

## Development of Phytophthora Root Rot of Alfalfa in the Field and the Association of Rhizobium Nodules with Early Root Infections

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### ABSTRACT

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Initial root infections of alfalfa, caused by *Phytophthora megasperma*, occurred 4-8 weeks after planting in naturally infested field soil and were primarily associated with nodules incited by *Rhizobium meliloti*. In pasteurized (temperature increased to 115 C over a 4-hour period) field soil artificially infested with *P. megasperma*, seedling death was 24% higher with *R. meliloti*-treated seed than in nontreated controls. Increases in seedling death were not observed when treated

and untreated seed were planted in field soil naturally infested with *P. megasperma* and *R. meliloti*. In a 4-month-old alfalfa stand, taproot lesions occurred from 4-25 cm below the soil surface with maximum lesion development at depths of approximately 17 cm. *Phytophthora megasperma* was recovered at soil depths as great as 56 cm in Mojave clay and 80 cm in Gila silt loam and Grabe clay loam.

*Additional key words:* *Medicago sativa*, microbial interactions, vertical distribution in soil.

Alfalfa (*Medicago sativa* L.) is grown on about 20% of the total irrigated crop land in Arizona. At the lower elevations where alfalfa is grown predominantly, the major problem is stand and yield decline. Although many factors undoubtedly contribute to this problem, studies by Hine et al. (15) indicated that root rot caused by *Phytophthora megasperma* Drechs. contributed significantly to the decline. This disease has been reported throughout the United States (2, 8, 10, 11, 16) as well as in Australia (20), Canada (3, 4), and Brazil (J. Albersio A. Lima, Plant Pathologist, Federal University of Ceara, School of Agriculture, Fortaleza, Ceara, Brazil, *personal communication*).

Several research workers have reported on the incidence and severity of *Phytophthora* root rot caused by *P. megasperma* in laboratory and greenhouse studies (2, 8, 10, 11, 16), but expanded studies on the development of the disease in the field have not been reported.

Information relating to the role of *Rhizobium* nodules, which are incited by *R. meliloti*, on *Phytophthora* root rot do not exist in the literature. However, closely related greenhouse studies with soybeans, conducted by Chou and Schmitthenner (5) indicated that more plants were killed by root rot, caused by *P. megasperma* var. *sojae*, when grown in sterile soil, than when *Rhizobium japonica* and *Endogene mosseae* were included. They pointed out that further studies on the effect of mycorrhizae and/or root nodules on pathogenicity of soilborne pathogens are needed.

This study deals with the development of *Phytophthora*

root rot in the field and the association of early root infections, caused by *P. megasperma*, with root nodules incited by *R. meliloti*. A preliminary investigation has been reported (12, 13).

### MATERIALS AND METHODS

**Disease development under field conditions.**—To determine the interval between initial infection and plant death, a plot naturally infested with both *P. megasperma* and *R. meliloti*, and located at The University of Arizona Campbell Avenue Farm, Tucson, Arizona, was seeded 1 October 1973, at a rate of 18 kg/ha with the nondormant cultivar Hayden. It was flood-irrigated (15-20 cm deep) weekly and 50 plants were removed at random 1, 4, 8, 16, 40, and 52 weeks after seeding. Infections by *P. megasperma* were determined by tissue isolation on a *Phytophthora*-selective medium (7). When lesions were not present, roots were washed and placed in petri dishes that contained distilled water to induce sporulation of *P. megasperma*. Both tissue isolations and submerged roots were incubated at room temperature (21-25 C) and examined after 3-5 days.

An additional time-study relating to *Phytophthora* infection was conducted in a naturally-infested field near Eden, Arizona. The soil type was Grabe clay loam. One border (0.6 ha) was seeded with the susceptible cultivar Hayden on 1 October 1973, at a rate of 18 kg/ha. One year after planting, 308 plants were randomly removed and percentage infection determined.

**Association of *Rhizobium* nodules with root infection.**—Greenhouse studies were conducted to determine if a seed treatment with *Rhizobium meliloti* influenced the incidence of *Phytophthora* root rot. The

test consisted of six treatments, replicated 10 times and placed in a randomized block design. Treatments consisted of placing Hayden seed, with and without a *Rhizobium* seed treatment in 15-cm pots (20 seeds/pot) containing pasteurized and nonpasteurized soil. Soil was taken from an alfalfa field naturally infested with *P. megasperma* and *R. meliloti*. The pasteurization process consisted of increasing the temperature of moist soil to 115 C within a 4-hour period. *Rhizobium meliloti* was applied to the seed using the Pelinoc method described by Nitragen Co. (1). Two weeks after seeding, one-half of the pots (20) with pasteurized soil were infested with a mycelial suspension of *P. megasperma* as described by Gray et al. (14). Isolations and observations on root disease were made after 4 months. In another study, 490 *Rhizobium* nodules were removed from the roots of 100, 16-week-old alfalfa plants growing in a naturally infested field plot. All plants exhibited symptoms of Phytophthora root rot. Both necrotic and healthy-appearing nodules were surface disinfested in a 0.05% sodium hypochlorite solution for 3 minutes, placed in distilled water, and observed for development of sporangia.

