Relationships of Increasing Levels of Resistance of Phytophthora Root Rot in Alfalfa to Stand Longevity and Yield

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ABSTRACT

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The relationships of increasing levels of resistance in alfalfa of Phytophthora root rot (PRR), incited by *Phytophthora megasperma* f. sp. *medicaginis*, to stand longevity and forage yield was studied over 4 yr (1981–1984) in a field naturally infested with *P. m.* f. sp. *medicaginis*. Cultivars Baker and Vernal, Futura and WL 220, Vancor and WL 312, and Peak and Agate were used to represent the PRR susceptible (S), low resistance (LR), moderate resistance (MR), and resistant (R) categories, respectively. Data from the two cultivars in each category were combined before analysis. Phytophthora root rot ratings (1–5; 1 = none, 5 = very severe), made 2 mo after seeding, for the S, LR, MR, and R cultivars were 3.6, 3.0, 2.7, and 2.9, respectively. Resistant cultivars produced significantly ($P = 0.05$) more forage than either the MR, LR, or S cultivars by the fourth year. Total forage yields of cultivars over 4 yr were: 30.2, 41.1, 43.6, and 46.9 t/ha for the S, LR, MR, and R cultivars, respectively. When the study was terminated after 4 yr, yields of the resistant cultivars were still increasing while the yields of the susceptible cultivars were declining. Stands remaining in the fall of 1984 for the S, LR, MR, and R cultivars were 21, 31, 46, and 54 plants/m^2, respectively. Disease and stand in 1981 were significantly related to total yield with correlation coefficients of $r = -0.51$ and $r = 0.53$. One hundred 2-yr-old transplants (50 healthy and 50 with severe root rot) of the PRR-susceptible cultivar Skyline 200, were studied for 2 yr to determine the effect of disease on yield and winter survival. A significant stand reduction occurred in diseased plants in the spring of the second year, following a severe winter. No loss occurred in the healthy plants. Yield of plants with PRR was reduced by 68% when compared with healthy plants over the 2 yr.

Phytophthora root rot (PRR) of alfalfa incited by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin (3,8) is a major contributor toward the failure to establish, as well as the decline, of established alfalfa stands in North America (5). Phytophthora root rot was recently shown to be a major disease of irrigated alfalfa in Wyoming (6). Control is best obtained through the use of resistant cultivars. Several researchers have shown the benefit of using resistant cultivars in the presence of *P. m.* f. sp. *medicaginis*, particularly when environmental conditions optimal for disease development exist. Leuchen et al (9) indicated that the most severe losses from PRR appear to occur during seedling establishment. They also pointed out that in *P. m.* f. sp. *medicaginis* infested fields, if severe stand loss does not occur during the seeding year, significant injury and yield loss will eventually occur in the remaining plants. They concluded that 60% resistant plants may be necessary to obtain maximum yield in a *P. m.* f. sp. *medicaginis* infested field. The advantage of host resistance was also shown by Faris and Sabo (4), who transplanted 5- and 7-wk-old plants of resistant and susceptible cultivars into a field followed by inoculation with *P. m.* f. sp. *medicaginis*. After 1 yr, cultivars with resistance to PRR had less disease and higher yields than susceptible cultivars. They found a more significant correlation between yield and percent of diseased plants than between yield and percent of dead plants. They indicated that infected plants could be considered as unproductive units, whereas yield loss due to dead plants may be compensated for by neighboring plants. It was indicated that diseased plants were probably predisposed to winterkill due to their weakened root system. Havey and Grau (7) monitored stand decline and root and crown weight of 10 commercial alfalfa cultivars that varied in their level of resistance to PRR over 3 yr in a Wisconsin field naturally infested with *P. m.* f. sp. *medicaginis*. Although there were differences among cultivars, statistical separation for PRR severity, stand, and yield could not be made among the resistant cultivars because of a large variability between blocks. They suggested reducing the replicate size or the establishment of a uniform *P. m.* f. sp. *medicaginis* nursery where high *P. m.* f. sp. *medicaginis* inoculum and favorable environmental conditions, not present in their field study, are maintained (7). There was a highly significant association between stand decline of entries in the field test and that of a laboratory seedling assay.

