Resistance to Phytophthora Root Rot in Selected Lines of Nondormant Alfalfa

R. B. Hine, F. A. Gray, M. H. Schonhorst, and J. S. Sanders

First, second, and fourth authors are Professor and Research Assistants, respectively, Department of Plant Pathology; third author is Professor, Department of Agronomy and Plant Genetics, University of Arizona, Tucson 85721.

Arizona Agricultural Experiment Station Journal Series Paper No. 2361.

The authors thank Mervin W. Nielson, Entomologist, U.S. Department of Agriculture, Tucson, for determination of resistance levels in the alfalfa cultivars to the spotted alfalfa aphid.

Accepted for publication 22 February 1975.

ABSTRACT

A high level of field resistance in alfalfa to root rot caused by *Phytophthora megasperma* was achieved in selections from a seedling screening of the nondormant alfalfa cultivar Hayden. In several greenhouse and field trials, a second-cycle polycross (Hayden PX II) had less root rot and higher stand density and plant vigor than a first-cycle polycross (Hayden PX I) or Lahontan, a semi-dormant cultivar with known resistance to root rot. In one field trial after 8.5 months of growth in infested soil, the percentage of disease-free plants was: Hayden PX II, 84; Hayden PX I modified, 62; Lahontan, 56; and Hayden, the cultivar from which the selections had been made, 28. In another field test, Hayden PX II, Hayden PX I, and Hayden had 67, 41, and 15% disease-free plants, respectively. The resistant synthetics, Hayden PX I and II, originally selected after seedling greenhouse exposure to a mixture of isolates from central Arizona, were equally resistant to other isolates from widely separated geographical areas in Arizona.

Phytopathology 65:840-844

Additional key words: Phytophthora megasperma, Medicago sativa L., breeding for disease resistance.

Alfalfa (*Medicago sativa* L.), a major crop in Arizona, is grown on approximately 20% of the irrigated crop land. Winter-dormant cultivars are grown successfully at elevations above 1,300 m. However, at lower elevations, where nonwinter-dormant cultivars are grown, a major problem is stand and yield decline. In some areas, fields are renovated and reseeded annually. Although a number of factors are involved in stand survival, such as soil fertility, soil compaction, cutting frequency, crown damage caused by equipment and grazing by sheep, scald injury, and insect problems, recent studies in Arizona (5) indicated that root rot caused by the soil fungus *Phytophthora megasperma* (Drechs) was a major factor contributing to stand and yield decline. It was also determined that the major nondormant-alfalfa cultivars grown in Arizona were susceptible to this disease. Later, a screening technique useful in selecting alfalfa plants for resistance to *P. megasperma* was described (4). Emphasis in this technique was placed on selection for seedling resistance to damping-off since this represented a faster and more economical method of screening large numbers of plants. Selections were made from Hayden, a University of Arizona-developed cultivar (9), because of its superior agronomic characteristics and high level of resistance to the spotted alfalfa aphid [*Therioaphis maculata* (Buckton)], a major insect pest in Arizona. First-cycle seed produced from interpollination of 200 survivors of the 10,000 Hayden alfalfa seedlings which were screened, had 59% less damping-off when compared with seedlings of certified Hayden (4).

The objectives of this study were to determine: (i) what level of field resistance to root rot had been achieved using synthetics obtained with a seedling greenhouse selection technique; (ii) if levels of resistance differed between first and second cycles of phenotypic recurrent selection in polycross material; and (iii) if these selections were resistant to isolates of *P. megasperma* from different geographical areas in Arizona.

MATERIALS AND METHODS.—*Field studies.*—Several experimental and commercial alfalfa entries were tested for resistance to *Phytophthora* root rot in three field trials at the University of Arizona Campbell Avenue Farm in Tucson, Arizona. Experimental University of Arizona entries developed in the previously described seedling greenhouse method included: Hayden PX I, a first-cycle polycross from certified Hayden; Hayden PX II, a second-cycle polycross from certified Hayden; Mesa-Sirsa PX I, a first-cycle polycross from field-selected plants; and Hayden polycross I modified (HPX I mod.). Certified Lahontan was included as a resistant check and certified Hayden and certified Mesa-Sirsa as susceptible checks. Hayden PX I seed was produced for these studies as previously described (4). This seed was again screened in the greenhouse using the same technique, and 125 of the most vigorous, disease-free seedlings were selected from a population of approximately 6,000 plants. These plants were interpollinated by bees in a greenhouse and the seed designated as Hayden PX II. These 125 clones were also propagated and transplanted in replicated plots in an infested field at Tucson. After 1 year, 35 of the vigorous and disease-free clones were allowed to interpollinate and the seed from them designated HPX I mod.

