Decline of Established Alfalfa in Soils Naturally Infested with *Phytophthora megasperma* f. sp. *medicaginis* and Level of Correlation by Seedling Assay

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ABSTRACT

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Decline of established alfalfa stands, as measured by changes in mean disease severity index, number of plants per square meter, and total root and crown dry weight per square meter was studied over 3 yr for 10 commercial cultivars varying in levels of resistance to *Phytophthora megasperma* f. sp. *medicaginis* (Pmm). Significant differences between cultivars were found for the three variables in analyses over 3 yr. Significant differences between cultivars were not found after the establishment year for analyses within years. A postemergence damping-off (PEDO) seedling assay was conducted for each of the 10 cultivars and two Pmm isolates. The proportion of seedling survivors after 11 days in the PEDO test correlated significantly with the results of the Minnesota Phytophthora root rot evaluations (0.94 and 0.95 for the two Pmm isolates) and the number of surviving plants per square meter after 3 yr in the field (0.73 and 0.87). The PEDO test was an accurate predictor of the relative rate of decline of alfalfa cultivars under conditions of natural Pmm infestation.

Phytophthora root rot (PRR) is a major disease of alfalfa (*Medicago sativa* L.) grown in soils that are frequently water-saturated (16,17,19). The disease was first described in California by Erwin (3) and was described later in the Upper Midwest (2,5). PRR is characterized by brown, water-soaked necrosis at defined sites on the taproots and lateral roots under conditions of prolonged soil wetness (7). The causal agent, *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin (Pmm) (15), is one of the major contributors to decline of established alfalfa stands (7,9,17).

Control of PRR has involved the use of host resistance currently available in commercial cultivars in varying levels (14). Several methods of evaluating alfalfa germ plasm for resistance to Pmm have been reported. Frosheiser and Barnes (6) described field and greenhouse evaluations and reported a correlation coefficient of at least 0.949 between the results of the two procedures. A greenhouse assay involving mature roots has been reported by Irwin et al (12). Several authors have described alfalfa seedling assays for rapid assessment of cultivar resistance or identification of resistant plants for use in a breeding program. Gray et al (8) and Irwin et al (13) described postemergence damping-off tests. Hine et al (10) successfully selected for resistance to Pmm in lines of nondormant alfalfa with a seedling assay. Hohrein et al (11) reported that a greenhouse seedling assay correlated significantly ($r = 0.78$) with the annual PRR evaluations conducted in the field at the Minnesota Agricultural Experiment Station at St. Paul (6), hereafter referred to as the Minnesota test.

Most studies on PRR to date have concentrated on the effects on seedling establishment (16,17,19). Field evaluations of alfalfa germ plasm under conditions of Pmm infestation usually involve supplemental irrigation to enhance disease development (4,6,20). The goal of this experiment was to monitor decline of established alfalfa stands of 10 commercial cultivars in field plots naturally infested with Pmm without supplemental irrigation over a 3-yr period. We wanted to conduct this experiment in a field plot that had not received supplemental inoculum (4,6) or irrigation (4,6,20) to promote PRR. We also used commercial cultivars with varying levels of resistance to Pmm to assess the impact of PRR on stand decline. The level of agreement between the results of the PEDO seedling assay (13) and actual field performance of the 10 cultivars was studied to assess the efficacy of a seedling assay as a predictor of decline of established alfalfa stands in Pmm-infested soils.

MATERIALS AND METHODS

Ten commercial cultivars varying in levels of resistance to Pmm were studied. Cultivars Answer, Apollo, Blazer, 545, Phytor, Trident, and WL318 were considered resistant; Iroquois, Saranac, and Vernal were considered susceptible (1). The origin and proportion of resistant plants (1) for each cultivar are listed in Table 1. Plots were established at the University of Wisconsin Experiment Station at Marshfield under conditions of natural Pmm infestation. The herbicide EPTC was preplant incorporated at a rate of 3.36 kg/ha. Cultivars were seeded in May 1980 in plots 0.9 × 7.6 m at a rate of 20.2 kg of seed per hectare with a self-propelled mechanical seeder sowing five rows spaced 15 cm apart. The experiment was a randomized complete block design with four replicates.