**Depth of taproot infection sites and related soil temperature studies.**—Two studies were initiated to determine the depth of infection sites on taproots of alfalfa growing in naturally infested soil. The first test was in Eden, Arizona, and the other at the Campbell Avenue Farm in Tucson.

At Eden, approximately 300 plants were removed from

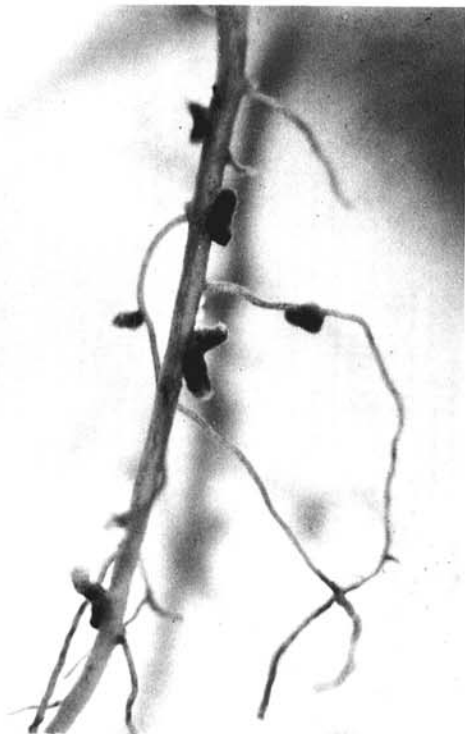


Fig. 1. Discolored nodules caused by *Rhizobium meliloti* on roots of a 3-month-old field-grown alfalfa plant, indicating early infections by *Phytophthora megasperma*.

a 3-year-old alfalfa stand showing severe losses from *Phytophthora* root rot. The distance from the crown to the upper portion of each *Phytophthora* lesion and the number of lesions per plant were recorded.

In the study conducted in Tucson, seeds of the susceptible cultivar Hayden were planted at the rate of 18 kg/ha on 1 March 1973 in a naturally infested field plot (0.1 ha). Random plants were removed periodically until taproot lesions were observed. Four months after seeding, when lesions were easily distinguishable but complete root decay had not occurred, approximately 300 plants were removed and lesion depths and number of lesions per plant were determined. Recorders with mercury-fitted probes were used to record soil temperatures at 8 and 23 cm. A hygrothermograph was used to record temperatures 30 cm above the soil surface. Temperatures were recorded over a 1-year period.

**Vertical distribution of *Phytophthora megasperma*.**—Vertical distribution of *P. megasperma* in naturally infested field soil was determined by removing samples from three randomly-selected sites located in Grabe clay loam (Eden, Arizona), Mojave clay (Higley, Arizona), and Gila silt loam (Wellton and Tucson, Arizona). Layers of soil (8 × 15 × 40 cm) were removed at 8-cm intervals to a depth of 96 cm and maintained separately. Each sample (approximately 1 kg of soil) was thoroughly mixed and stored in a sealed plastic bag at 23-25 C. Five 50-ml aliquots were removed and bioassayed for *P. megasperma* using a technique described by Marks and Mitchell (17, 19). Recorders, with mercury-fitted probes, were placed in the Tucson plot to record soil temperatures at 8 and 23 cm depths. A hygrothermograph was used to record temperatures 30 cm above the soil surface. Temperatures were recorded over a 1-year period.

## RESULTS

**Disease development under field conditions.**—Root infections of *P. megasperma* were first detected 8 weeks after seeding. Taproot lesions, that were easily distinguishable, were first noticed after 16 weeks. A few dead plants were present by 40 and 52 weeks but stand losses were negligible. The percentage of plants with diseased roots 1, 4, 8, 16, 40, and 52 weeks after planting was 0, 0, 9, 17, 34, and 43%, respectively.

Of 308 plants removed 1 year after planting from a naturally infested alfalfa field near Eden, Arizona, 63%

TABLE 1. Detection of *Phytophthora megasperma* in necrotic and healthy-appearing *Rhizobium* nodules from alfalfa roots

Nodule appearance	Nodules sampled <sup>a</sup>	Infected by <i>P. megasperma</i> <sup>b</sup>	
		(no.)	(%)
Necrotic	329	55	16.7
Healthy	161	11	6.8

<sup>a</sup>The 490 nodules were removed from approximately 100 field-grown alfalfa plants of the cultivar Hayden with severe symptoms of *Phytophthora* root rot.

