ALFALFA PHYTOPHTHORA ROOT ROT RESISTANCE SCREENING TECHNIQUES

By

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Finally, I dedicate this thesis to my wife Lorna, and my family for their constant support and quiet confidence.

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INTRODUCTION

Phytophthora root rot (PRR) is a major disease of alfalfa (Medicago sativa L.) in Oklahoma. It is caused by the soilborne fungus Phytophthora megasperma f. sp. medicaginis Kuan and Erwin (Pmm) in association with water-saturated soil at temperatures of 12-30 C. When left uncontrolled, PRR shortens alfalfa stand life, reduces yield, and encourages weed encroachment. Currently, the only control is to improve soil drainage and use genetically resistant cultivars.

Increasing resistance of alfalfa to PRR is accomplished by recurrent mass selection cycles (i.e., plant--screen material--intercross resistant selections--harvest seed and then repeat cycle). This selection cycle has been inefficient in Oklahoma due to the lengthy (10 months) screening period currently used to identify resistant plants. Completion of one selection cycle using the present screening technique requires up to 2 years.

Most field screening by breeders utilize spring plantings followed 3 or 4 months later with a fall screening. Conditions are such in Oklahoma that spring planting with fall screening does not always allow favorable moisture and temperature regimes for effective screening. Little work has been done with fall plantings, which is the traditional time to plant alfalfa in Oklahoma. Greenhouse screening techniques can also be used as a means of supplementing field screening to idenify PRR resistant plants.

Real progress in the development of PRR resistant alfalfa cultivars cannot be made until selection cycles for PRR resistance are made more efficient. The objectives of this research were to identify efficient field and greenhouse PRR resistance screening techniques in alfalfa which are suitable for Oklahoma climatic conditions.

CHAPTER II

Effects of Planting and Screening Dates on Severity of Phytophthora Root Rot in Alfalfa

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ABSTRACT

Porter, D. R., Caddel, J. L., and Singleton, L. L. 1984. Effects of planting and screening dates on severity of Phytophthora root rot in alfalfa. Plant Dis. 68:////.

Four field studies were conducted to identify efficient Phytophthora root rot (PRR) resistance screening techniques to minimize the time required for one cycle of selection in alfalfa (Medicago sativa L.). Disease index and percent resistant plants of two PRR resistant and two susceptible cultivars maintained under saturated soil conditions were used as indicators of screening effectiveness. In two studies, the effects of six and eight screening dates (Nov. 2 through July 15) on PRR symptoms of fall-planted alfalfa were evaluated in 1981 and 1982, respectively. Results indicated effective screening can be obtained by mid-May provided soil temperatures exceed 12 C prior to screening. In two separate studies, the effects of six spring planting dates (March 5 through May 13) were evaluated with an August screening in 1982 and

1983. A mid-April planting resulted in the most effective August PRR screening. One cycle of selection for PRR resistance per year can be achieved by utilizing either of these techniques.

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Phytophthora root rot (PRR) of alfalfa (Medicago sativa L.) occurs in nearly every area of the world where alfalfa is grown (9). It is caused by the fungus Phytophthora megasperma f. sp. medicaginis (Pmm), (10) and has been cited as a major factor in stand decline of alfalfa (4,6,7,9).

Wilkinson and Millar (13) reported Pmm activity only after soil temperatures reached 15 C in spring with an increase in activity at 18-20 C and no activity below 12 C in fall. Water saturation of the soil; 1) predisposes alfalfa to PRR (11), 2) is required for infection, and 3) promotes optimum disease development (4).

Marks and Mitchell (12) described a technique for detecting and isolating Pmm from naturally infested soils using 3-day-old alfalfa seedlings as bait. This methodology was useful for determining distribution of the pathogen in the field. They were able to detect Pmm in low drainage areas, but not on well-drained slopes.

Host plant resistance was first reported in 1966 (5). Resistance can be increased in breeding strains by recurrent mass selection cycles (i.e., plant - screen material - intercross selections - harvest seed and then repeat cycle). Starting populations with less than 10% resistant plants have been increased to 63% resistant plants after three cycles of selection (8). In Canadian field trials, plant losses of PRR resistant cultivars averaged only 21% compared to 44% for susceptible cultivars, and yield reductions were 21% and 55%, respectively (6).

A standard field test to characterize PRR resistance in alfalfa cultivars was developed at the University of Minnesota (1). In this test, seventy five viable seeds were planted in early May in Pmm naturally infested soils. One month after planting, soil was saturated for 2-3 weeks. If the disease was not severe enough for effective screen-

ing, one or two additional 3-week periods of soil saturation were imposed. At completion of test (first 2 weeks of September), plants were dug retaining as much taproot as possible. Roots were washed and individually scored using a 1-6 classification scale described by Frosheiser and Barnes (8). Bray and Irwin, (2) in Australia, modified the standard test by utilizing an August planting and delayed screening until September of the following year.

In the Southern Plains, fall planting of alfalfa is preferred to spring planting due to higher plant emergence and lower plant mortality after emergence (3). Conditions are such in Oklahoma that spring planting with fall screening does not always allow favorable moisture and temperature regimes for effective screening. The selecion cycle for PRR resistance has been inefficient in Oklahoma due to the lengthy (10 months) screening period currently used. Completion of one selection cycle using the present screening technique requires up to 2 years.

The purpose of this research was to evaluate the effects of planting and screening dates on severity of PRR expression in alfalfa in this region. Emphasis on fall planting with spring screening was to determine how soon cultivars could be screened. Emphasis on spring planting was to determine if there was an optimum date of planting which would result in the most effective screening in August.

MATERIALS AND METHODS

Date of Screening Studies. In study 1, one hundred viable seeds of each of two known PRR resistant (Agate, Apollo) and two susceptible (Arc, Vernal) cultivars (6) were planted 1 October 1981. In study 2, Apollo and Vernal were replaced with WL-318 (resistant) and Sarancac (susceptible) (6) and one hundred fifty viable seeds of each cultivar were planted 6 September 1982. In both studies, seed was planted in 2m long rows (one cultivar/row) with 30cm spacing between rows. The soil was a Port loam naturally infested with Pmm. Overhead irrigation was applied immediately following planting to ensure stand establishment. Plant counts were taken 2 weeks following planting in both studies. Rainfall combined with supplemental irrigation kept the soil at or near saturation in both studies during November and December, 1981/82, and March 1982/83. Plants in study 1 were dug and roots evaluated for PRR symptoms on April 1, 22, May 13, June 3, 24, and July 15, 1982. Plants in study 2 were dug November 2, December 20, 1982, and April 1, 22, May 13, June 3, 24 and July 15, 1983.

