The extremes of temperature and moisture in Oklahoma may be the main reason for the differences in severity and incidence of many of these diseases. The severity of Cytospora canker on cottonwood has been associated with water stress (8). Oak mortality during 1978 and 1980 in Arkansas, Mississippi, and Florida was reported to be triggered by drought (4). In addition, the natural decline of windbreaks planted during the PSFP may contribute to an increase in disease and decay organisms. Observations on disease incidence and severity indicate that the use of such trees as honeylocust, black locust, Austrian pine, ponderosa pine, and American elm as components in windbreaks in Oklahoma should be moderated in favor of trees more adapted to environmental stresses of the southern Great Plains. Additional information concerning disease incidence and severity in windbreaks in other areas of the southern Great Plains and on other tree species used in windbreak plantings would benefit agencies responsible for production of trees used in new windbreak plantings and renovation projects.

ACKNOWLEDGMENTS

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LITERATURE CITED


Phytophthora Root Rot of Alfalfa in Wyoming

F. A. GRAY, Associate Professor and W. H. BOHL and R. H. ABERNETHY, Assistant Professors, Plant Science Division, University of Wyoming, Laramie 82071

ABSTRACT


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.

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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 seeding 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 inoculation study were poured on the soil 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 seeding 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, mycelial characteristic of *Phytophthora* spp., could be seen growing in the PS medium from several root pieces. Ten-day-old subcultures of susceptible 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.
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 1). 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.

**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**

<table>
<thead>
<tr>
<th>Alalfa* cultivar tested</th>
<th>Known reaction to PRR*</th>
<th>Root disease rating (%)</th>
<th>Plant reaction 3 mo after inoculation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninoculated</td>
<td>Inoculated</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>Saranac</td>
<td>S</td>
<td>1.5 a</td>
<td>3.0 a</td>
</tr>
<tr>
<td>Agate</td>
<td>R</td>
<td>1.0 a</td>
<td>1.7 a</td>
</tr>
</tbody>
</table>
| *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.
| R = resistant, S = susceptible.
| *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.
| 7 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**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Reaction to Phytophthora root rot</th>
<th>Visual stand rating (%)</th>
<th>Disease rating (%)</th>
<th>Forage yield 6 Oct. 1981</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28 July 1981</td>
<td>28 July 1981</td>
<td>Dry wt (g)</td>
<td>12% Moisture (mt/ha)</td>
</tr>
<tr>
<td>WL-312</td>
<td>R</td>
<td>85</td>
<td>2.5</td>
<td>722 a</td>
</tr>
<tr>
<td>Vancor</td>
<td>R</td>
<td>70</td>
<td>2.9</td>
<td>694 ab</td>
</tr>
<tr>
<td>Armor</td>
<td>R</td>
<td>85</td>
<td>2.8</td>
<td>664 abc</td>
</tr>
<tr>
<td>Blazer</td>
<td>R</td>
<td>93</td>
<td>2.6</td>
<td>663 abc</td>
</tr>
<tr>
<td>Peak</td>
<td>R</td>
<td>80</td>
<td>2.9</td>
<td>638 abc</td>
</tr>
<tr>
<td>Futura</td>
<td>U</td>
<td>48</td>
<td>3.4</td>
<td>542 abcd</td>
</tr>
<tr>
<td>Lovelock 720</td>
<td>U</td>
<td>60</td>
<td>3.4</td>
<td>522 abcd</td>
</tr>
<tr>
<td>Agate</td>
<td>R</td>
<td>93</td>
<td>2.8</td>
<td>519 abcd</td>
</tr>
<tr>
<td>WL-220</td>
<td>R</td>
<td>75</td>
<td>2.6</td>
<td>513 abcd</td>
</tr>
<tr>
<td>Ranger</td>
<td>S</td>
<td>65</td>
<td>3.0</td>
<td>476 abc</td>
</tr>
<tr>
<td>Perry</td>
<td>S</td>
<td>40</td>
<td>3.4</td>
<td>412 abcd</td>
</tr>
<tr>
<td>Magnum</td>
<td>U</td>
<td>45</td>
<td>3.6</td>
<td>411 abcd</td>
</tr>
<tr>
<td>Baker</td>
<td>S</td>
<td>33</td>
<td>3.8</td>
<td>359 abcd</td>
</tr>
<tr>
<td>Ramsey</td>
<td>S</td>
<td>58</td>
<td>3.1</td>
<td>351 abcd</td>
</tr>
<tr>
<td>Vernal</td>
<td>S</td>
<td>35</td>
<td>3.3</td>
<td>298 abc</td>
</tr>
<tr>
<td>Lovelock 715</td>
<td>U</td>
<td>35</td>
<td>3.4</td>
<td>292 abc</td>
</tr>
<tr>
<td>Lovelock 705</td>
<td>U</td>
<td>26</td>
<td>3.4</td>
<td>275 bcd</td>
</tr>
<tr>
<td>Dawson</td>
<td>S</td>
<td>38</td>
<td>3.3</td>
<td>260 bcd</td>
</tr>
<tr>
<td>Spredor II</td>
<td>S</td>
<td>18</td>
<td>4.0</td>
<td>229 cd</td>
</tr>
<tr>
<td>Skyline 400</td>
<td>U</td>
<td>18</td>
<td>3.9</td>
<td>180 d</td>
</tr>
</tbody>
</table>

* = R = resistant, S = susceptible, and U = unknown.

All values are the means of four replicates.

*Numbers refer to the percentage of the stand remaining after 3 mo.

*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.

*Values followed by the same letter do not differ significantly at the 0.05 level, according to Duncan's multiple range test.

* Cultivar Agate is the USDA standard PRR-resistant check.

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![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.](image-url)
alfalfa are grown, showed that all cultivars reported were susceptible to PRR (W. H. Bohl, unpublished). This appears to be a major reason why this disease is so widespread in this area and probably in other areas of the state.

LITERATURE CITED