Greenhouse Technique to Evaluate Alfalfa Resistance to *Phytophthora megasperma* f. sp. *medicaginis*

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ABSTRACT

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A rapid, inexpensive, and reliable greenhouse technique was developed to evaluate large populations of alfalfa seedlings for resistance to *Phytophthora megasperma* f. sp. *medicaginis*. Seedlings were grown in a soil mix and inoculated when 12 days old. The percent stand 14 days later was used to rank entries. Rankings of entries for resistance to *P. megasperma* f. sp. *medicaginis* in the greenhouse were positively correlated with rankings in the Minnesota field test. Two populations of alfalfa derived as synthetic progenies from plants that survived the greenhouse test had higher percent stand compared with the original two parent populations when subjected to a second exposure to the fungus.

Phytophthora root rot caused by *Phytophthora megasperma* (Drechs.) f. sp. *medicaginis* (Kuan & Erwin) (6) occurs in most areas where alfalfa is grown (2). Evaluation of alfalfa (*Medicago sativa* L.) seedlings for Phytophthora root rot is valuable to plant breeders who want to develop resistant cultivars. Determination of resistance in seedlings would permit rapid identification of resistant germ plasm. Under field conditions, the standard test many alfalfa breeders use to evaluate prospective alfalfa cultivars for Phytophthora root rot resistance is a test developed by Frosheiser and Barnes (1) at the Minnesota Agriculture Experiment Station, St. Paul. In this test, greenhouse soil infested with *P. magasperma* f. sp. *medicaginis* is incorporated into the nursery in low-lying, poorly drained areas where Phytophthora root rot has not previously been a problem. In May, seed of alfalfa cultivars is planted in this area, which is periodically sprinkler irrigated during the 4–5 mo growing season. In September or October, plants are dug, washed, and evaluated for disease development. A greenhouse technique described in this paper provides a rapid, inexpensive, and reliable evaluation of resistance in alfalfa germ plasm to Phytophthora root rot.

MATERIALS AND METHODS

Inoculation Procedures. Three *P. megasperma* f. sp. *medicaginis* isolates were used for this study: California P1057 (D. C. Erwin, University of California, Riverside 92521), Minnesota 78-11 (F. I. Frosheiser, University of Minnesota, St. Paul 55108), and Maryland TN-1 (J. H. Graham, W-L Research Inc., Highland, MD 20777). All isolates were grown for 14 days on V-8 juice culture media (7) and maintained at room temperature (20–24 C).

Plastic tubs (45 cm wide × 60 cm long × 15 cm deep) were used in these experiments. A 1-cm-diameter hole was drilled in the bottom of each tub and sealed with a rubber stopper. Three centimeters of washed gravel was placed in the bottom to provide drainage with a 2-cm-diameter pipe placed in the center of the tub to facilitate adding water during inoculation. The gravel base was covered to within 3 cm of the top of the tub with a mixture of Pro-Mix (Premier Brands, Inc., New Rochelle, NY 10801), Palite (PA Perlite Corp., Lehigh Valley, PA 18001), and tap water (9:5:5; v/v/v).