In the first test, seed were planted at the rate of approximately 40 kg/ha in 3 × 3 m plots replicated five times. This site was naturally infested with *P. megasperma.* In test 2, the same entries were planted, but the field was artificially infested. Three isolates of *P. megasperma* (Buckeye, Gilbert, and Laveen) were grown on V-8 agar, blended in water, and applied to the seedbed prior to seeding. Sixty 5-week-old petri-dish cultures of each isolate were used to inoculate 0.04 ha. Each entry was replicated six times in 3 × 3 m plots. The third test consisted of 25 commercial and experimental entries planted in single-row plots replicated five times. The rows were 8 m long, and spaced 0.3 m apart. This site was artificially infested as in test 2. All three plots were established in fields composed of Gila Silt loam during October 1973, irrigated weekly, and evaluated in June 1974 for stand density, vigor, and incidence and severity of root rot. Approximately 200 plants were dug from each replication, washed, and observations made for root disease.

Pathogenicity studies.—Inoculum was grown at room temperatures (22-27 C) as standing cultures in liquid V-8 juice medium (100 ml Campbell's V-8 juice, 2 g $CaCO_3$/liter of distilled water) in 947-ml (32-ounce) medicine bottles containing 180 ml of the liquid medium. After 2 days of incubation, cultures were shaken vigorously for several seconds to fragment the mycelium and encourage development of a large quantity of mycelium per bottle. After 4 weeks the mycelial mats containing oospores were washed, placed in distilled water, and triturated in a Waring Blendor. The inoculum used in all tests consisted of oospores and mycelial fragments of *P. megasperma.*

For all pathogenicity experiments, a heat-pasteurized soil-sand mixture (1:1, v/v) with the following characteristics was used: pH 8.1; 83.0% sand; 13.0% silt; 4.0% clay; 0.06% organic matter; 90 μg/g NO_3; 6.9 μg/g PO_4; 1,050 μg/g soluble salts; and 3.2, 6.2, and 9.0% moisture retention at 15, 0.33, and 0.10 atm tension, respectively. Approximately 2.2 and 14.7 kg of the air-dried soil mixture were placed in 15.2-cm diameter plastic pots or in 38 × 54 cm galvanized metal flats, respectively. Growth chambers were maintained on a 12-hour light cycle at 24 C and a 12-hour dark cycle at 18 C. Greenhouse temperatures were maintained at 24-27 C. All plants were watered daily to ensure a high soil moisture. Saucers under all pots were kept flooded for 2 weeks and then removed. All experiments were replicated four or more times and repeated at least twice. Stand counts were taken 2-12 weeks after planting.

RESULTS.—In three field trials Hayden PX II was the entry most resistant to *Phytophthora* root rot. In one study conducted in a naturally infested field, Hayden PX II, Hayden PX I modified, and Hayden had 84, 62, and 28% resistant plants (root disease category 1), respectively, 8.5 months after planting (Table 1). Hayden PX II also had the highest stand-density and plant-height ratings. Both Hayden PX I modified and Hayden II had more resistant plants than Lahontan, which was used as a resistant check. Mesa-Sirsa, a commonly planted nondormant variety, was the most susceptible entry with

TABLE 1. Field resistance to *Phytophthora* root rot in two experimental alfalfa synthetics in comparison with one resistant (Lahontan) and two susceptible (Hayden, Mesa-Sirsa) cultivars

Entries	Stand density[a]	Plant height	Root disease rating[b] (Percentage of plants in each category) 1	2	3	4	5	Total plants examined
Synthetics:								
Hayden PX II	1.4	1.0	84	9	7	0	0	311
Hayden PX I modified	2.2	1.2	62	18	13	7	1	197
Susceptible:								
Hayden	2.6	2.2	28	32	23	16	1	176
Mesa-Sirsa	3.4	2.6	22	32	21	15	1	210
Resistant:								
Lahontan	3.0	2.8	56	29	9	6	0	227

[a]Score of 1.0 indicates excellent stand and growth; 5.0 indicates poor stand and stunted growth. Scores are means of five replications.

