

Phytophthora Root Rot Resistant Alfalfa for Arizona

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During 1968 and 1969, in several large field trials in Arizona, Schonhorst, et al. (4), demonstrated that their new alfalfa cultivar Hayden, which was developed by crossing two top forage-producing clones of the cultivars Sonora-70 with two clones of Mesa-Sirsa, outyielded Mesa-Sirsa, El-Unico, and Sonora, three of the most widely planted nondormant alfalfas in Arizona, by approximately 10 to 20%. This new cultivar was also shown by Nielson, et al. (3), to have a high de-

gree 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 plantings in the State. Studies initiated in 1970, however, indicated that this popular cultivar, as well as other nondormant alfalfas, was susceptible to root rot caused by the fungus *Phytophthora megasperma* (2,3). This disease was shown to be an important factor in stand and yield decline of alfalfa

in Arizona. 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. (1), 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 germplasm derived from this screening method. However, it was emphasized that the efficacy of the screening method was dependent upon reaction of the resistant germplasms to root rot in field situations.

Studies were initiated, then, to determine: (i) what level of field resistance to root rot had been achieved using germplasm obtained from the seedling greenhouse selection technique; (ii) if levels of resistance differed between first- and second-cycle selections; and (iii) if these selections were resistant to isolates of *P. megasperma* from different geographical areas in Arizona.

Field studies — Several experimental and commercial alfalfa entries were tested for resistance to *Phytophthora* root rot in three field trials



Figure 1. A comparison of growth and survival of the cultivar Hayden (rows A, C, E) with Hayden PX II (rows B, D, F) after 10 weeks of growth in soil infested with *Phytophthora megasperma*.

at the University of Arizona Campbell Avenue Farm in Tucson. 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. Lahontan and Certified Hayden, and Certified Mesa-Sirsa,

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

Entries	Stand ^a Density	Plant Height	Root disease rating ^b (Percent of plants in each category)					Total Plants Examined
			1	2	3	4	5	
Hayden PX II	1.4	1.0	83.9	8.7	7.1	0.0	0.3	311
Hayden PX I, modified	2.2	1.2	62.4	17.8	12.7	6.6	0.5	197
Hayden	2.6	2.2	28.4	31.8	22.7	15.9	1.1	176
Lahontan	3.0	2.8	55.9	28.6	9.3	6.2	0.0	227
Mesa-Sirsa	3.4	2.6	22.4	31.9	21.4	14.8	9.5	210

^a Score of 1.0 indicates excellent stand and growth; 5.0 indicates poor stand and stunted growth. These 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.

were included as resistant and susceptible checks, respectively. Hayden PX I seed was produced by interpollinating 100 surviving seedlings of approximately 10,000 original Hayden seedlings exposed to the *Phytophthora* fungus. Hayden PX I 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 6000 plants. These plants were interpollinated by bees in the greenhouse and the seed designated as Hayden PX II. These 125 clones were also propagated and planted in replicated plots in an infested field at Tucson. After 1 year, 35 of the most vigorous and disease-free clones were allowed to interpollinate and the seeds from them designated HPX I, mod.

In the first test, seeding was made at the rate of approximately 40 lbs./acre in 10 x 10 sq. ft. plots replicated five times. This site was naturally infested with *P. megasperma*. In test two the same entries were planted, but the field was artificially infested with isolates of *P. megasperma* from diseased alfalfa from Buckeye, Gilbert, and Laveen. Each entry was replicated six times in 10 x 10 sq. ft. plots. The third test consisted of 25 commercial and experimental entries planted in single-row plots replicated five times. All three plots were established 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 on root disease.

A heat-pasteurized soil-sand mixture was used for all pathogenicity experiments in the greenhouse. In most studies, growth chambers were maintained on a 12-hour light cycle

at 24 C and a 12-hr. dark cycle at 18 C. Temperatures in glasshouse studies were maintained at 24-27 C. Daily watering of all experiments insured a high soil-moisture condition. Saucers placed 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 to 12 weeks after planting.