A study was initiated in west central Wyoming in the spring of 1981 in a field naturally infested with *P. m.* f. sp. *medicaginis* to determine the relationships of increasing levels of PRR resistance to stand establishment, stand longevity, and forage yield in alfalfa. A second study, initiated in 1982, was designed to determine the effect of severe PRR in alfalfa on forage yield and survival in field transplants. This paper reports the findings in both field studies.

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MATERIALS AND METHODS

Experiment 1 was established on 29 April 1981 in a field near Riverton, WY. The soil was a clay loam containing 30.2% sand, 34.6% silt, 35.2% clay, and

0.7% organic matter. The test consisted of 20 alfalfa entries planted at a seeding rate of 11.2 kg live seeds/ha and arranged in a randomized complete block design with four replications. Plots were 0.6 (five rows spaced 12.5 cm apart) × 4.6 m in size. Several weeks after the test was established, the remainder of the field was planted with the alfalfa cultivar Skyline 200. The field was flood-irrigated and corregations were formed after 1 mo to ensure even dispersal of water in the field and plots. Two months after seeding, symptoms typical of PRR were present in the test site. Symptoms included chlorosis and reddening of leaves, stunting, and death of plants. Eight certified cultivars (two cultivars for each level) were selected on the basis of statistical similarity for yield and disease from the original 20 entries to represent increasing levels of PRR resistance (Table 1). Cultivars Baker and Vernal, Futura and WL 220, Vancor and WL 312, and Peak and Agate were selected to represent the susceptible (S), low resistance (LR), moderate resistance (MR), and PRR resistant (R) categories, respectively. On 28 July 1981, plots were given a subjective rating for PRR, on a scale of 1–5 (1 = no symptoms or plant death present, 2 = slight symptoms with minor plant death, 3 = moderate, 4 = severe, and 5 = very severe symptoms with major plant death). Plots were harvested once in 1981 and three times each in 1982–1984 at 10% bloom and forage yields were determined. To determine the effect of disease on stand decline, a 1-m section of the center row, representative of the plot, was marked with metal spikes and surveyor flags and the number of live plants was determined in the fall of 1981, spring of 1982, and fall of 1984, at which time plants were removed and final counts were made. Accurate counts could not be made between the spring of 1982 and fall of 1984 due to overlapping plant crowns, particularly in the resistant cultivars. Plants were removed, washed free of soil, and roots were rated for PRR on a scale of 1–5 (1 = none; 2 = slight, minor lesion on root; 3 = moderate, distinct lesion; 4 = severe, one or more distinctive lesions; and 5 = very severe, taproot rotted off or plant death) and were observed for other disease.

Experiment 2 was established on 15 October 1982, and was adjacent to Experiment 1. The experiment was conducted to compare the productivity and longevity of severely diseased and healthy plants under field conditions. Many of the plants remaining in plots of the S and LR cultivars in Experiment 1 showed severe stunting and were apparently infected with *P. m.* f. sp. *medicaginis*. Because these plants could not be removed, we selected plants from the surrounding field for the experiment. Fifty plants of the cultivar Skyline 200 with severe root rot (taproot completely rotted off approximately 10 cm below the soil line) and 50 plants free of PRR were removed and placed in paired plots, 10 plants/row, with five replicates, and were observed for 2 yr. The number of live plants was recorded in the fall of 1982 after transplanting, and in the spring and fall of 1983 and 1984. Plots were harvested twice in 1983 and 1984.

Data from both studies were analyzed using standard analysis of variance techniques, and mean separation was determined using Duncan's new multiple range test. In Experiment 1, data for the two cultivars within each resistance category were combined before analysis. Correlation coefficients were calculated for all data in Experiment 1.

