Distribution of Phytophthora Root Rot of Alfalfa in Central Mexico and Development of Disease Resistance in Mexican Cultivars of Alfalfa

R. J. AGUIRRE, Graduate Student, Department of Plant Sciences; R. B. HINE, Professor, Department of Plant Pathology; and M. H. SCHONHORST, Professor, Department of Plant Sciences, University of Arizona, Tucson 85721

ABSTRACT

Aguirre, R. J., Hine, R. B., and Schonhorst, M. H. 1983. Distribution of Phytophthora root rot of alfalfa in central Mexico and development of disease resistance in Mexican cultivars of alfalfa. Plant Disease 67:91-94.

Surveys during the summers of 1977 and 1978 in central Mexico (18–22° N latitude) indicated that root rot of alfalfa (*Medicago sativa*), caused by *Phytophthora megasperma* f. sp. *medicaginis*, was widespread in irrigated fields at elevations between 1,600 and 2,000 m in the states of Aguascalientes, Estado de Mexico, Guanajuato, Hidalgo, Michoacan, and Queretaro, Mexico. The pathogen was recovered from eight of 28 soil samples taken from infested fields by use of an alfalfa seedling-bait technique and the antibiotics pimaricin and hymexazol. Observations in the field and pathogenicity studies in the greenhouse demonstrated that all commonly planted alfalfa cultivars in Mexico, including Moapa, Mesa-Sirsa, and Joaquin 11 (all from the United States), Aragon (from Spain), and INIA-76, Bajio-76, Puebla-76, and Mixteca-76 (from Mexico), were susceptible to the pathogen. The level of resistance to the pathogen in the widely planted Mexican cultivar INIA-76 increased from 5.6 to 27.8% survival levels following two cycles of phenotypic recurrent selection. Two isolates of *P. megasperma* f. sp. *medicaginis* from central Mexico were similar to each other in pathogenicity but differed from the typical isolate from Arizona.

Alfalfa (*Medicago sativa* L.) was introduced into Mexico in the 16th century by the Spaniards. Today, approximately 200,000 ha are grown in Mexico with major production (100,000 ha) concentrated at elevations above 1,600 m and between 18 to 20° north latitude in central Mexico. An ideal climate exists for the growth of cultivars of alfalfa throughout the year in this area, and many fields are cut as frequently as 10 times each year. Preliminary field observations showed that root rot caused by *Phytophthora megasperma* Drech. f. sp. *medicaginis* was a significant problem in stand establishment and persistence.

The disease affected all commonly planted cultivars including Moapa, Mesa-Sirsa, Joaquin 11 (all from the United States), and Aragon (from Spain), and local varieties such as INIA-76, Bajio-76, Puebla-76, and Mixteca-76.

Because the disease is important and because it has not been described previously in Mexico, surveys were made during the summers of 1977 and 1978 to determine the distribution and significance of Phytophthora root rot as well as to seek out other important soilborne fungal pathogens in the major alfalfa areas in central Mexico.

In this report we present the results of these surveys and outline the research done to find resistance to Phytophthora root rot in Mexican cultivars of alfalfa. An abstract of some of this work was published previously (1).

Portion of a Ph.D. thesis by the senior author. Journal Series Paper No. 3518 of the Arizona Agricultural Experiment Station, Tucson.

The first author wishes to thank the University of Arizona, Consejo Nacional de Ciencia y Tecnología (CONACYT), and Instituto Nacional de Investigaciones Agrícolas (INIA), all of whom contributed to the successful completion of this dissertation.

Accepted for publication 30 May 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/83/01009104/$03.00/0

MATERIALS AND METHODS

Distribution of Phytophthora root rot of alfalfa in central Mexico. Approximately 45,000 ha of alfalfa in central Mexico were surveyed for the occurrence of Phytophthora root rot and the occurrence of other soilborne pathogens during the summers of 1977 and 1978 in the states of Aguascalientes (Pabellón); Estado de México (Texcoco); Guanajuato (Abasolo, Celaya, El Refugio, San Juan, San Miguel de Allende); Hidalgo (Actuopan); Michoacan (Antunes, La Pieded, Yurecuaro, Zacapu); and Queretaro (Cadereyta, El Colorado, Queretaro). Locations consisted of irrigated and unirrigated fields ranging from 400 to 2,600 m altitude at approximately 18–22° north latitude in central Mexico. Plants with typical aboveground symptoms were removed from the soil, and taproots were examined for lesions typical of Phytophthora root rot. Isolations were made from infected roots and pure cultures obtained by use of a hymexazol and pimaricin medium described by Tsao (12).