Plots were sampled during October of 1980, 1981, and 1982. On each sampling date, two 0.09-m^2 samples were dug from each plot and combined. Roots were washed and scored on a disease severity index (DSI) of 1–5, where 1 = taproot healthy, no lesions present; 2 = non-girdling lesions on lower one-half of taproot or small girdling lesions permissible on lower one-third of taproot; 3 = girdling lesions on lower one-half of taproot; 4 = girdling lesions on the top one-half of taproot; and 5 = taproot rotted, aboveground parts necrotic. Number of roots and dry weight in grams of the roots and crowns were

Table 1. Origin and proportion of Phytophthora root rot-resistant plants of 10 commercial cultivars used in studies of stand decline under conditions of natural infestation with *Phytophthora megasperma* f. sp. *medicaginis*

Cultivar	Developer or owner	Proportion of resistant plants[a]
Answer	Midland Cooperatives, Inc.	0.66
Apollo	NAPB	0.36
Blazer	Land O'Lakes	0.26
545	Pioneer Hi-bred International, Inc.	0.30
Iroquois	Cornell University	0.01
Phytor	Northrup King Co.	0.30
Saranac	Cornell University	0.03
Trident	PAG Seeds	0.71
Vernal	Wisconsin Agricultural Experiment Station and USDA	0.05
WL318	W. L. Research, Inc.	0.21

[a] Results reported by Barnes and Frosheiser (1).

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also recorded. From these measurements, mean DSI, number of plants per square meter, and total dry weight of roots and crowns per square meter were calculated.

The PEDO test assessed decrease in seedling survivors over time and served as a possible predictor of stand decline in the field. The test was conducted as described by Irwin et al (13). Briefly, the test involved growing 40 alfalfa seedlings in a Pmm-infested sand-vermiculite-perlite (1:1:1, v/v) mixture in 946-ml plastic cups under a lighting regime of 14 hr of light (21 C, 10,070 lux) and 10 hr darkness (16 C). The cups were flooded 3 days after sowing and the planting medium was maintained in a saturated state. The number of seedling survivors was recorded each day until the survival of the susceptible cultivar Vernal was less than 10%. The PEDO test was conducted on the 10 cultivars with each of two Pmm isolates: 5b4, a single-zoospore isolate collected from the PRR nurseries in St. Paul; and Mf-245, a single-zoospore isolate obtained from diseased alfalfa roots from the same location on the Marshfield Experiment Station as the previously described field plots. Entries in the PEDO test were replicated three times.

RESULTS

Assessment of field stand decline. Analyses over years were first completed to assess the significance of stand decline throughout the 3-yr sampling period. Three experimental units were lost and missing plot values were estimated (22). The error degrees of freedom were therefore reduced from 90 to 87. Years were highly significant ($P<0.01$) in the analyses of mean DSI, plants per square meter, and total root and crown dry weight per square meter (Table 2). Therefore, significant stand decline, as measured by plants per square meter and total root and crown dry weight per square meter, was observed from 1980 through 1982. Highly significant differences in mean DSI were also found over years. Means for each of these variables for each cultivar and year are listed in Table 3.

Single degree of freedom comparisons for response surfaces were generated for years in the analyses of mean DSI, plants per square meter, and total root and crown dry weight per square meter. The linear component was highly significant in all three analyses. The quadratic component was also highly significant for mean DSI and for total root and crown dry weight per square meter. The quadratic effect was not significant for plants per square meter. Because much of the variability over years in plants per square meter can be explained by linear regression, estimates of linear regression coefficients were calculated for each cultivar. In spite of the significant linear component, only five of the 10 cultivars demonstrated significant ($P<0.05$) regression coefficients.