RESULTS

Experiment 1. For 1981, disease ratings and yields of the original 20 entries were reported earlier (6). Symptoms of PRR (seedling blight) were first noticed in early July and had become severe by mid-July 1981. Plants were 10 weeks old at this time, and those that had died or had severe root rot could easily be lifted out of the soil because taproots were rotted. Twenty-five live plants with moderate to severe symptoms were removed from border rows, and *P. m.* f. sp. *medicaginis* was readily isolated from them on a selective medium (2). The presence of *P. m.* f. sp. *medicaginis* in a composite soil sample collected from the test site was confirmed using the standard seedling assay (10). Plots were rated for PRR on 28 July 1981 when maximum disease occurred (Table 2). The R and MR cultivars had significantly ($P = 0.05$) less disease than S cultivars. Disease ratings of LR cultivars were not statistically different from ratings of other cultivars.

When Experiment 1 was terminated in the fall of 1984, there was no significant difference in PRR severity among categories in the remaining plants (Table 2). Plants were examined and found to be free of disease symptoms, other than those of PRR.

The relationship of increasing levels of host resistance to stand survival are given in Table 2. Plant counts in the fall of 1981 for the R, MR, and LR cultivars were not significantly different from each other, but all were significantly higher than the S cultivars. In the spring of 1982, plant counts of cultivars in the R category were significantly higher than in the other three categories. The MR and LR cultivars were not different from each other, but were significantly higher than the S cultivar. In the fall of 1984, plant counts in the R and MR cultivars were not different from each other, but were

Table 1. Origin, category of resistance to Phytophthora root rot (PRR), and percent of resistant plants of the eight alfalfa test cultivars

Cultivar	Developer or owner	Resistance category[z]	Resistant plants[z] (%)
Baker	USDA & Nebraska Agric. Exp. Stn.	Susceptible	<5
Vernal	USDA & Wisconsin Agric. Exp. Stn.	Susceptible	
Futura	Dairyland Seed Co.	Low resistance	6–14
WL 220	WL Research, Inc.	Low resistance	
Vancor	Northrup King Co.	Moderate resistance	15–30
WL 312	WL Research, Inc.	Moderate resistance	
Peak	Research Seeds, Inc.	Resistant	31–50
Agate	USDA & Minnesota Agric. Exp. Stn.	Resistant	

[z] Categories of PRR resistance and percent of resistant plants are assigned by the National Alfalfa Certification Review Board.

Table 2. Relationships of increasing levels of resistance of Phytophthora root rot (PRR) to disease and plant stands[x]

	PRR ratings[y]		Stand (plants/m^2)[z]		
Resistance category	1981	1984	Fall 1981	Spring 1982	Fall 1984
Resistant	2.9 a	1.5 a	85 aA	66 aB	54 aB
Moderate resistance	2.7 a	1.6 a	95 aA	47 bB	46 aB
Low resistance	3.0 ab	1.7 a	83 aA	40 bB	31 bB
Susceptible	3.6 b	2.2 a	53 bA	22 cB	21 bB

[x] Values followed by the same letter (lowercase letters for columns and uppercase letters for rows) do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

[y] Values for 1981 were taken on 28 July, 3 mo after planting, and are a visual whole plot rating for PRR (1–5, where 1 = none, 5 = very severe). Values for 1984 were taken on 28 August and are a visual rating for PRR of roots of plants removed from 1 m of row in each plot. All values are the mean of four replications.

[z] Values are the number of plants in the 1 m of row converted to plants/m^2 and are the mean of four replications.

significantly higher than cultivars in either the LR or S categories. There was a significant reduction in plant stands in all categories from the fall of 1981 to the spring of 1982, but not from the spring of 1982 to the fall of 1984.

