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

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ABSTRACT

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 México, 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-Sirs, and Joaquin 11 (all from the United States), Aragon (from Spain), and INIA-76, Bajojo-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 Drechs. f. sp. medicaginis was a significant problem in stand establishment and persistence.

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

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 (Pabellon);


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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 silo 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 a lettered 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, Bajo-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.

**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 irrigated 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 Bajo 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 (Bajo-76, INIA-76, Mixteca-76, Puebla-76) were susceptible to the Bajo 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 (Bajo).

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%), Bajo-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 Bajo-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 Bajo-76, two lines were better than the checks with 14.0

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

<table>
<thead>
<tr>
<th>Inoculum Level</th>
<th>Transmittance</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>27</td>
<td>4.5</td>
</tr>
<tr>
<td>Medium</td>
<td>80</td>
<td>22.7</td>
</tr>
<tr>
<td>Low</td>
<td>97</td>
<td>72.8</td>
</tr>
<tr>
<td>Check</td>
<td>100</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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 differ at the 0.01 probability level, according to Student-Newman-Keul's range test.
Table 2. Resistance of intercross progeny lines of Hayden PX-III, INIA-76, Puebla-76, and Bajo-76 to Phytophthora megasperma f. sp. medicaginis (Bajo strain) after one cycle of selection in the greenhouse at 20-25 C and natural light conditions

<table>
<thead>
<tr>
<th></th>
<th>Hayden PX-III</th>
<th>INIA-76</th>
<th>Puebla-76</th>
<th>Bajo-76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>Survival*</td>
<td>Lines</td>
<td>Survival</td>
<td>Lines</td>
</tr>
<tr>
<td>38</td>
<td>60.3%</td>
<td>97</td>
<td>61.0%</td>
<td>85</td>
</tr>
<tr>
<td>81</td>
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<td>44</td>
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<td>50.5%</td>
<td>115</td>
<td>41.0%</td>
<td>57</td>
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<td>57</td>
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<td>37.3%</td>
<td>84</td>
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<td>51</td>
<td>47.3%</td>
<td>48</td>
<td>35.7%</td>
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<td>114</td>
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<td>33.7%</td>
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<td>102</td>
<td>32.3%</td>
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</tr>
<tr>
<td>7</td>
<td>29.7%</td>
<td>Check</td>
<td>13.7%</td>
<td>Check</td>
</tr>
</tbody>
</table>

*Percentage of surviving plants 8 wk after planting in infested soil.
2 Lines significantly better than the unselected parental check at the 0.05 probability level; LSD = 11.05.
3 Lines significantly better than parental check at the 0.01 probability level according to Student-Newman-Keuls' range test.

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 Phytophthora 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 reduced by increasing 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 seedings (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-I 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


