculated	Inoculated
Saranac	S	1.5 a	3.0 a	12.0 a	68.2 a
Agate	R	1.0 a	1.7 a	0.0 a	32.0 a

[w] Cultivars Saranac and Agate are the standard PRR-susceptible and -resistant checks recommended by the USDA. Twenty-five plants were inoculated and 25 plants uninoculated for each cultivar.
[x] R = resistant and S = susceptible.
[y] Values are the average of five replicates. Values in columns followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.
[z] Plants were rated for root rot on a scale of 1–5 (1 = none, 5 = very severe disease).

Table 3. Reaction of 20 alfalfa cultivars to Phytophthora root rot in a field naturally infested with *Phytophthora megasperma*

Cultivar	Reported[u] reaction to Phytophthora root rot	Plot data collected[v]			
		Visual stand[w] rating (%) 28 July 1981	Disease[x] rating 28 July 1981	Forage yield 6 Oct. 1981[y]	
				Dry wt (g)	12% Moisture (mt/ha)
WL-312	R	85	2.5	722 a	2.82
Vancor	R	70	2.9	694 ab	2.72
Armor	R	85	2.8	664 abc	2.60
Blazer	R	93	2.6	663 abc	2.60
Peak	R	80	2.9	638 abc	2.50
Futura	U	48	3.4	542 abcd	2.12
Lovelock 720	U	60	3.4	522 abcd	2.04
Agate[z]	R	93	2.8	519 abcd	2.03
WL-220	R	75	2.6	513 abcd	2.01
Ranger	S	65	3.0	476 abcd	1.86
Perry	S	40	3.4	412 abcd	1.61
Magnum	U	45	3.6	411 abcd	1.61
Baker	S	33	3.8	359 abcd	1.41
Ramsey	S	58	3.1	351 abcd	1.37
Vernal	S	35	3.3	298 abcd	1.16
Lovelock 715	U	35	3.4	292 abcd	1.14
Lovelock 705	U	28	3.4	275 bcd	1.07
Dawson	S	38	3.3	260 bcd	1.02
Spredor II	S	18	4.0	229 cd	0.89
Skyline 400	U	18	3.9	180 d	0.70

[u] R = resistant, S = susceptible, and U = unknown.
[v] All values are the means of four replicates.
[w] Numbers refer to the percentage of the stand remaining after 3 mo.
[x] Plots were given a disease rating of 1–5 (1 = none, 5 = very severe) on 28 July 1981. Ratings were based on overall appearance, which included stunting, dieback, and stand loss.
[y] Values followed by the same letter do not differ significantly at the 0.05 level, according to Duncan's multiple range test.
[z] Cultivar Agate is the USDA standard PRR-resistant check.