Forage yields for 1981–1984 are shown in Table 3. In 1981, the year of seeding, yields were significantly higher in the R, MR, and LR cultivars than in the S cultivars. In 1982, the R and MR cultivars could not be separated from each other, but both had significantly higher yields than the S cultivars. The LR cultivars were not different from the MR cultivars, but had significantly higher yields than the S cultivars. In 1983, the only separation occurred between the R and S cultivars. The R cultivars had significantly higher yield than the S cultivars. By 1984, the R cultivars had obtained significantly higher yields than cultivars in all other categories. Yields in the R cultivars were 2.2, 3.1, and 6.7 t/ha higher than the MR, LR, and S cultivars, respectively. Although the MR and LR cultivars could not be separated, both were significantly higher than the S cultivars. The R cultivars yielded 3.4, 5.9, and 16.9 t/ha more than the MR, LR, and S cultivars, respectively, over the 4 harvest years. Total yields were correlated with initial disease and stand with correlation coefficients of $r = -0.51$ and $r = 0.53$, respectively.

Experiment 2. There was no significant difference in plant mortality within treatments in either the fall of 1982 or the spring and fall of 1983 (Table 4). However, in the spring of 1984, significantly higher mortality occurred in the diseased as compared with healthy plants. No plant mortality occurred over the first winter (fall 1982–spring 1983). A significant ($P = 0.05$) loss (20%) occurred over the second winter (fall 1983–spring 1984). No further loss had occurred by the fall of 1984 when the experiment was terminated. Total forage yield of rows with diseased and healthy plants were 94 (diseased) and 359 (healthy) grams (dry wt) for 1983, and 348 (diseased) and 904 (healthy) grams (dry wt) for 1984.

DISCUSSION

Planting susceptible alfalfa cultivars in soil conducive to PRR followed by an extended period of cool, rainy weather resulted in severe seedling blight and root rot. Stands had stabilized by the spring of the second year and, because most susceptible plants had apparently died, no further decline occurred through the remainder of the study. Yield loss was associated with both an early reduction in stand and with reduced yields of the surviving infected plants.

It became obvious that many of the remaining plants in the S and LR cultivars of Experiment 1 showed severe stunting and were apparently infected with *P. m.* f. sp. *medicaginis*. The results of our transplant study showed that PRR-infected plants produced 68% less forage (average of 2 yr) than plants free of PRR. This agrees with Faris and Sabo (4), who reported an increase in forage yield as high as 101% (percent of uninoculated check) from field transplants of PRR-resistant cultivar Agate. Since *P. m.* f. sp. *medicaginis* infected plant transplants were shown to be predisposed to winterkill, many of the plants in Experiment 2 that survived the early seedling blight attack and that were either infected with *P. m.* f. sp. *medicaginis* or later became infected, were most likely predisposed to winterkill. Previous studies by Boelter et al (1) reported that alfalfa plants severely infected with the alfalfa stem nematode *Ditylenchus dipsaci* Kühn were predisposed to winterkill. They indicated that field-grown plants infected with *D. dipsaci* had lower total nonstructural carbohydrates in the uppermost 15 cm of the taproot than did healthy plants and that the stem nematode apparently interfered with the normal hardening process. In our study, carbohydrate analyses were not determined for PRR-diseased and healthy plants. However, it appears that the most probable explanation for the winterkill was due to the elimination of sufficient upper taproot tissue needed for carbohydrate storage. Losses occurred only during the second winter when sub-zero temperatures were common. Average monthly temperatures recorded in Riverton, WY, during the first winter (October 1982 through March 1983) were 6, −2, −12, −7, −3, and 3 C, and during the second winter (October 1983 through March 1984) were 7, −3, −19, −14, −11, and −2 C. Therefore, a more rapid decline of stands of cultivars that are susceptible or have only a low level of resistance to PRR may occur in colder alfalfa-producing areas or in certain years following a particularly hard winter where PRR is present.

Increasing levels of resistance to PRR resulted in less disease, better stand establishment and persistence, and increased forage yield. When the study was terminated after 4 yr, yields of cultivars in the R, MR, and LR categories were still increasing, whereas yields of cultivars in the S category were declining.