Date of Planting Studies. In studies 3 and 4, one hundred fifty viable seeds of each of two known PRR resistant (Agate, WL-318) and two susceptible (Arc, Saranac) cultivars were planted in 2m long rows (one cultivar/row). Planting dates were March 5, 17, April 2, 16, 29, and May 13, 1982 (study 3) with same six dates in 1983 (study 4). Irrigation was applied following each planting date to ensure stand establishment. Plant counts were taken two weeks following each planting date. All treatments were irrigated daily from June 13 to August 17, 1982 (study 3) and 1983 (study 4). Plants were clipped and roots dug and

evaluated for PRR symptoms on August 24, 1982 (study 3) and 1983 (study 4).

Split-plot designs were used for all studies with screening, (or planting) dates as main plots in randomized complete blocks and four cultivars as subplots with six replications. Evaluation of individual plant roots for PRR symptoms was based on a 6-class scale (1= no symptoms, 6= dead plants) where plants in classes 1 and 2 are considered resistant (8). An average disease severity index (ASI) was calculated for each subplot by use of the following formula;

$$\frac{\text{Summation(Class No. X \% in class)}}{100}$$

Significant differences between resistant and susceptible cultivars for PRR severity, as expressed in ASI's and percent resistant plants (classes 1 and 2), were used as indicators of screening effectiveness.

RESULTS AND DISCUSSION

Date of Screening Studies. Cultivars planted in October, 1981 (study 1) showed significant differences ($P<0.05$) in mean ASI and percent resistant plants among screening dates (Tables 1,2). ASI's generally increased, and percent resistant plants generally decreased with subsequent screening dates indicating an increase in PRR severity (Tables 1,2). Differences among cultivar types (resistant, susceptible) for ASI and percent resistant plants were significant within each screening date even at the first date of screening (April 1). Differentiation of resistant and susceptible cultivars at April 1 indicates effective screening had been achieved by that date. Magnitude of ASI's are similar to those obtained in other breeding programs (8). No significant ($P>0.05$) date X cultivar interaction was detected. Six days (March 16-22) with soil temperatures at or above the 12 C Pmm activity threshold (13) were observed about 2 weeks prior to the first date (April 1) as indicated in Figure 1.

Two late fall dates (November 2, December 20) were added in study 2 to determine if the screening process could be further shortened. However, significant differences among cultivars for ASI and percent resistant plants could not be detected until May 13, 1983 (Tables 3,4). Mean ASI and percent resistant plants of all cultivars at May 13 were significantly different than those of preceeding dates (Tables 3,4). No significant date X cultivar interaction was detected. Unlike 1982, soil temperatures (Fig. 1) for 1983 did not exceed the 12 C Pmm activity threshold (13) until about 2 weeks prior to the May 13 screening date. By July 15, ASI's and percent resistant plants for the resistant cultivars could not be distinguished from the susceptible cultivars due to

severity of PRR damage from prolonged screening under favorable Pmm disease development conditions (Tables 3,4).

Wilkinson and Millar (13) report that soil temperatures significantly affect Pmm activity in early spring and late autumn. Results of this study support their finding. Differences in effective screening dates in study 1 (April 1, 1982) and study 2 (May 13, 1983) may be attributed to differing soil temperatures prior to screening dates. Soil temperatures for 1983 were cooler than 1982 which suppressed Pmm activity thus increasing the time required for disease development. Monitoring soil temperatures in early spring would appear to be an important factor in deciding when screening would be effective. Prolonged periods of soil-saturation under higher soil temperatures during June and July, 1983 resulted in severe PRR damage even to the resistant cultivars.

Utilization of either screening date (April 1 or May 13) will facilitate completion of one selection cycle per year. Breeding material can be planted in September/October and screened in April-May after soil temperatures reach 12 C or higher at least 2 weeks prior to screening. Selected resistant plants could then be transplanted in greenhouse for intercrossing in June-August, seed could be harvested and planted to begin another selection cycle in September.

Date of Planting Studies. In study 3, an August, 1982 screening produced no useable significant differences among ASI means and percent resistant plants of the six spring and summer 1982 planting dates (Tables 5,6). Only the third planting date (April 2) resulted in significant differences in ASI's and percent resistant plants betweeen cultivar types (resistant, susceptible) which indicates planting on this date resulted in the most effective August screening. The ASI mean for

the fourth planting date (April 16) in study 4 was significantly different from other planting dates when cultivars were screened in August (Table 7). However, there was no significant difference in ASI's detected between cultivar types at that date. Analysis of percent resistant plants indicated significant differences between cultivar types at the third date of planting (April 2) which confirms results of study 3 (Tables 3,4)

Results from these two studies indicate that there is an optimum time period (early April), rather than a particular date, in which alfalfa should be planted to obtain effective screening in August. No significant ($P>0.05$) cultivar X date interaction was detected in either study.

Stand establishment of all cultivars at all spring planting dates was poor. Average number of plants for both years counted 2 weeks after each planting date were as follows: March 5 - 29 plants, March 17 - 18 plants, April 2 - 47 plants, April 16 - 61 plants, April 29 - 33 plants, May 13 - 28 plants. Small numbers of plants being screened resulting from planting dates 1, 2, 5, and 6 may have biased the results from those dates. El-Tomi (3) reports that for spring planting in Oklahoma, an April 1 planting resulted in best establishment as measured by top and root growth. Results of this study support his finding. Relationship between good spring plant establishment and effective August screenig does exist but cannot be explained. Soil temperatures (Fig. 1) during period of soil-saturation (June 13-August 17) remained above 18 C. Wilkinson and Millar (13) report Pmm activity is affected more by changes in soil moisture than temperature at 18 C or above. Pmm activity during soil-saturation period should not have been a limiting

factor. Why the period of planting (April 1-16) results in the best August screening is unclear.

Early April plantings with August screening could facilitate completion of one selection cycle per year. Breeding material could be planted in early April, screened in August, selections transplanted in greenhouse and intercrossed September-March, seed harvested and planted to begin another selection cycle in April.

The objective of minimizing the time required for one cycle of selection for PRR resistance in alfalfa can be fulfilled by utilizing either or both selection cycles based on screening techniques discussed earlier. The screening technique based on fall planting and spring screening will likely be preferred in Oklahoma due to favorable fall climatic conditions conducive to stand establishment. In addition, this technique resulted in more consistent data. However, the availabiltiy of a screening technique utilizing spring planting and late summer screening adds flexibility to the breeding program in addition to allowing the breeder the opportunity to screen more material per calendar year.

LITERATURE CITED

1. Barnes, D. K., Frosheiser, F. I., Sorensen, E. L., Elgin, Jr., J. H., Nielson, M. W., Lehman, W. F., Leath, K. T., Ratcliffe, R. H., and Buker, R. J. 1974. Standard test to characterize pest resistance in alfalfa varieties. U.S. Dep. Agric. ARS NC-19. 23pp.