<sup>b</sup>Presence of *Phytophthora megasperma* was determined by placing nodules in distilled water after surface disinfesting in a 0.05% sodium hypochlorite solution for 3 minutes. Nodules were observed for sporangia after 3-5 days.

had visible root lesions. Although counts were not made, a visible loss of stand was evident.

**Association of Rhizobium nodules with root infection.**—In one field study, initial root infections of *P. megasperma* primarily were associated with discolored *Rhizobium* nodules (Fig. 1, Table 1). Infections of root tips not associated with *Rhizobium* nodules were also noticed but were minimal. In field soil pasteurized and artificially infested with *P. megasperma* seedling death at 12 weeks was 24% higher in treatments with *Rhizobium*-treated seed compared to treatments without added *Rhizobium* (Table 2). The pasteurization process did not eliminate, but drastically reduced root nodulation. Root symptoms produced in this artificial system (pasteurized field soil with *R. meliloti* and *P. megasperma* added) were similar to those observed from the nonpasteurized soil treatment and also to plants removed from naturally infested alfalfa fields. *Phytophthora megasperma* was readily recovered from marginal areas of necrotic root lesions and from discolored *Rhizobium* nodules (Table 2).

In contrast to results in pasteurized soil, infection was high (25-27%) in naturally infested field soil (in the greenhouse) regardless of whether the seed was treated or not treated with *Rhizobium*.

**Depth of taproot infection sites and related soil temperature studies.**—In the 3-year-old alfalfa stand in Eden, taproot lesions occurred from 1-40 cm below the soil surface with the majority occurring between 3-20 cm. In the 4-month-old stand in Tucson, lesions occurred from 4-25 cm below the soil surface with the majority between 6-20 cm (Fig. 2). The 3-year-old and 4-month-old stands had an average of 1.4 and 1.7 lesions per plant, respectively. A distinct zone of root lesions was most evident in the 4-month-old stand where 28% of all taproot lesions occurred approximately 17 cm below the soil surface.

The high and low monthly mean temperatures for 1974-1975 at 30 cm above the soil and 8 and 23 cm below the soil surface are shown in Fig. 3. The soil at 23 cm had a great capacity to buffer against temperature changes. The mean high air temperature in June was 36.8 C compared to 22.7 C at 23 cm depth. This represented approximately a 0.6 C decrease in temperature for every 1 cm of soil depth. Optimum temperature range for disease development of *P. megasperma* is 21-27 C (2, 9). Thus, temperatures within this favorable range existed 23 cm

under the soil surface during months when the highest air temperatures were recorded.

**Vertical distribution of Phytophthora megasperma.**—*Phytophthora megasperma* was readily

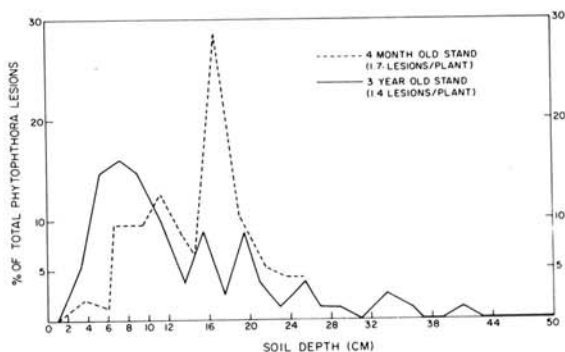


Fig. 2. Vertical distribution of taproot lesions caused by *Phytophthora megasperma* in two naturally-infested alfalfa fields.

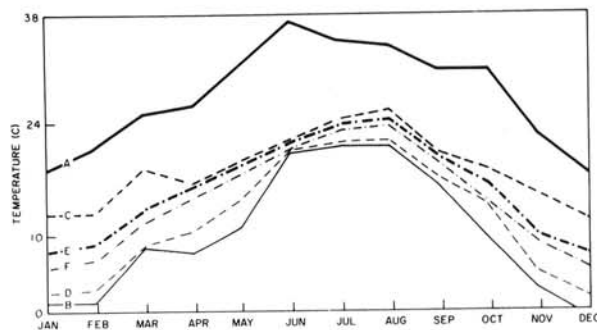


Fig. 3. Average monthly high and low temperatures 30 cm above and 8 and 23 cm below the soil surface for 1 year in an Arizona alfalfa field. (A) Mean high air temperature, 30 cm above the soil surface; (B) Mean low air temperature, 30 cm above the soil surface; (C) Mean high soil temperature, 8 cm below the soil surface; (D) Mean low soil temperature, 8 cm below the soil surface; (E) Mean high soil temperature, 23 cm below the soil surface; (F) Mean low soil temperature, 23 cm below the soil surface.