The highly significant quadratic effect in the analysis of mean DSI resulted from higher DSI values in 1981 than in 1980 or 1982. The lower mean DSI values in 1982 are a result of selection in 1981 and 1982 for plants showing some level of resistance. Therefore, this selection would be expressed as a significant nonlinear (ie, quadratic) effect.

A highly significant quadratic effect was observed for total root and crown dry weight per square meter. A large decrease in total root and crown dry weight was found from the 1980–1981 sampling dates, followed by a smaller decrease from 1981 to 1982. A nonlinear effect would be possible when decline in plant number and increase in individual root weights are occurring at the same time.

Cultivar effects were significant in the analyses of the three variables over the 3-yr sampling period (Table 2). Single degree of freedom comparisons between resistant (Answer, Apollo, Blazer, 545, Phytor, Trident, and WL318) and susceptible (Iroquois, Saranac, and Vernal) cultivars were significant for all three variables. With regard to measured variables, the PRR-resistant cultivars performed better over time than the PRR-susceptible cultivars in Pmm-infested soils. The significant year × cultivar interaction for plants per square meter (Table 2) may have resulted from the higher stand counts in 1981 for cultivars Answer and Vernal.

In the establishment year (1980), a significant difference between cultivars was observed for number of plants per square meter (Table 3); significant differences between cultivars were also found at the 10% level for mean DSI. No significant differences were observed for total root and crown dry weight per square meter. This was in agreement with the observation that plots with fewer numbers of roots tended to have larger roots and crowns, whereas plots with greater numbers of roots had smaller roots and crowns. Block differences were not significant in 1980.

In 1981 and 1982, there were no

Table 2. Analyses of variance and significance of mean squares for years and cultivars for the variables mean disease severity index (DSI), number of plants per square meter, and total root and crown dry weight per square meter

		Mean squares		
Source of variation	df	DSI	No. of plants/m^2	Total root and crown weight (g/m^2)
Years	2	7.6**[a]	105,455**	993,185**
Linear	1	5.2**	210,535**	1,872,720**
Quadratic	1	10.1**	375[ns]	113,650**
Cultivars	9	0.6**	16,714**	31,976*
R vs. S[b]	1	5.2**	26,291*	110,883**
Interaction	18	0.2[ns]	7,683*	11,508[ns]
Error	87[c]	0.2	3,392	13,206
Total	116	...	...	...

[a] ns = Not significant, * = significant ($P<0.05$), and ** = highly significant ($P<0.01$) mean square.
[b] R = resistant cultivars Answer, Apollo, Blazer, 545, Phytor, Trident, and WL318; S = susceptible cultivars Iroquois, Saranac, and Vernal (1).
[c] Error degrees of freedom equal 87 because three experimental units were estimated.

Table 3. Mean disease severity index (DSI), mean number of plants per square meter, and mean total root and crown dry weight per square meter for 10 commercial cultivars sampled over 3 yr

	Mean DSI			Mean no. of plants/m^2			Mean root and crown dry weight (g/m^2)		
Cultivar	1980	1981	1982	1980	1981	1982	1980	1981	1982
Answer	1.1	1.9	1.5	168	173	98	575	432	265
Apollo	1.2	2.1	1.5	149	143	91	462	354	360
Blazer	1.2	1.8	1.6	93	85	70	634	397	258
545	1.1	2.3	1.4	368	149	97	630	335	327
Iroquois	1.2	2.3	2.2	195	130	47	602	392	238
Phytor	1.2	1.8	1.5	157	108	94	672	365	338
Saranac	1.7	2.2	2.0	196	138	52	625	319	218
Trident	1.1	2.0	1.5	219	149	99	627	391	318
Vernal	1.1	2.6	2.7	61	99	56	446	220	152
WL318	1.3	1.8	1.6	210	150	83	622	408	235
Grand Mean	1.2	2.1	1.8	182	145	79	590	370	271
LSD (0.10)	0.3	ns[a]	0.7	86	ns	ns	ns	ns	ns
LSD (0.05)	ns	ns	ns	103	ns	ns	ns	ns	ns