2. Bray, R. A., and Irwin, J. A. G. 1978. Selection for resistance to Phytophthora megasperma var. sojae in Hunter River lucerne. Aust. J. Exp. Agric. Anim. Husb. 18:708-713.

3. El-Tomi. O. A. 1982. Influence of sowing dates on establishment of alfalfa in Oklahoma. M.S. Thesis. Okla. State Univ. 50pp.

4. Erwin, D. C. 1954. Root rot of alfalfa caused by Phytophthora cryptogea. Phytopathology 44:700-704.

5. Erwin, D. C. 1966. Varietal reaction of alfalfa to Phytophthora megasperma and variation in virulence of the causal fungus. Phytopathology 56:653-657.

6. Faris, M. A., and Sabo, F. E. 1981. Effect of Phytophthora megasperma on yield and survival of resistant and susceptible alfalfa cultivars. Can. J. Plt. Sci. 61:955-960.

7. Frosheiser, F. I. 1980. Conquering Phytophthora root rot with resistant alfalfa cultivars. Plant Dis. 64:909-912.

8. Frosheiser, F. I., and Barnes, D. K. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:714-717.

9. Graham, J. H., Stuteville, D. L., Frosheiser, F. I., and Erwin, D. C. 1979. Phytophthora root rot. Pages 29-30 in: A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, Mn. 65pp.

10. Kuan, T. L., and Erwin, D. C. 1980. Formae speciales differentiation of Phytophthora megasperma isolates from soybeans and alfalfa. Phytopathology 70:333-338.

11. Kuan, T. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.

12. Marks, G. C., and Mitchell, J. E. 1970. Detection, isolation, and pathogenicity of Phytophthora megasperma from soils and estimation of inoculum levels. Phytopathology 60:1687-1690.

13. Wilkinson, H. T., and Millar, R. L. 1982. Effects of soil temperature and moisture on activity of Phytophthora megasperma f. sp. medicaginis and alfalfa root rot in the field. Phytopahtology 72:790-793.

Table 1. Phytophthora root rot average disease severity index (ASI) of four alfalfa cultivars planted October, 1981 and screened at six dates in 1982.

		Screening dates and ASI[v]						
Cultivar	PRR[w] reaction	4-1	4-22	5-13	6-3	6-24	7-15	Mean
Agate	R	2.33a[x]	2.52a	2.54a	2.50a	2.46a	3.33a	2.61a[y]
Apollo	R	2.43a	2.89a	2.33a	2.82a	2.88a	2.98a	2.72a
Vernal	S	3.58b	4.00b	3.80b	4.49b	4.42b	4.49b	4.13b
Arc	S	4.48c	4.85c	4.39c	4.64b	4.89b	4.98b	4.70c
Mean		3.20a[z]	3.57ab	3.26a	3.61b	3.66bc	3.94c	

[v]Average disease severity index; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 0.52.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.21.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.32.

Table 2. Percent Phytophthora root rot reistant plants of four alfalfa cultivars planted October, 1981 and screened at six dates in 1982.

		Screening dates and resistant plants (%)[v]						
Cultivar	PRR[w] reaction	4-1	4-22	5-13	6-3	6-24	7-15	Mean
Agate	R	74.1a[x]	69.1a	58.0b	68.1a	59.3a	37.7a	61.0a[y]
Apollo	R	73.3a	59.3a	73.5a	58.4a	46.4a	39.0a	58.3b
Vernal	S	45.3b	38.1b	33.0c	16.0b	9.1b	11.7b	25.5c
Arc	S	26.7c	20.5c	21.3c	10.3b	6.4b	1.9b	14.5d
Mean		54.8a[z]	46.7b	46.4b	38.2c	30.3d	22.6e	

[v]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 13.7.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 5.6.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 7.5.

Table 3. Phytophthora root rot average disease severity index (ASI) of four alfalfa cultivars planted September, 1982 and screened at eight dates in 1982-83.

		Screening dates and ASI[v]								
Cultivar	PRR[w] reaction	11-2	12-20	4-1	4-22	5-13	6-3	6-24	7-15	Mean
Agate	R	2.43a[x]	2.87a	2.52a	2.77a	2.69a	3.30b	3.04a	4.13a	2.97a[y]
WL-318	R	2.56a	2.83a	2.38a	2.90a	2.93a	2.81a	3.22a	3.94a	2.95a
Saranac	S	2.31a	3.00a	2.98ab	3.20a	3.59b	3.82bc	4.05b	4.60ab	3.44b
Arc	S	2.48a	2.99a	2.60a	3.08a	3.67b	3.52b	4.04b	4.10a	3.31b
Mean		2.44a[z]	2.92b	2.61a	2.99b	3.22c	3.36c	3.59d	4.19c	

[v]Average disease severity index; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 0.48.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.17.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.22.

Table 4. Percent Phytophthora root rot resistant plants of four alfalfa cultivars planted September, 1982 and screened at eight dates in 1982-83.

		Screening dates and resistant plants (%)[v]								
Cultivar	PRR[w] reaction	11-2	12-20	4-1	4-22	4-13	6-3	6-24	7-15	Mean
Agate	R	71.6a[x]	59.5a	77.6a	70.7a	64.7a	51.7a	54.4a	18.2a	58.5a[y]
WL-318	R	67.2a	58.4a	75.9a	63.7a	54.4a	60.7a	43.3b	14.0a	54.7a
Saranac	S	74.9a	55.5a	64.0b	60.5a	40.4b	36.7b	30.5c	9.0a	46.4b
Arc	S	67.9a	54.1a	71.1ab	59.9a	35.5b	39.0b	25.0c	13.6a	45.7b
Mean		70.4a[z]	56.9c	72.1a	63.7b	48.7d	47.0d	38.3e	13.7f	

[v]Percent resistant plants = % of plants in classes 1 and 2 based on a scale of 1-6; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 10.8.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 3.8.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 5.6.

Table 5. Phytophthora root rot average disease severity index (ASI) of four alfalfa cultivars screened August, 1982 resulting from six spring and summer 1982 planting dates.

		Planting dates and ASI[v]						
Cultivar	PRR[w] reaction	3-5	3-17	4-2	4-16	4-29	5-13	Mean
Agate	R	2.64a[x]	2.53a	2.91a	2.97a	2.05a	1.36ab	2.41a[y]
WL-318	R	3.07ab	3.39b	3.25a	3.40a	2.73b	1.96b	2.96b
Saranac	S	2.76a	3.39b	4.06b	3.60ab	2.42ab	1.27a	3.03b
Arc	S	3.46b	3.64b	3.99b	4.12b	2.80b	1.95b	3.21b
Mean		2.98b[z]	3.24bc	3.55bc	3.52bc	2.50b	1.64a	

[v]Average disease severity index; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 0.64.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.26.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.57.