Forty-five seeds of each alfalfa entry were seeded 1 cm deep in 17.5-cm rows 2.5 cm apart. Seeds were sprayed with 1.6 ml 29 EC pentachloronitrobenzene per liter of tap water to control *Rhizoctonia* spp., then covered with Pro-Mix and the media surface sprayed with tap water. Emerged seedlings were counted 7 days after seeding, then thinned to 40/row. Seedlings were inoculated 12 days after planting with a mixture of two of the three *P. megasperma* f. sp. *medicaginis* isolates. In a preliminary experiment, we were unable to detect differences in pathogenicity of the three isolates so mycelial mats were selected at random. The inoculum was prepared by blending two 2-wk-old mycelial mats from 95-mm culture plants in 1 L of water for 10 sec in a Waring Blendor. Sixty-five milliliters of inoculum was poured into 2-cm-deep furrows made between every other row of seedlings. The inoculum consisted solely of mycelium. Uniform inoculum suspension was maintained by repeated stirring during application. No attempt was made to quantify inoculum. Immediately after inoculation, 200 ml of tap water was poured over the inoculum in each furrow to enable the inoculum to infiltrate the Pro-Mix-Palite medium. The furrows were covered with Pro-Mix and sprayed with tap water. Tap water was added through the tube in the center of the tub until free water reached the surface of the Pro-Mix-Palite medium. After 3 days, water was drained and subsequent water added only as needed to maintain normal seedling growth. In a preliminary experiment, we did not detect any adverse effects from flooding uninoculated seedlings for 3 days. Fourteen days after inoculation, the plants were examined for disease development. Only plants with no symptoms were counted and retained and all diseased plants were discarded. The percent stand was calculated and used to compare the response of alfalfa synthetics to *P. megasperma* f. sp. *medicaginis*.

Consistency among greenhouse inoculation experiments. Three populations of experimental alfalfa germ plasm developed by the research staff at W-L Research, Inc., and two commercial cultivars were used in these comparisons. The three experimental populations were T1 (resistant to *P. megasperma* f. sp. *medicaginis*), T22 (intermediate resistance), and T27 (susceptible). The cultivar Agate was used as the resistant check and Saranac AR as the susceptible check cultivar. Each experiment consisted of one tub divided into six replicates, with each replicate separated by a 5-cm alleyway. Each replicate contained 40 plants each of Agate, Saranac AR, T1, T22, and T27.

Correlation between greenhouse experiments and Minnesota field tests. In this study, 25 experimental alfalfa populations developed by W-L Research, Inc., previously evaluated for Phytophthora root rot in the Minnesota field test were evaluated for resistance to *P. megasperma* f. sp. *medicaginis* using greenhouse inoculation techniques. The percent resistance (or tolerance) of each experimental population was compared with the percent resistance of Agate. The resulting percentage was the figure used to compare results of the greenhouse experiments with those of the Minnesota

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field test. To evaluate larger numbers of more experimental populations per experiment, the inoculation technique was modified. Each experiment consisted of two tubs divided into two replicates. Each replicate contained 80 plants each of Agate, Saranac, and six experimental alfalfa populations. The amount of inoculum used was decreased. Mycelial mats of each of the three *P. megasperma* f. sp. *medicaginis* isolates were blended together in 600 ml of tap water for 10 sec; the resulting suspension was divided equally and water added to each suspension to equal 1 L. The susceptible check was Saranac, which is more susceptible to *P. megasperma* f. sp. *medicaginis* than Saranac AR.

Testing of progeny from surviving plants. During winter 1980–1981, 231 plants of T22 and 218 plants of T27 were selected for absence of disease symptoms after inoculation with *P. megasperma* f. sp. *medicaginis*. These plants were transplanted into a greenhouse soil/peat mix and maintained in the greenhouse for 3–4 mo. In spring 1981, the roots and tops of each plant were clipped and the plants sent to the W-L Research Station, Bakersfield, CA. The two populations were planted separately in field isolation cages in which honeybees were allowed to pollinate. Seeds from these plants were returned to the W-L Research Station, Highland, MD, during fall 1981.

Progenies of selected plants of T22 and T27 subjected to one cycle of selection for Phytophthora root rot resistance were designated T22 PYR and T27 PYR, respectively. To compare Phytophthora root rot resistance of the original populations T22 and T27 with the Syn_1 generations of T22 PYR and T27 PYR, seedlings of these synthetics were inoculated as described before.

RESULTS

Symptomatology. When seedlings were examined, roots of susceptible plants showed characteristic Phytophthora root rot symptoms, red to brown discolored roots and often decay of the entire root. Tops of affected plants were stunted. Resistant or tolerant plants had healthy top growth and an extensive root system free of discoloration. Other plants were placed in an intermediate class when the taproot was decayed but the seedlings had regenerated secondary roots.