[b]Score of 1 = disease free; 2 = no obvious root lesions but fine roots destroyed, leaving small lesions at point of attachment; 3 = distinct lesions on taproot; 4 = many elongated lesions on taproot; 5 = nearly all of taproot rotted.

only 22% resistant plants. Plants in root disease categories 3, 4, and 5 were considered highly susceptible. The percentage of plants in these categories was: Hayden PX II, 7; Hayden PX I modified, 20; Hayden, 40; Lahontan, 16; and Mesa-Sirsa, 46 (Table 1).

In another study consisting of two 8.5-month field tests, Hayden PX II, Lahontan, Hayden PX I, and certified Hayden had 67, 60, 41, and 15% resistant plants in a naturally infested field site and 79, 78, 59, and 42% resistant plants in an artificially infested site, respectively (Table 2). In a general-appearance rating (1.0 indicates excellent stand and growth, and 5.0 indicates poor stand and stunted growth), Hayden PX II, Hayden PX I, Lahontan, and certified Hayden had ratings of 1.25, 2.50, 3.00, and 3.75, respectively (Table 2). In this test, Mesa-Sirsa polycross I, which had originally been selected from a 4-year-old thin stand of Mesa-Sirsa growing in an infested area, had 29% resistant plants of a total of 395 plants dug from the four replications.

Several studies were designed to determine if the Hayden polycross synthetic, which was resistant to isolates of *P. megasperma* used in the original screening work from central Arizona (Buckeye, Laveen, Gilbert), would show similar resistance to isolates from other areas in Arizona. Isolates used in these studies were recovered from diseased taproots of alfalfa from widely separated geographical areas at different elevations in Arizona: Parker (west-central), 140 m elevation; Yuma (southwest), 47 m; Snowflake (north-central), 1,860 m; Safford (southeast), 973 m; Tucson (south-central), 796 m; and Many Farms (northeast), 1,766 m elevation. None of these isolates was used in the original greenhouse screening studies.

Inoculum for the flat studies consisted of triturated mycelial mats from nine culture bottles (947 ml) placed in 5 liters of distilled water. One liter of this inoculum was blended into the soil of each flat. The inoculum of each isolate was standardized by adjusting the wet weight of the mycelial mats prior to trituration. In the pot studies, each pot received 50 ml of a prepared inoculum suspension consisting of mycelial mats from three culture bottles (947 ml) blended in 660 ml of distilled water. Each flat was seeded with three rows each of scarified seed of CH and HPX II (100 seeds/row). Each flat was considered a replication. In a test typical of several, we showed that Hayden PX II was as resistant to the isolate from Snowflake as it was to the isolate from Buckeye. The average number of surviving seedlings/row after 10 weeks in the Hayden PX II resistant synthetic was 40 and 39 with the isolates from Snowflake and Buckeye, respectively. In the susceptible cultivar Hayden, surviving seedlings/row were 9 and 5, with the isolates from Snowflake and Buckeye (Fig. 1). Most surviving seedlings of Hayden were stunted with numerous taproot lesions whereas the surviving seedlings of Hayden PX II were vigorous and essentially free of root rot. Similar data were collected from several pot tests with different isolates of *P. megasperma*. Results of one typical test are shown in Table 3. In this test, the pathogenicity of equal mixtures of the three isolates from Buckeye, Laveen, and Gilbert were compared with the isolates from Snowflake and Tucson. Hayden PX II was as resistant to isolates from Tucson and Snowflake as it was to the three isolates from Buckeye, Laveen, and Gilbert when data were taken 6 weeks after inoculation of 11-day-old seedlings. In this study, which was repeated twice, the Tucson isolate was more virulent than the other isolates. Similar results were obtained in pot studies with isolates from Safford, Yuma, Parker, and Many Farms.