Table 6. Percent Phytophthora root rot resistant plants of four alfalfa cultivars screened August, 1982 resulting from six spring and summer 1982 planting dates.

Cultivar	PRR[w] reaction	Planting dates and resistant plants (%)[v] 3-5	3-17	4-2	4-16	4-29	5-13	Mean
Agate	R	46.3a[x]	53.7a	46.7a	52.4a	80.6a	90.4a	61.7a[y]
WL-318	R	34.9ab	27.8a	39.5a	44.6a	63.4ab	77.3a	47.9b
Saranac	S	42.3a	26.1a	20.8b	41.1a	68.5a	80.1a	46.5b
Arc	S	22.0b	27.1a	17.7b	22.0b	59.9b	94.0a	40.4c
Mean		36.4c[z]	33.7c	31.2c	40.0c	68.1b	85.4a	

[v]Percent resistant plants = % of plants in classes 1 and 2 based on a scale of 1-6; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 16.9.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 6.9.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 13.2.

Table 7. Phytophthora root rot average disease severity index (ASI) of four alfalfa cultivars screened August, 1983 resulting from six spring and summer 1983 planting dates.

		Planting dates and ASI[v]						
Cultivar	PRR[w] reaction	3-5	3-17	4-2	4-16	4-29	5-13	Mean
Agate	R	2.61a[x]	2.35a	2.58a	3.05a	2.64a	2.43a	2.61a[y]
WL-318	R	2.79ab	2.59a	2.77ab	3.35a	2.52a	2.74a	2.79b
Saranac	S	2.99ab	2.96ab	3.14ab	3.67ab	2.88a	2.64a	3.05c
Arc	S	3.30b	3.22b	3.20b	3.98b	2.91b	2.86a	3.24d
Mean		2.92a[z]	2.78a	2.92a	3.51b	2.74a	2.67a	

[v]Average disease severity index; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 0.43.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.17.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 0.28.

Table 8. Percent Phytophthora root rot resistant plants of four alfalfa cultivars screened August, 1983 resulting from six spring and summer 1983 planting dates.

		Planting dates and resistant plants (%)[v]						
Cultivar	PRR[w] reaction	3-5	3-17	4-2	4-16	4-29	5-13	Mean
Agate	R	61.1a[x]	76.3a	61.2a	49.7a	69.3a	63.9a	63.6a[y]
WL-318	R	44.2b	55.8b	49.6b	37.6b	69.4a	37.6c	52.4b
Saranac	S	45.7b	48.6b	37.4c	32.0bc	56.1b	49.8b	44.9c
Arc	S	33.4c	29.1c	37.6c	24.0c	56.4b	52.2b	38.6d
Mean		45.8bc[z]	52.4ab	46.4bc	35.8c	62.8a	56.0ab	

[v]Percent resistant plants = % of plants in classes 1 and 2 based on a scale of 1-6; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at the 0.05 level; LSD= 10.7.

[y]Means followed by same letter are not significantly different at the 0.05 level; LSD= 6.0.

[z]Means followed by same letter are not significantly different at the 0.05 level; LSD= 14.8.

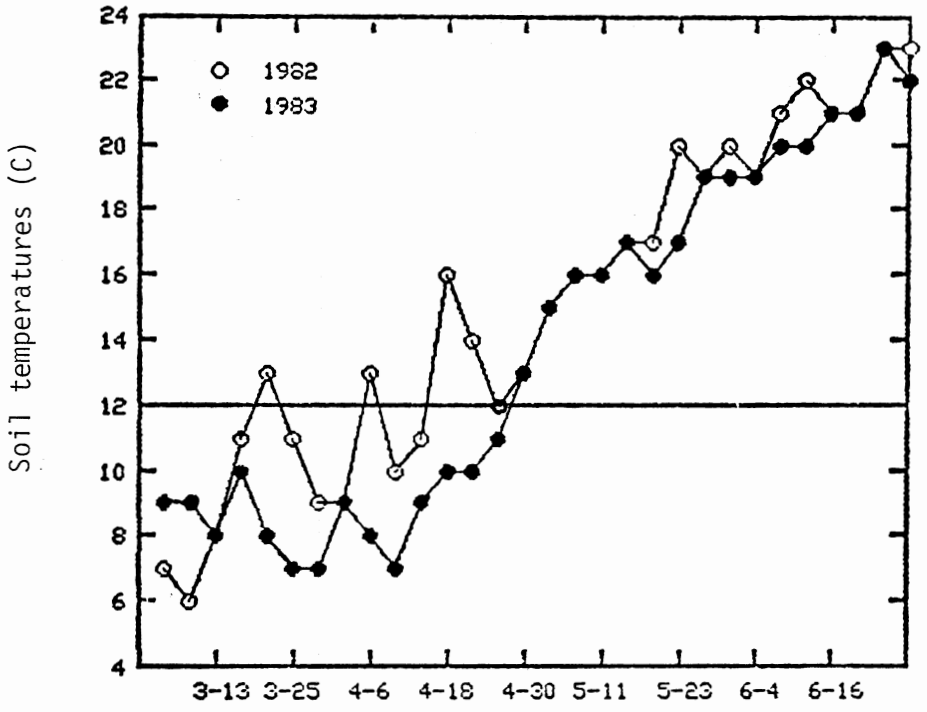

Figure 1. Mean weekly soil temperatures (C) at the 15 cm depth from March 1 through June 30, 1982 and 1983.

CHAPTER III

Greenhouse Studies of Phytophthora Root Rot Resistance Screening Techniques in Alfalfa

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ABSTRACT

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Greenhouse studies were conducted to develop a simple, reliable screening technique for Phytophthora root rot (PRR) resistance in alfalfa (Medicago sativa L.). Disease index and percent resistant plants of two PRR resistant (Agate, Wl-318) and two susceptible (Saranac, Arc) cultivars maintained under saturated soil conditions were used as indicators of screening effectiveness. Five soil treatments of varying percentages of Phytophthora megasperma f. sp. medicaginis Kuan and Erwin (Pmm) naturally infested field soil (1%, 3%, 10%, stratified (steamed sand layer over field soil), and 100% field soil) were evaluated as media for screening.

Results indicate that 1% Pmm naturally infested field soil by volume in steamed sand gave best results in screening for differentiating between PRR resistant and susceptible cultivars. This technique offers

a simple alternative to use of artificially cultured Pmm isolates as inoculum source. More work is needed to perfect the screening procedure to obtain optimum screening results.