Soil samples were collected from areas adjacent to infected taproots of alfalfa, placed in plastic bags, and taken to the Department of Plant Pathology, University of Arizona, Tucson. Soil texture was determined by the hydrometer method of particle-size analysis. *P. megasperma* f. sp. *medicaginis* was isolated from the soil samples using an alfalfa seedling-bait technique (9,10).

The geographical distribution of the disease, elevation, and latitude of the selected fields, cropping history, cultivar, irrigation practices, and soil textures were recorded.

Pathogenicity and Phytophthora root rot resistance studies. Three isolates of *P. megasperma* f. sp. *medicaginis* from soil, infected taproots of alfalfa from central Mexico, and one isolate from Arizona were maintained on V-8 agar (100 ml Campbell's V-8 juice, 900 ml of distilled water, and 20 g Difco agar). Inoculum was produced by transferring mycelium to 500-ml flasks containing 100 ml of sterilized V-8 liquid medium (100 ml of V-8 juice and 900 ml of distilled water per liter). Flasks were stored as standing cultures at room temperature for 4–5 wk to allow growth of the fungus. Mycelial mats were washed three times in 500 ml distilled water and comminuted in a Waring Blendor. Inoculum used in all

tests consisted of aqueous suspensions of oospores and mycelial fragments. Inoculum levels were diluted with water until light transmittance, using a Spectronic 20 spectrophotometer (550 nm), was 27, 80, or 97% to correspond to "high," "medium," and "low" inoculum levels, respectively. In some studies, oospore counts were made with a Neubauer hemacytometer. The fungal inoculum was mixed 1:4 with water. A 5-L volume was then thoroughly mixed into 35 kg of air-dry, pasteurized soil (Gila silt loam-sand 1:1, v/v).

Infested soil was then placed in greenhouse flats (60 × 40 × 8 cm high). Each flat (containing six rows, each row considered a plot) was seeded with 100 seeds of an alfalfa cultivar. The tests were conducted in a greenhouse at 20–25 C under natural light.

In some studies, the irrigation frequency was adjusted to increase seedling survival, particularly with the high inoculum level. The twice-daily saturation of the flats (which were allowed to drain after each watering) was terminated 2 wk after seeding; thereafter, flats were irrigated once daily until completion of the test. Basic procedures used in these studies were similar to those described by Gray et al (4).

Initial screening tests involved four Mexican cultivars, Bajio-76, INIA-76, Mixteca-76, and Puebla-76, as well as two cultivars from Arizona, Hayden, and Hayden PX-III, which was resistant to the pathogen. The experimental design was a randomized block composed of eight replicates with a high level of inoculum. A strain of the fungus from Mexico (labeled Bajio), which in preliminary studies was similar in pathogenicity to other strains isolated from plants in central Mexico, was used as the inoculum. In some experiments eight to 10 flats (each containing approximately 2,000 seedlings of each of the four Mexican cultivars) were infested with the high level inoculum of the Bajio strain.

In all studies, 8–10 wk after sowing, the most vigorous and apparently pathogen-free seedlings were transplanted into pasteurized soil and grown in the greenhouse for 40 days. Selected plants were then labeled and transplanted into crossing blocks in the field. Each block, consisting of selected plants from each cultivar, was caged with a colony of honeybees for pollination. Mature seed was harvested from individual plants for subsequent progeny testing. An equal quantity of seed from each plant was composited to represent the first cycle of selection for resistance to Phytophthora root rot from each cultivar. Similar techniques were used to develop the second cycle of selection.

All experimental data were recorded as percent plant survival and transformed to arc sine for statistical analyses using standard analysis of variance procedures.