[a] ns = Not significant.

significant differences between cultivars for number of plants per square meter or total root and crown dry weight per square meter. Cultivar differences in mean DSI were not significant in 1981 but were significant at the 10% level in 1982. Cultivars differed significantly at the 10% level for mean DSI and number of plants per square meter only during the establishment year. Significant differences were found, however, between blocks for plants per square meter in 1981 and 1982 as well as for total root and crown dry weight per square meter in 1982. The significant block variances in 1981 and 1982 are problematic because stand decline (as measured by plants per square meter and total root and crown dry weight per square meter) progressed at different rates in the individual blocks. Large differences between observations from the four blocks resulted in large standard deviations and reduced the precision in tests for differences between cultivars.

Assessment of decline with PEDO test. The PEDO test assessed the proportion of seedling survivors over time. The PEDO test was conducted over a period of 11 days until the proportion of seedling survivors for Vernal was less than 10%. Regression analyses were completed on the proportion of seedling survivors over time for each of the 10 cultivars and two Pmm isolates. All regression coefficients were highly significant. The proportion of seedling survivors on day 11 and the regression coefficients are listed in Table 4.

Field-PEDO correlations. Two methods were used to assess the level of agreement between stand decline in the field and the results from the PEDO test. The first was to compare the proportion of survivors in the PEDO test for each cultivar with the number of survivors in the field in 1982. Significant or highly significant correlations were found between the proportion of PEDO survivors (with Pmm isolates 5b4 and Mf-245), number of plants per square meter in the field in 1982, and proportion of resistant plants in the Minnesota test (Table 5). A greater level of significance was found between the Marshfield field data and the PEDO test using the Marshfield isolate (Mf-245) than with the PEDO test using the Minnesota isolate (5b4).

The second method used to assess the level of agreement between the PEDO test and field performance of cultivars resistant and susceptible to PRR was to consider stand decline over time. The rate of decrease in the proportion of seedling survivors in the PEDO test is expected to depend on the level of resistance to Pmm in the cultivars. Therefore, the regression of proportion of seedling survivors over time can be an estimate of stand decline caused by Pmm. Although highly significant regression coefficients were observed in the PEDO test, only five of the 10 cultivars demonstrated significant regression coefficients for stand decline in the field. Attempts were made to correlate the regression coefficients from the PEDO test with the significant regression coefficients from the field data. No significant correlation was found.

DISCUSSION

The inability to detect cultivar differences in number of plants per square meter and total root and crown dry weight per square meter after the establishment year was due to the large variability between blocks. This illustrates a potential problem with evaluating alfalfa germ plasm under conditions of natural Pmm infestation. Different levels of disease can result in adjacent plots, possibly because of nonrandom inoculum distribution or microenvironmental effects. The result can be an ineffective response when selecting alfalfa germ plasm for PRR resistance in field plots naturally infested with Pmm. Therefore, it becomes necessary to limit the size of replicates in the field or to establish a uniform Pmm nursery similar to the one maintained at the University of Minnesota. Although significant differences were not found between cultivars for number of surviving plants per square meter after the establishment year, significant correlations were found between survivors in the field in 1982 and the proportion of survivors after 11 days in the PEDO test. A greater level of agreement was found, however, between the results of the PEDO test and the proportion of resistant plants in each cultivar in the annual PRR trials at the University of Minnesota (Table 5).