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Phytophthora root rot (PRR) of alfalfa (Medicago sativa L.) caused by the fungus Phytophthora megasperma f. sp. medicaginis Kuan and Erwin (Pmm), (11) is a major factor in stand decline (3). PRR occurs in nearly every area of the world where alfalfa is grown (8).

Zoospores of Pmm sporangia are motile in free soil water and are attracted to alfalfa roots where they encyst, germinate, and penetrate the cells in the zone of cell extension, thus initiating the root rot infection process (7). MacDonald and Duniway (13) reported that fine-textured soil may reduce the ability of the spores to swim through such soils. However, water-saturated soil conditions predispose alfalfa roots to Pmm through increasing root damage and through increased exudation of nutrients that increases the attraction of zoospores to the roots (12).

Cardinal temperatures for growth of Pmm in culture are reported to be: minimum, 8 C; optimum 25 C; maximum 30-33 C (3). Erwin (4) reported severity of PRR damage in greenhouse tests was similar at 17, 21, 24, and 27 C, and was slightly less at 30 C. Wilkinson and Millar (18) reported Pmm activity was affected more by changes in soil moisture than by temperature when soil temperature was 18 C or above.

Marks and Mitchell (14) described a baiting technique for detecting and isolating Pmm from naturally infested soils using 3-day-old alfalfa seedlings. Pratt and Mitchell (16) used the same baiting technique to determine the surivial of Pmm in field soils and found Pmm remained infective in naturally infested soils stored for 3.5 years at 25 C. They also reported growth of susceptible cultivars in infested soils resulted in increased infective activity.

Host plant resistance was first reported in 1966 (4). Resistance is increased in breeding strains by recurrent mass selection cycles (i.e., plant - screen material - intercross selections - harvest seed and repeat cycle). Starting populations with less than 10% resistant plants have been increased to 63% resistant plants after three cycles of selection (6). In Canadian field trials, plant losses of PRR resistant cultivars averaged only 21% compared to 44% for susceptible cultivars, while yield reduction was 21% and 55%, respectively.

Greenhouse techniques have been developed to screen alfalfa populations to identify resistant plants. All techniques described were similar in that Pmm isolates were artificially cultured and used to inoculate seedlings grown in controlled environments (1,2,6,9,10,15,17). A standard greenhouse test is described by Barnes et al (1) in which seed is planted directly into Pmm infested sand in water-tight containers. The seedlings are watered sparingly for about 4 weeks. Then, drain-holes in the container are plugged and water is added daily to raise the water level to the surface. Sand temperature is maintained at 20-24 C. After 4 weeks, plant roots are evaluated for PRR symptoms (1). Field and greenhouse evaluations were correlated ($r = 0.99$ and 0.95) in two tests (6). Hohrein et al (10) added Pmm inoculum to 12-day-old seedlings, saturated soil for 3 days, and screened 18 days later. Frosheiser and Barnes (6) inoculated 14-day-old seedlings, saturated the soil for 2-3 weeks, and then screened. Rogers et al (17) inoculated 12-week-old seedlings, flooded soil for 6 weeks, and then screened. Miller et al (15) planted seeds in artificially infested soil. After 3 days, soil was saturated for 1 week and plants screened.

All reported techniques rely on artificially cultured Pmm isolates as a source of inoculum. Little work has been done on using Pmm naturally infested soil as a source of inoculum for greenhouse screening purposes. The purpose of this paper is to report the results of using varying percentages of Pmm naturally infested field soils for screening for PRR resistance in greenhouse tests.

MATERIALS AND METHODS

Three greenhouse benches (2.4 X 0.9 X 0.2 m) with metal bottoms and wooden sides were partitioned into four sections each (0.6 X 0.9 X 0.2 m). Ten holes (1 cm dia.) were drilled in the bottom of each section. Each section bottom was lined with a 2 cm layer of gravel (6 cm dia.) to promote drainage. Soil known to be naturally infested with Pmm collected from the Agronomy Research Station, Stillwater, Ok. and passed through a 9-mesh screen was used as Pmm inoculum source throughout studies.

Four soil treatments were evaluated during study one (steamed field soil (check treatment), 1% infested field soil, 10% infested field soil, 100% infested field soil). Soil treatments of 1% and 10% infested field soil were thoroughly mixed with sand steamed at 95 C for 8 hours at ambient pressure to obtain volumes equal to other soil treatments. Fertilizer (N, P, K) and lime were mixed with individual soil treatments as needed on the basis of soil analysis results.

Each of the four sections per bench were filled with equal quantity (0.1 m^3) of soil treatment. One hundred viable seeds of each of two known PRR resistant (Agate, WL-318) and two susceptible (Saranac, Arc) cultivars (5) were planted in 90 cm long rows (two rows/cultivar, two border rows).

Study one. Seeds were planted 6 February 1982. Plant counts were taken 16 February 1982. Plants were clipped 8 April 1982. Beginning 10 April 1982, all treatments were irrigated daily to maintain soil saturation. Periodic checks of PRR disease development were made by uprooting plants of resistant and susceptible cultivars in border rows. All

plants were uprooted and evaluated for PRR symptoms on 14 June 1982. Soil treatment temperatures ranged from 10-20 C.

Study two. Study one was repeated with the following revisions; 1) steamed field soil treatment was replaced with 50% steamed field soil and steamed sand, 2) 10% infested field soil treatment was replaced with 3% infested field soil, 3) quantity of unsteamed field soil was halved, and an equal amount of steamed sand was placed over the field soil (stratified), and 4) one hundred fifty viable seeds were planted per row. Seeds were planted 13 July 1982 and plant counts taken 1 week later. All treatments were irrigated daily beginning 8 August 1982. Plants were clipped 8 September 1982, uprooted and evaluated for PRR symptoms on 14 September 1982. Soil treatment temperatures ranged from 20-30 C.

Study three. Study two was repeated with a 9 October 1982 planting date. Plant counts were made 18 October, plants were clipped and daily irrigation began 9 November 1982. Plants were uprooted and evaluated 9 December 1982. Soil treatment temperatures ranged from 15-25 C.

Study four. Study three was repeated with a 7 January 1983 planting date. Plant counts were taken 18 January, and plants were clipped 17 February, 17 March, and 1 April 1983. Daily irrigation began 2 April and plants were uprooted and evaluated 10 May 1983. Soil treatment temperatures ranged from 15-30 C.

Study five. Study four was repeated with a 22 May 1983 planting date. Plant counts were taken 31 May, and daily irrigation began 22 August 1983. All plants were uprooted and evaluated for PRR symptoms on 22 September 1983. Soil treatment temperatures ranged from 20-30 C.