Table 1. Effect of inoculum level of *Phytophthora megasperma* f. sp. *medicaginis* (Bajio strain) on disease severity in the alfalfa cultivar INIA-76 in the greenhouse at 20–25 C and natural light conditions

Inoculum Level	Transmittance[a] level (%)	Survival[b] (%)
High	27	4.5 x[c]
Medium	80	22.7 y
Low	97	72.8 z
Check	100	100.0

[a] Percent light transmittance of an aqueous suspension of oospores and mycelial fragments as shown by a Spectronic 20 spectrophotometer.

[b] Percentage of surviving seedlings (of 800) after 6 wk of growth in infested soil.

[c] Percent survival rates not followed by the same letter are different at the 0.01 probability level, according to Student-Newman-Keul's range test.

RESULTS

Distribution of Phytophthora root rot in central Mexico. The fungus was widespread and was a major factor in stand decline in the states of Aguascalientes, Estado de México, Guanajuato, Hidalgo, Michoacan, and Queretaro, but only in irrigated fields at elevations between 1,600 and 2,000 m. Although soil in these areas ranged from heavy clay to sandy loam, there was no correlation between soil type and occurrence of the disease. In infested fields, clay content ranged from 53.4 to 7.7%, sand content from 73.6 to 24.0%, and silt content from 27.9 to 13.8%. The average annual temperature was approximately 20 C. The disease was most commonly expressed as a rot of the mature taproots of plants 1–3 yr of age; however, high incidence of Phytophthora root rot was also noted in several recently seeded fields. The postemergence damping-off of seedlings reduced stands in these fields.

Pathogenicity and resistance studies. An initial study was made to determine the effect of inoculum level on seedling survival. Cultivar INIA-76, because of its excellent agronomic characteristics and adaptation to the high elevations of central Mexico, was used as the test cultivar. The Bajio isolate of *P. megasperma* f. sp. *medicaginis* was grown in liquid media and mixed (at three inoculum levels) into the greenhouse soil. Survival was lowest at the high level of inoculum after 6 wk of growth (Table 1). Surviving plants had root systems free of lesions. Almost all seedling deaths resulted from postemergence killing occurring primarily during the first and second week after emergence. Root systems of seedlings susceptible to Phytophthora root rot were rotted and showed abundant oospore production in the cortical tissues. Only the high level inoculum was used for subsequent studies.

The pathogenicity of isolates collected from two geographical areas in Mexico and one in Arizona was compared. Isolates were grown and tested in the greenhouse. Seedling survival of INIA-76, 6 wk after emergence, was 6.5% with the Bajio isolate and 5.3% with the Refugio isolate but with no significant difference at the 0.01 probability level (Student-Newman-Keul's range test).

The Bajio strain from Mexico was compared to a typical isolate of *P. megasperma* f. sp. *medicaginis* from Douglas, AZ, because earlier studies had indicated that strains of the fungus even from widely separated geographical areas in Arizona were similar in pathogenicity (4,5). All of the Mexican cultivars of alfalfa (Bajio-76, INIA-76, Mixteca-76, Puebla-76) were susceptible to the Bajio isolate. Percent survival after 6 wk of growth in infested soil in several tests was less than 1%. Postemergence damping-off was the most common expression of this disease. The Arizona isolate was significantly less pathogenic to the Mexican cultivars with survival percentages ranging from 3.7 to 8.7%. Hayden PX-III with resistance to this pathogen was developed in Arizona, had high resistance to the Arizona isolate (33.0% survival), but was susceptible (1.5% survival) to the Mexican isolate (Bajio).

Less frequent irrigations of infested soils increased percent seedling survival of the alfalfa cultivars. In one trial, typical of several, Puebla-76 had the highest survival level (16.8%) followed by INIA-76 (9.9%), Certified Hayden (9.1%), Bajio-76 (8.5%), and Mixteca (6.5%). The survival level of Puebla-76 was significantly greater ($P = 0.01$) than the other entries, which were not different from each other. Vigorous, apparently pathogen-free, seedlings were selected from each population for transplanting into crossing blocks in the field for progeny seed production.

Progeny testing of Hayden PX-III, INIA-76, Puebla-76, and Bajio-76 was undertaken to identify individual plants with high levels of resistance to Phytophthora root rot and to eliminate possible "disease-escapes." Eight of the 15 progeny lines of Hayden PX-III showed seedling survival rates ranging from 47.7 to 60.3%. These rates were significantly higher ($P = 0.05$) than the 19.0% survival level in the unselected parental check.