DISCUSSION.—During 1968 and 1969, in several large field trials in Arizona, Schonhorst et al. (9) demonstrated that their new alfalfa cultivar, Hayden, outyielded Mesa-Sirsa, El-Unico, and Sonora, three of the most widely planted nondormant alfalfas in Arizona, by 10-20%. Hayden was developed by making a two-clone or "single-cross" combination between the two parent clones of the cultivar Mesa-Sirsa which were highest in general combining ability for forage production. Similarly, a second single-cross was made between the two highest yielding parents of the cultivar Sonora-70. A 1:1 blend of seed from the two single-crosses is classed as Breeder seed. This new cultivar was also shown by Nielson et al. (8) to have a high degree of resistance to the most common biotypes of the spotted alfalfa aphid. Hayden, presently, is the most widely planted cultivar in Arizona, comprising approximately 50% of all new

TABLE 2. A comparison of field resistance in two experimental alfalfa synthetics to *Phytophthora* root rot in naturally and artificially infested sites

Sites[a]	Entries			
	HPX II	HPX I	Lahontan	Hayden
1 - Resistant plants	505[b]	360	762	209
Susceptible plants	131	255	210	295
Percentage resistant plants	79	59	78	41
2 - Resistant plants	314	185	273	58
Susceptible plants	157	271	183	334
Percentage resistant plants	67	41	60	15
General-appearance score	1.25[c]	2.50	3.00	3.75

[a]Site 1 was seeded and artificially infested 1 October 1973 with *Phytophthora megasperma* as described in the text and evaluated 14 June 1974. Site 2 was seeded 2 October 1973 and evaluated 13 June 1974. This site was naturally infested, and disease incidence was high in a previous planting.

[b]Total number of plants dug from five replications in site 1 and four replications in site 2. Resistant plants were free of taproot lesions.

[c]Score of 1.0 indicates excellent stand and growth; score of 5.0 indicates poor stand and stunted growth. The scores are means of four replications.

TABLE 3. Comparative effects of isolates of *Phytophthora megasperma* on survival and growth of certified Hayden and Hayden PX II in greenhouse pot studies

Isolates	Entries	Average Plants/Pot[a]	Average Fresh Wt/Pot (g)[b]
Tucson	CH	3	1
	HPX II	7	14
BLG[c]	CH	4	5
	HPX II	8	16
Snowflake	CH	6	6
	HPX II	10	16
CK (Noninoculated)	CH	10	26
	HPX II	10	24

[a]Based on ten replications. Plants inoculated at 11 days of age. Readings made 6 weeks after inoculation. Plants thinned to ten per pot.
[b]Plants cut off 20 mm above soil line.
[c]Inoculum consisted of an equal mixture of the isolates from Buckeye, Laveen, and Gilbert.

plantings in the State. Studies initiated in 1970, however, indicated that this popular cultivar, as well as other nondormant alfalfas, was susceptible to *Phytophthora* root rot. Because of the excellent agronomic characteristics of Hayden, research was initiated in 1970 to increase resistance in this cultivar to *Phytophthora* root rot. Gray et al. (4) demonstrated that incidence of seedling disease caused by *P. megasperma* could be altered by manipulating inoculum level and temperature. Increased resistance to seedling disease was described in a synthetic derived from this screening method. However, it was emphasized that the efficacy of the screening method was dependent upon reaction of the resistant synthetic to root rot in field situations. Data in this paper validate the greenhouse seedling screening technique as an effective method for increasing the level of *Phytophthora* resistance of mature plants in the field. Under optimum conditions for disease in the field, resistance was increased from less than 15% in the starting population to about 60% in the first cycle of selection and then to about 83% after two cycles of selection. These results are similar to those from Minnesota described by Frosheiser and Barnes (3), who increased resistance in a dormant alfalfa from 10% in starting populations to about 63% after three cycles of selection. Their studies resulted in a new cultivar, Agate, described by Barnes and Frosheiser (1).