Split-plot designs were used for all studies with soil treatments as main plots in randomized complete blocks and four cultivars as subplots with three replications. Evaluation of individual plant roots for PRR symptoms was based on a 6-class scale (1= no symptoms, 6= dead plants) with plants in classes 1 and 2 considered resistant (6). An average disease severity index (ASI) was calculated for each subsample by use of the following formula;

$$\frac{\text{Summation(Class No. X \% in class)}}{100}$$

Significant differences between resistant and susceptible cultivars for PRR severity, as expressed in ASI's and percent resistant plants, were used as indicators of screening effectiveness in each soil treatment.

RESULTS AND DISCUSSION

Significant ($P<0.05$) differences were detected among soil treatments for ASI and percent resistant plants means of all cultivars in studies 1 and 2 (Tables 1,2). Significant ($P<0.05$) differences for ASI means were detected among cultivars in studies 1, 2, 4, and 5 (Tables 1,2,4,5). Significant differences for percent resistant plants among cultivars were detected only in study 1 (Table 1). No significant soil treatment X cultivar interaction for ASI was detected throughout these studies. Significant soil treatment X cultivar interaction for percent resistant plants was detected only in study 3.

Significant differences among cultivars for ASI and percent resistant plants were detected within various soil treatments throughout these studies (Table 1-5). However, differences detected among cultivars in four soil treatments (FS, 3%, 10%, and STRAT.) are neither consistent nor useable in a screening procedure due to non-differentiation of cultivar types (resistant, susceptible). Severe PRR damage of resistant cultivars (Agate, WL-318), as indicated by high ASI's and low percent resistant plants, is evident in studies 2-5 in soil treatments containing Pmm infested field soil (Tables 2-5). The severe PRR damage obtained may be explained by the prolonged exposure to optimum conditions of Pmm infestation and disease development under which the plants were grown. Soil treatment temperatures varied considerably within studies, and from study to study, but remained within the range of temperatures (17-30 C) at which Pmm is active. These conditions may have enabled Pmm to overcome the resistance of Agate and WL-318. Frosheiser (7) reported PRR resistance does not confer immunity, and any plant may succumb to Pmm attack under certain conditions. Cooler soil

temperatures (10-20 C) in study 1 may have suppressed Pmm activity resulting in increased percent resistant plants in the 1% infested field soil treatment compared to results obtained in the same treatment in subsequent studies

Soil treatment consisting of 1% infested field soil mixed with 99% steamed sand (1%) resulted in best separation of cultivar types (resistant, susceptible) for ASI and percent resistant plants in studies 1, 3, and 4 (Tables 1,3,4). Even at this low percentage of Pmm infested field soil, PRR damage appears to be too severe for effective screening due to prolonged duration of tests under optimum conditions for PRR development.

It is interesting to note no significant differences appeared in ASI means of all cultivars among the 1%, 3% and stratified field soil (STRAT.) treatments in studies 2-5 (Tables 2-5). Pmm contamination of the check treatments (SFS/SS) in studies 4 and 5 resulted in no significant differences among soil treatments in those studies (Tables 4,5).

Bray and Erwin (2) reported greenhouse PRR screening of alfalfa is generally more severe than under field conditions. Results of these studies confirm their finding as indicated by high ASI's and low percent resistant plants obtained for all cultivars (Tables 2-5).

Greenhouse screening for PRR resistance in alfalfa utilizing Pmm naturally infested field soil as an inoculum source can be used as a simple alternative to artificially cultured Pmm isolate inoculum. In addition to ease of handling and preparing Pmm naturally infested field soil screening media, possibility of truncation selection for resistance to other naturally-occuring soilborne pests does exist. Soil treatment consisting of as little as 1% Pmm naturally infested field soil by

volume resulted in adequate PRR disease development for PRR resistance screening purposes. More work is needed in the areas of soil temperature control and duration of screening time to perfect the technique.

LITERATURE CITED

1. Barnes, D. K., Frosheiser, F. I., Sorensen, E. L. , Elgin, Jr., J. H., Nielson, M. W. , Lehman, W. F., Leath, K. T., Ratcliffe, R. H., and Buker, R. J. 1974. Standard test to characterize pest resistance in alfalfa varieties. U.S. Dep. Agric. ARS NC-19. 23pp.

2. Bray, R. A., and Irwin, J. A. G., 1978. Selection for resistance to Phytophthora megasperma var. sojae in Hunter River lucerne. Aust. J. Exp. Agric. Anim. Husb. 18:708-713.

3. Erwin, D. C. 1954. Root rot of alfalfa caused by Phytophthora cryptogea. Phytopathology 44:700-704.

4. Erwin, D. C. 1966. Varietal reaction of alfalfa to Phytophthora megasperma and variation in virulence of the causal fungus. Phytopathology 56:653-657.

5. Faris, M. A., and Sabo, F. E. 1981. Effect of Phytophthora megasperma on yield and survival of resistant and susceptible alfalfa cultivars. Can. J. Plt. Sci. 61:955-960.

6. Frosheiser, F. I., and Barnes, D. K. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.

7. Frosheiser, F. I. 1980. Conquering Phytophthora root rot with resistant alfalfa cultivars. Plant Dis. 64:90-912.

8. Graham, J. H., Stuteville, D. L., Frosheiser, F. I., and Erwin, D. C. 1979. Phytophthora root rot. Pages 29-30 in: A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, Mn. 65pp.

9. Gray, F. A., Hine, R. B., Schonhorst, M. H., and Naik, J. D. 1973. A screening technique useful in selecting for resistance in alfalfa to Phytophthora megasperma. Phytopathology 63:1185-1188.

10. Hohrein, B. A., Bean, G. A., and Graham, J. H. 1981. A rapid method for screening alfalfa for resistance to Phytophthora megasperma. Phytopathology (Abstr.) 71:76.

11. Kuan, T. L., and Erwin, D. C. 1980. Formae speciales differentiation of Phytophthora megasperma isolates from soybeans and alfalfa. Phytopathology 70:333-338.

12. Kuan, R. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.

13. MacDonald, J. D., and Duniway, J. M. 1978. Influence of soil texture and temperature on the motility of Phytophthora cryptogea and P. megasperma zoospores. Phytopathology 68:1627-1630.

14. Marks, G. C., and Mitchell, J. E. 1970. Detection, isolation, and pathogenicity of Phytophthora megasperma from soils and estimation of inoculum levels. Phytopathology 60:1687-1690.

15. Miller, S. A., Irwin, J. A. G., and Maxwell, D. P. 1979. An alfalfa seedling assay for detection of resistance to Phytophthora megasperma. Phytopathology (Abstr.) 69:1039.

16. Pratt, R. G., and Mitchell, J. E. 1975. The survival and activity of Phytophthora megasperma in naturally infested soils. Phytopathology 65:1267-1272.