From INIA-76, 14 of 15 lines had significantly higher ($P = 0.05$) survival levels (30.3–61.0%) than the parental check (13.7%) (Table 2). Of the five progeny lines tested from Puebla-76, three had higher survival levels (19.3–24.0%) than the parental check (8.3)% ($P = 0.01$). From Bajio-76, two lines were better than the checks with 14.0

Table 2. Resistance of intercross progeny lines of Hayden PX-III, INIA-76, Puebla-76, and Bajio-76 to *Phytophthora megasperma* f. sp. *medicaginis* (Bajio strain) after one cycle of selection in the greenhouse at 20–25 C and natural light conditions

Hayden PX-III		INIA-76		Puebla-76		Bajio-76	
Lines	Survival[a]	Lines	Survival	Lines	Survival	Lines	Survival
38	60.3[b]	97	61.0[b]	85	24.0[c]	134	16.0[c]
81	55.0[b]	34	51.3[b]	24	24.0[c]	133	14.0[c]
113	54.0[b]	147	48.0[b]	44	19.3[c]	30	9.0
52	53.3[b]	112	47.0[b]	20	18.0	136	8.8
94	52.3[b]	98	42.3[b]	Check	8.3	Check	7.8
39	50.3[b]	115	41.0[b]				
57	47.7[b]	15	37.3[b]				
84	47.7[b]	48	35.7[b]				
51	47.3	103	35.3[b]				
8	45.3	16	35.0[b]				
114	45.0	50	33.7[b]				
115	44.0	111	32.3[b]				
9	33.7	102	32.3[b]				
6	32.7	101	30.3[b]				
77	29.7	Check	13.7				
Check	29.0						

[a] Percentage of surviving plants 8 wk after planting in infested soil.
[b] Lines significantly better than the unselected parental check at the 0.05 probability level; LSD = 11.05.
[c] Lines significantly better than parental check at the 0.01 probability level according to Student-Newman-Keul's range test.

Table 3. Increase in resistance to *Phytophthora megasperma* f. sp. *medicaginis* (Bajio strain) after two selection cycles in the greenhouse at 20–25 C and natural light conditions in the alfalfa cultivars INIA-76 and Hayden PX-III

Parental source			
INIA-76		Hayden PX-III	
Cycle[x]	Survival[y] (%)	Cycle	Survival (%)
0	5.6 a	0	12.8 a
1	12.2 b	1	15.8 a
2	22.8 c	2	30.6 b

[x] 0 = The unselected population, 1 = the first cycle of selection, and 2 = the second selection cycle.
[y] Percent survival levels not followed by the same letters are different at the 0.01 probability level according to Student-Newman-Keul's range test. Percentage based on a total plant population of 500.

and 16.0% survival ($P = 0.01$) (Table 2). Superior plants from these evaluations were intercrossed within parental sources to obtain material for the second cycle of selection for resistance to Phytophthora root rot.

Following two cycles of phenotypic recurrent selection, the level of resistance to Phytophthora root rot was increased from 5.6 to 22.8% in the new population originating from the cultivar INIA-76 and from 12.8 to 30.6% in the new population from Hayden PX-III (Table 3). These germ plasm populations will be tested in the field, and further selections will be made in Celaya, Guanajuato, Mexico.

DISCUSSION

In a survey of soilborne fungal pathogens of alfalfa in central Mexico, only three significant diseases were noted: Phytophthora root rot (*P. megasperma* f. sp. *medicaginis*), Phymatotrichum root rot (*Phymatotrichum omnivorum* (Shear) Duggar), and Southern blight (*Sclerotium rolfsii* Sacc.). Phytophthora root rot was a serious problem only in irrigated fields at elevations above 1,600 m, whereas Phymatotrichum root rot and Southern blight were more common at lower elevations where higher soil temperatures were more favorable for fungal activity.