Consistency among greenhouse inoculation experiments. In all five greenhouse experiments, entries ranked in the same order. Agate and T1 were always the most resistant, with Agate slightly more resistant than T1 (51 vs. 40% disease-free plants). Both Agate and T1 were significantly more resistant than Saranac AR (12% disease-free plants), which was consistently low in all experiments. T22 and T27 (26 and 20% disease-free plants, respectively) always ranked intermediate between Agate and Saranac AR, with T22 more resistant than T27.

Correlation between greenhouse experiments and Minnesota field tests. When the percent Phytophthora root rot resistance of each of the 25 experimental populations was compared with the percent resistance of Agate both in the greenhouse experiments and the Minnesota field tests, a highly significant correlation was obtained ($r = +0.78$). The modified inoculation technique was effective in producing characteristic Phytophthora root rot symptoms and in separating resistant, intermediate, and susceptible plants into distinct classes.

Development of alfalfa synthetics with Phytophthora root rot resistance. The increase in resistance to *P. megasperma* f. sp. *medicaginis* in the experimental populations T22 and T27 is shown in Table 1. After one cycle of selection, 42% of the T22 PYR population (progeny of the T22 synthetic) was resistant compared with 22% of T22 population and 36% of the T27 PYR population (progeny of the T27 synthetic) was resistant compared with 8% of T27. In addition, both the top growth and root systems of synthetic populations that had undergone selection (T22 PYR and T27 PYR) were considerably more vigorous than the original populations (T22 and T27).

Table 1. Percent stand of alfalfa synthetics and their progenies after one cycle of selection

Entry	Percent resistance[y]
Agate	46 a
T22 PYR[z]	42 a
T27 PYR	36 b
T22 (original	22 c
T27 (original)	8 d
Saranac	4 d

[y] Numbers are the means of three replicates. Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] T22 PYR and T27 PYR are progeny from seed of the original T22 and T27 synthetic populations of plants that had been exposed to the *Phytophthora* seedling test.

DISCUSSION

The greenhouse inoculation technique described in this paper is a rapid, reliable, and inexpensive way to evaluate large numbers of plants and select for resistance of alfalfa to Phytophthora root rot. This procedure has several qualities that make it easy to do this. The containers are lightweight, durable, and require little space in the greenhouse. An even distribution of water and inoculum is maintained throughout the tubs because each tub can be leveled, which is extremely important; if drainage is not uniform, the seedlings in one area may be more severely damaged by *P. megasperma* f. sp. *medicaginis*, giving variable results when seedlings are evaluated.

Also, the technique provides for rapid evaluation of large numbers of alfalfa experimental populations for Phytopthora root rot resistance. For example, in the winter and spring of 1981–1982, 83 tests were conducted in which 152 alfalfa experimental populations were evaluated for their resistance to *P. megasperma* f. sp. *medicaginis*. This represented more than 200,000 seedlings evaluated in a 7-mo period.

Several greenhouse techniques have been developed to identify alfalfa plants with resistance to *P. megasperma* f. sp. *medicaginis* (1,3–5). Whereas the greenhouse method developed by Frosheiser and Barnes (1) took about 6–8 wk to complete and used a 1–5 rating system, this method takes about 26 days and used only a resistant or susceptible rating system. The method of Gray et al (3) required that seedlings be flooded for 2 wk after inoculation in contrast to 3 days with this method. In another greenhouse study by Irwin et al (4), 8-wk-old plants were used for root inoculation and 10-wk-old plants were used for stem inoculation. A 1–5 rating system was used for root inoculation and a 1–4 rating system for stem inoculation. The method we have developed uses only seedlings and a simplified percent stand-rating system. This method thus offers several advantages over the currently used field and greenhouse inoculation procedures for developing alfalfa cultivars with Phytophthora root rot resistance.

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