Faris and Sabo (4) stated that plants infected with *P. m.* f. sp. *medicaginis* could be considered as unproductive units within the plant population. Leuchen et al (9) stated that as high as 60% resistant plants may be necessary to obtain maximum yield in alfalfa exposed to high disease pressure. Our studies are in agreement with those of Faris and Sabo (4) and Leuchen et al (9). Data from these studies indicate that emphasis in breeding programs should be placed both on the selection of highly resistant plants and on increasing the percentage of resistant plants within the population. Although cultivars with a highly resistant (HR) rating (⩾51% resistant plants) were not available for inclusion in our study, further increase in yield above that obtained in the R cultivars would be expected to occur.

Table 3. Relationships of increasing levels of resistance of Phytophthora root rot (PRR) to yields in alfalfa[z]

Resistance category	Forage yield (t/ha)				
	1981	1982	1983	1984	Total
Resistant	2.4 a	13.2 a	14.6 a	16.8 a	46.9 a
Moderate resistance	2.7 a	12.8 ab	13.4 ab	14.7 b	43.6 ab
Low resistance	2.1 a	11.9 b	13.4 ab	13.7 b	41.1 b
Susceptible	1.3 b	7.9 c	10.8 b	10.2 c	30.2 c

[z] Values are the amount of forage removed from plots, converted to 12% moisture, and are the mean of four replications. Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

Table 4. Relationship of Phytophthora root rot to mortality and yield in alfalfa field transplants

Plant status	Live plants[z]					Yield/row (g, dry wt)	
	Winter 1		Winter 2				
	Fall 1982	Spring 1983	Fall 1983	Spring 1984	Fall 1984	1983	1984
Healthy	10 aA	9.4 aA	9.2 aA	9.2 aA	9.0 aA	359 aA	904 aB
Diseased	10 aA	9.2 aA	9.0 aA	7.2 bB	7.2 bB	94 bB	348 bB

[z] Values are the remaining plants from 10 plants per row and are the mean of four replications. Values followed by the same letter (lowercase for columns and uppercase for rows) do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

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LITERATURE CITED

1. Boelter, R. H., Gray, F. A., and Delaney, R. H. 1985. Effect of *Ditylenchus dipsaci* on alfalfa mortality, winterkill and yield. J. Nematol. 17:140-144.
2. Eckert, J. W., and Tsao, P. H. 1962. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. Phytopathology 52:771-777.
3. Erwin, D. C. 1965. Reclassification of the causal agent of root rot of alfalfa from *Phytophthora cryptogea* to *P. megasperma*. Phytopathology 55:1139-1143.
4. Faris, M. A., and Sabo, F. E. 1981. Effect of *Phytophthora megasperma* on yield and survival of resistant and susceptible alfalfa cultivars. Can. J. Plant Sci. 61:955-960.
5. Graham, J. H., Frosheiser, F. I., Stuteville, D. L., and Erwin, D. C. 1979. A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, MN. 65 pp.
6. Gray, F. A., Bohl, W. H., and Abernethy, R. H. 1983. Phytophthora root rot of alfalfa in Wyoming. Plant Dis. 67:291-294.
7. Havey, M. J., and Grau, C. R. 1985. Decline of established alfalfa in soils naturally infested with *Phytophthora megasperma* f. sp. *medicaginis* and level of correlation by seedling assay. Plant Dis. 69:221-224.
8. Kuan, T. -L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
9. Leuchen, W. E., Barnes, D. K., Rabas, D. L., Frosheiser, F. I., and Smith, D. M. 1976. Field performance of alfalfa resistant and susceptible to Phytophthora root rot. Agron. J. 68:281-285.
10. Pratt, R. G., and Mitchell, J. E. 1973. Conditions affecting the detection of *Phytophthora megasperma* in soils of Wisconsin alfalfa fields. Phytopathology 63:1374-1379.