Phytophthora root rot (both seedling disease and root rot of mature plants) is a particularly serious problem in Mexico's elevations above 1,600 m because 1) favorable soil temperatures exist throughout the year for pathogenic activity of *P. megasperma* f. sp. *medicaginis*, 2) cultivars susceptible to Phytopthora root rot are planted throughout the area, 3) excessive irrigation is practiced, and 4) soil drainage is poor in many fields. These observations contrast with those in the low-elevation desert area of California and Arizona where disease occurrence is more seasonal because high soil temperatures during the summer and fall inhibit activity of the pathogen. Seedling disease in the desert areas also is extremely rare because planting takes place during late summer and fall.

Our observations, based on studies with isolates of the fungus from widely different geographical areas in Arizona (*unpublished*), support the conclusions of Kuan and Erwin (7) on the host specificity of isolates of the fungus to alfalfa. Consequently, we have followed their suggestion in identifying the pathogen as *P. megasperma* f. sp. *medicaginis*.

Seedling survival in the system described in this paper is greatly influenced by soil moisture, level of inoculum, virulence of the isolate, and the reaction of the cultivar. For example, the number of surviving seedlings can be significantly increased by simply reducing soil moisture or decreased by increasing inoculum level. Furthermore, differences occur depending upon isolates used for the tests.

Several techniques have been described to find resistance in alfalfa to Phytophthora root rot, including inoculating cotyledons of seedlings (11), inoculating plants 1–12 mo of age (2,3), and selecting plants in the field with favorable disease conditions (3,8). We feel, however, that the methods described in this paper further substantiate the original study of Gray et al (4) and the recent study by Irwin et al (6) concerning the usefulness of a seedling assay method to find resistance in alfalfa to Phytophthora root rot easily and rapidly in a wide range of genetic material.

The success of the methods described depends upon understanding the parameters involved in postemergence damping-off. Each researcher should work out details for screening seedlings under his unique set of conditions.

The difference in disease reaction of Hayden PX-III, a cultivar developed in Arizona with a high degree of resistance to isolates of the fungus from Arizona, but susceptible to Mexican isolates, demonstrates that cultivars from an area where resistance is known to the pathogen of that area are not necessarily resistant to the same pathogens in other areas. It is anticipated that the cultivars selected in our screening tests will show mature plant resistance in the field to Phytophthora root rot as earlier studies have indicated (5,6).

We hope that the synthetic-1 seed produced on plants identified in this study as resistant to Phytophthora root rot will be used by the alfalfa improvement program in the high-plains area of central Mexico where this disease is a major factor suppressing production of this important forage crop.

LITERATURE CITED

1. Aguirre, R. J., Hine, R. B., and Schonhorst, M. H. Some important root and crown diseases of alfalfa in central Mexico. 1978. (Abstr.). Annu. Proc. Am. Phytopathol. Soc. 68:165.
2. Erwin, D. C. 1966. Varietal reaction of alfalfa to *Phytophthora megasperma* and variation in virulence of the causal fungus. Phytopathology 56:653-657.
3. Frosheiser, F. I., and Barnes, D. K. 1973. Field and greenhouse selection for *Phytophthora* root rot resistance in alfalfa. Crop Sci. 13:735-738.
4. 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.
5. Hine, R. B., Gray, F. A., Schonhorst, M. H., and Sanders, J. S. 1975. Resistance to *Phytophthora megasperma* root rot in selected lines of non-dormant alfalfa. Phytopathology 65:840-844.
6. Irwin, J. A. G., Miller, S. A., and Maxwell, D. P. 1979. Alfalfa seedling resistance to *Phytophthora megasperma*. Phytopathology 69:1051-1055.
7. Kuan, T. L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
8. Lueschen, W. E., Barnes, D. K., Rabas, D. L.,

Frosheiser, F. I., and Smith, D. M. 1976. Field performance of alfalfa cultivars resistant and susceptible to *Phytophthora* root rot. Agron. J. 68:281-285.

9. Marks, G. C., and Mitchell, J. E. 1970. Detection, isolation and pathogenicity of *Phytophthora megasperma* from soil and estimation of inoculum levels. Phytopathology 60:1687-1690.
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.
11. Pratt, R. G., Mitchell, J. E., and Willis, D. A. 1975. Resistance and susceptibility to *Phytophthora megasperma* expressed in alfalfa cotyledons. Phytopathology 65:365-369.
12. Tsao, P. H., and Guy, S. O. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora* isolation medium containing Hymexazol. Phytopathology 67:796-801.