Greater correlations between the PEDO test and field data were obtained with the Marshfield isolate than with the Minnesota isolate. This result may reflect a greater predictability of the PEDO test with an isolate from the field location where the alfalfa cultivar is grown. Another possible explanation is that the Minnesota isolate (5b4) had been maintained in culture by repeated transfer over a period of 4 yr. Reisolation of 5b4 from inoculated alfalfa seedlings was completed numerous times, but reduced aggressiveness of the isolate is possible. The Marshfield isolate (Mf-245) was obtained from diseased alfalfa roots about 4 mo before the PEDO tests were completed. The higher level of correlation between Mf-245 and the field data may reflect the greater aggressiveness of a newly isolated Pmm culture.

Frosheiser and Barnes (6) reported very high correlations between the results of a greenhouse and field PRR evaluations. Hohrein et al (11) also reported significant agreement between their greenhouse evaluation and the annual PRR tests at Minnesota. Results

Table 4. Final proportion of seedling survivors and the regression coefficients for rate of change in seedling survival over 11 days in PEDO test of 10 cultivars to two *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) isolates, 5b4 and Mf-245

	Pmm isolates					
	5b4			Mf-245		
Cultivars	Proportion of survivors on day 11	Regression coefficient	r^2[a]	Proportion of survivors on day 11	Regression coefficient	r^2
Answer	0.65	−0.039**[b]	53.5	0.58	−0.048**	79.3
Apollo	0.49	−0.054**	90.0	0.33	−0.078**	85.5
Blazer	0.38	−0.066**	89.4	0.21	−0.092**	93.2
545	0.44	−0.062**	85.7	0.33	−0.074**	78.5
Iroquois	0.36	−0.066**	96.1	0.09	−0.109**	88.6
Phytor	0.43	−0.066**	82.7	0.25	−0.084**	86.2
Saranac	0.33	−0.081**	89.6	0.09	−0.106**	91.2
Trident	0.74	−0.029**	64.3	0.44	−0.059**	73.8
Vernal	0.19	−0.093**	96.9	0.08	−0.104**	80.4
WL318	0.33	−0.078**	95.2	0.26	−0.085**	88.3
LSD (0.05)	0.12	...	...	0.18	...	...
LSD (0.01)	0.23	...	...	0.25	...	...

[a] r^2 = Coefficient of determination.
[b] ** = Highly significant regression coefficient ($P < 0.01$).

Table 5. Correlation coefficients and level of significance between proportion of survivors in the PEDO test using two *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) isolates, 5b4 and Mf-245, proportion of resistant plants in the Minnesota test, and number of survivors after 3 yr in field plots infested with Pmm for 10 cultivars

	PEDO test with Pmm isolates		No. of plants/m² in field in 1982
	5b4	Mf-245	
PEDO test with Pmm isolate Mf-245	0.874**[a]	...	...
Number of plants/m² in field in 1982	0.730*	0.872**	...
Proportion of resistant plants in Minnesota test	0.936**	0.949**	0.845**

[a] * = Significant at $P < 0.05$ and ** = significant at $P < 0.01$.

presented in this report show a significant correlation between the PEDO test and decline in plant number over a 3-yr period under conditions of natural Pmm infestation for 10 commercial cultivars. Although our results show that performance of cultivars in the PEDO test can be used as an accurate predictor of stand decline under field conditions, one must recognize that we are only assessing the relationships between the rates of decline of the cultivars. Both the PRR-resistant and PRR-susceptible cultivars are experiencing stand decline, but at different rates. Other factors may be playing a role in stand decline in Pmm-infested soils, and the lower correlations observed in this study may be due to the presence of other pathogens in the Marshfield soil. Schmitthenner (21) and McKeen and Traquair (18) have reported that an *Aphanomyces* sp. is a pathogen of alfalfa. Recently, an *Aphanomyces* sp. pathogenic to alfalfa was isolated from the site of this experiment on the Marshfield Experimental Farm (C. R. Grau, *unpublished*). Breeding alfalfa for resistance to Pmm alone may not lead to increases in stand longevity in poorly drained soils because other pathogens, such as *Aphanomyces* sp., contribute to stand decline of PRR-resistant germ plasm.

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