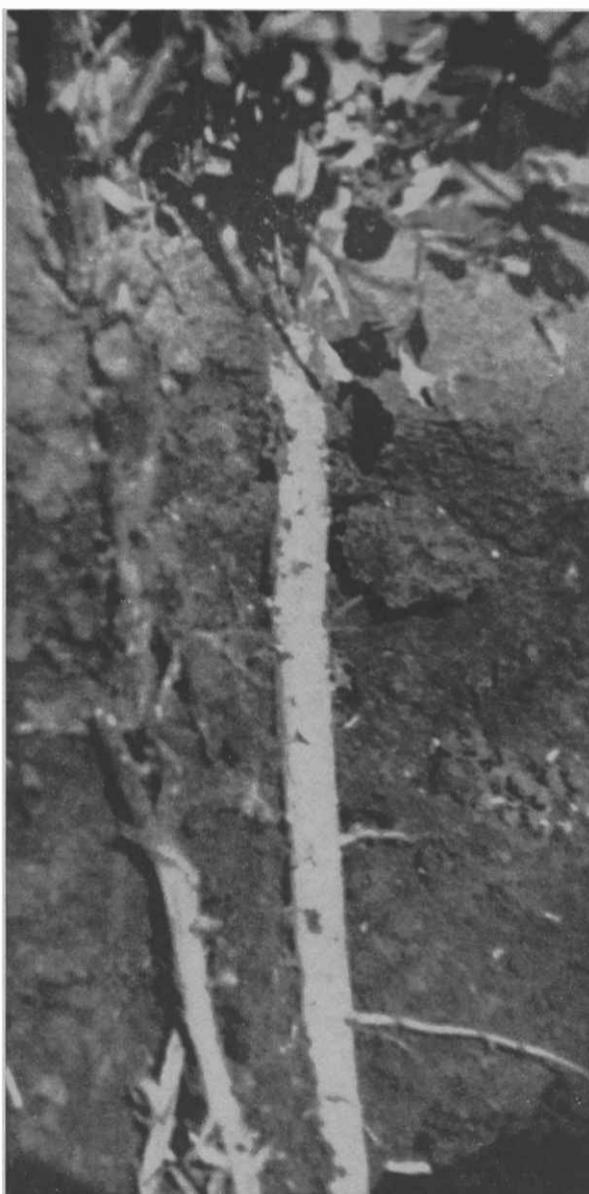


Figure 2. Infected and healthy roots of alfalfa plants grown under field conditions.

RESULTS AND DISCUSSION — In three field trials Hayden PX II was the entry most resistant to *Phytophthora* root rot. In one study Hayden PX II, Hayden PX I, and Hayden had 83.9, 62.4, and 28.4% resistant plants, respectively, 8 months after planting (Table 1). Hayden PX II also had the highest density and plant height ratings. Both Hayden PX I and 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 only 22.4% resistant plants. Plants in root disease categories 3, 4, and 5 were considered to be highly susceptible. The percentage of plants in these categories was: Hayden PX II, 7.4; Hayden PX I, modified, 19.8; Hayden, 39.7; Lahontan, 15.5, and Mesa-Sirsa, 45.7 (Table 1).

In another study consisting of two field tests, after 8 months Hayden PX II, Lahontan, Hayden PX I, and Certified Hayden had 66.7, 59.9, 40.6, and 14.8% resistant plants in a naturally infested field site and 79.4, 78.4, 58.5, and 41.5% 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).

Several studies were designed to determine if the Hayden polycross germplasm, which was resistant to isolates of *P. megasperma* used in the original screening work (Buckeye, Laveen, Gilbert), would show similar resistance to isolates from other areas in Arizona. Isolates used in these studies

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were recovered from diseased taproots of alfalfa from widely separated geographical areas in Arizona: Parker (west-central), 140 m elevation; Yuma (southwest), 47 m elevation; Snowflake (north-central), 1860 m elevation; Safford (southeast), 973 m elevation; Tucson (south-central), 796 m elevation; and Many Farms (northeast), 1766 m elevation. None of these isolates were used in the original greenhouse screening studies.

Each of the flats 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, it was shown 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 germplasm was 40.4 and 38.8 with the isolates from Snowflake and Buckeye, respectively. In the susceptible cultivar Certified Hayden, surviving seedlings/row were 8.8 and 5.3, with the isolates from Snowflake and Buckeye. Surviving seedlings of Certified Hayden were mostly 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*. In one test the pathogenicity of equal mixtures of the three isolates from Buckeye, Laveen, and Gilbert was compared with the isolates from Snowflake and Tucson. Hayden PX II was equally resistant to the isolates from Tucson and Snowflake as it was to the three isolates from Buckeye, Laveen, and Gilbert when data were taken 8 weeks after inoculation of 11-day-old plants. 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.

The information presented in this paper validates the greenhouse seedling screening technique as an effective method for increasing the level of resistance of mature alfalfa plants in the field to *Phytophthora* root rot. 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

Table 2. A comparison of field resistance in two experimental alfalfa germplasms 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
Percent resistant plants	79.4	58.5	78.4	41.5
2 — Resistant plants	314	185	273	58
Susceptible plants	157	271	183	334
Percent resistant plants	66.7	40.6	59.9	14.8
General appearance score	1.25 ^c	2.50	3.00	3.75

^a Site 1 was seeded and artificially infested with *PHYTOPHTHORA MEGASPERMA* as described in the text on 1 Oct. 1973 and evaluated on 14 June 1974. Site 2 was seeded on 2 Oct. and evaluated on 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.

83% after two cycles of selection.

According to researchers in Minnesota a low frequency of resistant genes occurs in many susceptible alfalfa cultivars. This probably explains our ability to successfully select resistant seedlings in large populations of a susceptible cultivar, such as Hayden. Our work in Arizona is similar to that in Minnesota in that our resistant germplasms are resistant to isolates of *Phytophthora* from different areas in Arizona, an important factor in adaptability of a new alfalfa cultivar to the various geographical locations in Arizona. Presently, seed of the resistant germplasms are being produced at the University Farm at Yuma and Marana so that large-scale field trials for forage production and disease resistance can be made in several areas in Arizona.

Hayden PX II was shown to have the same level of resistance as Certi-

fied Hayden (unpublished data) 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.

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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	0.7
	HPX II	6.9	14.2
BLG ^c	CH	4.2	5.1
	HPX II	7.8	16.3
Snowflake	CH	6.1	6.4
	HPX II	9.6	15.8
CK	CH	10.0	25.8
	HPX II	10.0	24.1

^a Inoculum consisted of an equal mixture of the isolates from Buckeye, Laveen, and Gilbert.

^b Plants cut off 20 mm above soil line.

^c Inoculum consisted of an equal mixture of the isolates from Buckeye, Laveen, and Gilbert.