17. Rogers, V. E., Irwin, J. A. G., and Stovold, G. 1978. The development of lucerne with resistance to root rot in poorly aerated soils. Aust. J. Exp. Agric. Anim. Husb. 18:434-441.

18. Wilkinson, H. T., and Millar, R. L. 1982. Effects of soil temperature and moisture on activity of Phytophthora megasperma f. sp. medicaginis and alfalfa root rot in the field. Phytopathology 72:790-793.

Table 1. Phytophthora root rot average disease severity index (ASI) and percent resistant plants of four alfalfa cultivars grown 4 months in four soil treatments (study 1).

		Soil treatments[q]				
	PRR[s]	ASI[r]				
Cultivars	reaction	SFS	1%	10%	FS	Mean
Agate	R	2.65a[t]	2.57a	4.86a	4.70a	3.70a[u]
WL-318	R	3.58b	2.82a	5.06ab	5.08ab	4.14b
Saranac	S	3.57b	3.17ab	5.52b	5.30b	4.39b
Arc	S	3.37b	3.54b	5.31ab	5.23ab	4.36b
Mean		3.29a[v]	3.03a	5.19b	5.07b	
		Percent resistant plants[w]				
Agate	R	65.5b[x]	68.0b	14.5a	12.9a	40.2c[y]
WL-318	R	46.1a	63.0ab	14.1a	11.8a	33.8b
Saranac	S	46.9a	54.8a	5.9a	5.3a	28.2a
Arc	S	51.2a	49.4a	7.9a	8.3a	29.2ab
Mean		52.4b[z]	58.8b	10.6a	9.6a	

[q]Soil treatments; SFS= steamed field soil, 1%= 1% infested field soil, 10%= 10% infested field soil, FS= 100% infested field soil.

[r]Average disease severity index; 1= no symptoms, 6= dead plants.

[s]PRR reaction; R= resistant; S= susceptible.

[t]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 0.50.

[u]Means followed by same letter are not significantly different at 0.05 level; LSD= 0.25.

[v]Means followed by same letter are not significantly different at 0.05 level; LSD= 0.62.

[w]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[x]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 10.4.

[y]Means followed by same letter are not significantly different at 0.05 level; LSD= 5.2.

[z]Means followed by same letter are not significantly different at 0.05 level; LSD= 14.1.

Table 2. Phytophthora root rot average disease severity index (ASI) and percent resistant plants of four alfalfa cultivars grown 2 months in four soil treatments (study 2).

		Soil treatments[r]				
		ASI[s]				
Cultivar	PRR[s] reaction	SFS/SS	1%	3%	STRAT.	Mean
Agate	R	1.98a[u]	4.09a	4.23b	3.35a	3.41b[v]
WL-318	R	1.83a	4.00a	4.31b	3.18a	3.33ab
Saranac	S	1.83a	3.98a	3.78a	3.27a	3.21a
Arc	S	1.94a	4.11a	4.20b	3.35a	3.40b
Mean		1.89a[w]	4.05b	4.13b	3.29b	
		Percent resistant plants[x]				
Agate	R	80.5a[y]	12.7a	20.2a	21.3a	33.7 NS
WL-318	R	83.4a	7.2a	24.1a	19.0a	33.4 NS
Saranac	S	83.5a	10.0a	30.1a	23.4a	36.7 NS
Arc	S	81.2a	11.7a	28.4a	23.6a	36.2 NS
Mean		82.1b[z]	10.4a	25.7a	21.8a	

[r]Soil treatments; SFS/SS= steamed field soil and steamed sand, 1%= 1% infested soil, 3%= 3% infested field soil, STRAT.= infested field soil topped with steamed sand layer.

[s]Average disease severity index; 1= no symptoms, 6= dead plants.

[t]PRR reaction; R= resistant; S= susceptible.

[u]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 0.35.

[v]Means followed by same letter are not significantly different at 0.05 level; LSD= 0.17.

[w]Means followed by same letter are not significantly different at 0.05 level; LSD= 1.06.

[x]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[y]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 6.9.

[z]Means followed by same letter are not significantly different at 0.05 level; LSD= 31.2.

Table 3. Phytophthora root rot average disease severity index (ASI) and percent resistant plants of four alfalfa cultivars grown 2 months in four soil treatments (study 3).

Cultivar	PRR[w] reaction	Soil treatment[u]				
		ASI[v]				
		SFS/SS	1%	3%	STRAT.	Mean
Agate	R	1.93ab[x]	3.32ab	3.26ab	4.16a	3.17 NS
WL-318	R	1.74a	3.06a	3.44b	4.29a	3.13 NS
Saranac	S	1.97ab	3.75bc	2.89a	4.08a	3.17 NS
Arc	S	2.26b	3.85c	3.47b	4.46a	3.51 NS
Mean		1.97NS	3.49NS	3.26NS	4.24NS	
		Percent resistant plants[y]				
Agate	R	78.9ab[z]	30.1ab	36.3a	6.7ab	38.0 NS
WL318	R	82.1b	35.8b	36.7a	3.4a	39.5 NS
Saranac	S	77.3ab	21.6a	50.0b	13.3b	40.5 NS
Arc	S	70.7a	29.9a	38.7a	4.7ab	36.0 NS
Mean		77.2NS	29.3NS	40.4NS	7.0NS	

[u]Soil treatments; SFS/SS= steamed field soil and steamed sand, 1%= 1% infested soil, 3%= 3% infested field soil, STRAT.= infested field soil topped with steamed sand layer.

[v]Average disease severity index; 1= no symptoms, 6= dead plants.

[w]PRR reaction; R= resistant; S= susceptible.

[x]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 0.49.

[y]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[z]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 9.0.

Table 4. Phytophthora root rot average disease severity index (ASI) and percent resistant plants of four alfalfa cultivars grown 2 months in four soil treatments (study 4).

Cultivar	PRR[v] reaction	Soil treatments[t]				
		ASI[u]				
		SFS/SS	1%	3%	STRAT.	Mean
Agate	R	3.61a[w]	4.17a	4.04b	3.99a	3.95a[x]
WL-318	R	3.82a	4.25a	3.35a	3.97a	3.84a
Saranac	S	3.71a	4.87b	4.27a	4.17a	4.25b
Arc	S	4.08a	4.76b	4.38b	4.26a	4.37b
Mean		3.81NS	4.51NS	4.01NS	4.10NS	
		Percent resistant plants[y]				
Agate	R	41.0a[z]	13.0a	13.5a	20.4a	22.0 NS
WL-318	R	35.1a	16.3a	29.5b	19.5a	25.1 NS
Saranac	S	36.2a	7.9a	15.3a	17.7a	19.3 NS
Arc	S	33.4a	8.9a	14.5a	17.7a	18.6 NS
Mean		36.4NS	11.5NS	18.2NS	18.8NS	

[t]Soil treatments; SFS/SS= steamed field soil and steamed sand, 1%= 1% infested field soil, 3%= 3% infested field soil, STRAT.= infested field soil topped with steamed sand layer.