Lehman et al. (6) in California released two synthetics with an intermediate level of resistance to *Phytophthora* root rot after earlier work by Erwin (2) had indicated that many of the cultivars commonly planted in southern California were highly susceptible to this fungus. They have also described a new cultivar, Salton, which has resistance to the summer disease complex and some tolerance to *Phytophthora* root rot in the low desert valley areas of California. According to Lu et al. (7), resistance to *Phytophthora* is simply inherited, with susceptibility conditioned by one tetrasomic gene (Pm) with incomplete dominance. They also suggested that resistance genes occurred in low frequency in other alfalfa cultivars. Our research in Arizona supports their observation since we successfully selected resistant plants from a relatively large population of a susceptible cultivar, such as Hayden. Our results on fungal strain differences were similar to those observed in Minnesota in that our resistant synthetics are resistant to a number of isolates of *Phytophthora* from different areas in Arizona. Hayden PX I seed was also sent to Australia and shown to possess moderate levels of resistance to isolates of *P. megasperma* from Queensland. Hayden PX II is presently being evaluated for resistance to root rot (*personal communication* with J. A. G. Irwin, Plant Pathology Branch, Department of Primary Industries, Brisbane, Australia).

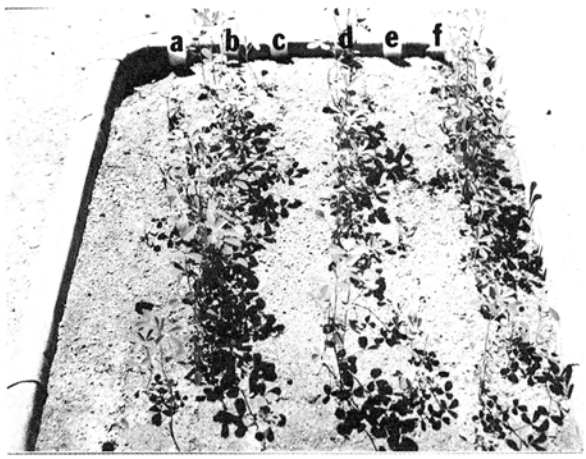

Fig. 1. A comparison of the growth and survival of the susceptible cultivar Hayden (rows a, c, e) with the resistant Hayden PX II (rows b, d, f) after 10 weeks of growth in soil infested with *Phytophthora megasperma*.

Hayden PX II was shown to have the same level of resistance as certified Hayden (Mervin W. Nielson, *unpublished*) when seedlings were exposed to biotypes of the spotted alfalfa aphid. This level of insect resistance is necessary for release of a new cultivar in Arizona.

LITERATURE CITED

1. BARNES, D. K., and F. I. FROSHEISER. 1973. Registration of Agate alfalfa. Crop Sci. 13:768-769.
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 D. K. BARNES. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.
4. GRAY, F. A., R. B. HINE, M. H. SCHONHORST, and J. D. NAIK. 1973. A screening technique useful in selecting for resistance in alfalfa to Phytophthora megasperma.

Phytopathology 63:1185-1188.
5. HINE, R. B., F. A. GRAY, and M. H. SCHONHORST. 1972. Phytophthora root rot of alfalfa in Arizona. Plant Dis. Rep. 56:472-473.
6. LEHMAN, W. F., D. C. ERWIN, and E. H. STANFORD. 1969. Registration of Phytophthora tolerant alfalfa germplasm, U.C. 38 and U. C. 47. Crop Sci. 9:527.
7. LU, N. S., D. K. BARNES, and F. I. FROSHEISER. 1973. Inheritance of Phytophthora root rot resistance in alfalfa. Crop Sci. 13:714-717.
8. NIELSON, M. W., M. H. SCHONHORST, H. DON, W. F. LEHMAN, and V. L. MARBLE. 1971. Resistance in alfalfa to four biotypes of the spotted alfalfa aphid. J. Econ. Entomol. 64:506-510.
9. SCHONHORST, M. H., R. K. THOMPSON, and R. E. DENNIS. 1971. Hayden alfalfa. Arizona Agri-File Field Crops 272.1. College of Agriculture, University of Arizona, Tucson. 4 p.