[u]Average disease severity index; 1= no symptoms, 6= dead plants.

[v]PRR reaction; R= resistant; S= susceptible.

[w]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 0.48.

[x]Means followed by same letter are not significantly different at 0.05 level; LSD= 0.24.

[y]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[z]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 9.4.

Table 5. Phytophthora root rot average disease severity index (ASI) and percent resistant plants of four alfalfa cultivars grown 4 months in four soil treatments (study 5).

Cultivar	PRR[v] reaction	Soil treatments[t] ASI[u] SFS/SS	1%	3%	STRAT.	Mean
Agate	R	3.57a[w]	3.79a	4.38a	4.11ab	3.96a[x]
WL-318	R	3.55	4.32b	4.31a	3.96a	4.03a
Saranac	S	3.66a	4.27b	4.37a	3.92a	4.06a
Arc	S	3.87a	4.48b	4.60a	4.42b	4.34b
Mean		3.66NS	4.22NS	4.41NS	4.10NS	
		Percent resistant plants[y]				
Agate	R	29.8ab[z]	15.6a	0.0a	10.9ab	14.1 NS
WL-318	R	34.6b	12.0a	0.0a	9.9ab	14.1 NS
Saranac	S	29.3ab	14.1a	3.2a	15.3b	15.5 NS
Arc	S	26.3a	9.2a	0.0a	7.5a	10.8 NS
Mean		30.0NS	12.7NS	0.8NS	10.9NS	

[t]Soil treatments; SFS/SS= steamed field soil and steamed sand, 1%= 1% infested field soil, 3%= 3% infested field soil, STRAT.= infested field soil topped with steamed sand.

[u]Average disease severity index; 1= no symptoms, 6= dead plants.

[v]PRR reaction; R= resistant; S= susceptible.

[w]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 0.35.

[x]Means followed by same letter are not significantly different at 0.05 level; LSD= 0.18.

[y]Percent resistant plants = % of plants in classes 1 and 2 based on scale of 1-6; 1= no symptoms, 6= dead plants.

[z]Means in same column followed by same letter are not significantly different at 0.05 level; LSD= 6.5.

CHAPTER IV

SUMMARY AND CONCLUSION

The objectives of this research were to identify efficient Phytophthora root rot (PRR) field and greenhouse screening techniques to minimize the time required for one cycle of selection in alfalfa (Medicago sativa L.). An average disease index (ASI) and percent resistant plants of two PRR resistant and two susceptible cultivars maintained under saturated soil conditions were used as indicators of screening effectiveness. In two field studies, (1981, 1982) the effects of six and eight screening dates (Nov. 2 through July 15) on PRR observations of a fall-planted nursery were evaluated. In two separate studies, (1982, 1983) the effects of six spring planting dates (March 5 through May 13) were evaluated with an August screening. Split-plot designs were used for all studies with screening, (or planting) dates as main plots in randomized complete blocks, and four cultivars as subplots with six replications. In greenhouse studies, the effects of using five soil treatments of varying percentages of Phytophthora megasperma f. sp. medicaginis Kuan and Erwin (Pmm) naturally infested field soil as sources of inoculum for greenhouse screening purposes were evaluated.

In the first date of screening field study (study 1), cultivars planted October, 1981 showed significant differences in mean ASI and percent resistant plants among screening dates. Differences among cultivar types for ASI mean and percent resistant plants were significant within each screening date even at the first date of screening. Magnitude of ASI's

were similar to those obtained in other breeding programs. No significant date X cultivar interaction was detected. Soil temperatures for 1982 revealed 5 consecutive days of temperatures at or above the 12 C Pmm activity threshold 2 weeks prior to the first screening date.

In study 2, cultivars planted September, 1982 showed no significant differences until the May 13 screening date. Mean of all cultivars at that date was significantly different from those of preceeding dates. No significant date X cultivar interaction was detected. Soil temperatures for 1983 did not exceed the 12 C Pmm activity threshold until about 2 weeks prior to the May 13 screening date. By July 15, ASI means for the resistant cultivars were too high to distinguish from the susceptible cultivars.

In the first date of planting study (study 3), an August, 1982 screening produced no useable significant differences among ASI means and percent resistant plants of the six spring and summer 1982 planting dates. Only the April 2 planting date produced significant differences between cultivar types (resistant, susceptible) when screened in August. In study 4, the ASI mean for the April 16 planting date was significantly different from other planting dates when cultivars were screened in August. No significant cultivar X date interaction was detected.

Results from the greenhouse studies showed significant differences for ASI means and percent resistant plants of all cultivars among the soil treatments in studies 1 and 2. Significant differences for ASI means among cultivars were detected in studies 1, 2, 4, and 5. Significant differences for percent resistant plants among cultivars were detected only in study 1. No significant soil treatment X cultivar

interaction for ASI was detected throughout studies. Significant differences among cultivars were detected within the FS, 3%, 10%, and STRAT. soil treatments throughout studies, but differences detected were neither consistent nor useable in a screening procedure due to non-differentiation of PRR cultivar types (resistant, susceptible). Soil treatment consisting of 1% infested field soil by volume mixed with 99% steamed sand resulted in best separation of cultivar types (resistant, susceptible) in studies 1, 3, and 4. Significant differences among cultivars were not detected in study 2.

From the above results some conclusions may be summarized as follows:

1. Effective PRR screening of a fall planted nursery can be obtained by mid-May even under cooler soil temperatures.
2. An early April planting resulted in the most effective August PRR screening.
3. Both screening techniques can be used to complete one cycle of selection for PRR resistance per year.
4. The screening technique based on fall planting and spring screening will likely be preferred in Oklahoma due to favorable fall climatic conditions conducive to stand establishment, in addition to producing more consistent data.
5. Pmm naturally infested field soil can be used as a simple alternative for inoculum source when screening in the greenhouse.
6. A mixture of 1% Pmm infested field soil by volume in 99% steamed sand gave best results in differentiating between resistant and susceptible cultivars.

To facilitate further studies, some suggestions are summarized as

follows:

1. Monitoring of soil temperatures during late fall and early spring should be a requirement for screening techniques under Oklahoma conditions to better judge when screening would be most effective.
2. More work in the areas of soil temperature control and duration of screening time is needed to perfect screening for PRR resistance in Pmm naturally infested soil treatments in the greenhouse.

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