TABLE 2. Effect of seed treatment with *Rhizobium meliloti* on the incidence of alfalfa seedling death caused by *Phytophthora megasperma* in Gila silt loam naturally infested with *Phytophthora megasperma* and *Rhizobium meliloti*

Seed treatment <sup>a</sup>	Seedling death (%) in soil:		
	Pasteurized (check)	Not pasteurized <sup>b</sup>	Pasteurized and reinfested <sup>c</sup> with <i>Phytophthora megasperma</i>
<i>Rhizobium</i>	0 <sup>d</sup>	27	33
No <i>Rhizobium</i>	0	25	9

<sup>a</sup>Seeds were inoculated with *R. meliloti* using the Pelinoc method described by the Nitragen Company.

<sup>b</sup>Soil was obtained from an alfalfa field cropped with alfalfa for 8 consecutive years with a natural infestation of *P. megasperma* and *R. meliloti*.

<sup>c</sup>Each pot received 40 ml of a *P. megasperma* mycelial suspension, 2 weeks after seeding. The suspension consisted of 2-week-old mycelial mats from five 956-ml (32-ounce) culture bottles blended in 1 liter of distilled water.

<sup>d</sup>Stand counts were made 4 months after planting. Values are expressed as a percentage of the check and represent the average number of surviving seedlings of 10 replications, each containing 20 seeds.

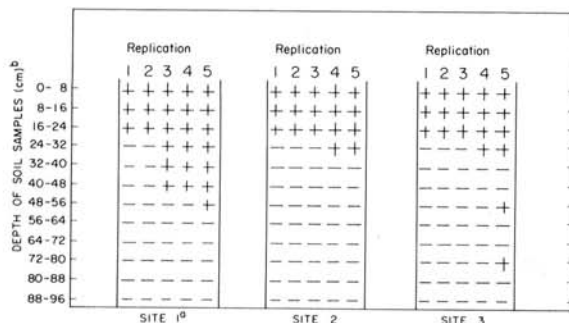


Fig. 4. Vertical distribution of *Phytophthora megasperma* in soil from a naturally infested field. Each field site was 1.0 m wide  $\times$  2.0 m long  $\times$  1.0 m deep. Soil samples (8  $\times$  15  $\times$  40 cm) were removed in layers down to 96 cm and maintained separately. Five 50-ml aliquots were removed from each 8-cm sample, placed in petri dishes, and flooded with 50 ml of distilled water. To bioassay for the presence of *P. megasperma*, five 3-day-old alfalfa seedlings were floated on the water surface in each dish. After 3-5 days, readings were taken and a plus (+) or minus (-) given to each 50-ml sample.

recovered at depths of 16, 24, 32, and 56 cm and occasionally as deep as 48, 64, 80, and 80 cm in the Mojave clay, Gila silt loam (Tucson), Gila silt loam (Wellton) and Grabe clay loam (Eden). Mojave clay loam appeared to restrict, to a limited extent, the downward movement of *P. megasperma*; otherwise recovery was similar for all soils. Recovery depths in the Tucson site in Gila silt loam are shown in Fig. 4.

#### DISCUSSION

Our studies indicate that under field conditions, initial root infections may occur as early as 4-8 weeks after seeding. Within 1 year after planting, in one field which showed an observable stand loss, approximately 63% of all plants exhibited taproot lesions incited by *P. megasperma*. Previous observations in Arizona had indicated that losses do not occur until 2-3 years after planting. Most lesions were found between 4 to 40 cm depth beneath the soil surface, but some occurred as deep as 80 cm. However, some stratification was evident as a distinct zone of taproot lesions at approximately 17 cm below the soil surface in the 4-month-old alfalfa stand at Tucson. Presumably the depth of such a zone varies with soil type, plow depth, moisture, temperature, inoculum depth, and other factors. *Phytophthora megasperma* was readily detected, using an alfalfa seedling bioassay, from the soil surface to depths varying from 16-56 cm, depending on the soil type.

The soil temperature studies indicate that optimal and reasonably constant temperatures for *P. megasperma* existed at the 23 cm soil depth from June through August 1973. This combination of optimal soil temperatures and available inoculum may partially explain the high numbers of taproot lesions near the 17-cm soil depth.

Few studies have dealt with the possible role of *Rhizobium* nodules in relation to the incidence or severity of root diseases. Drapeau et al. (6), using in vitro studies, implied that legumes may derive possible disease

protection from their association with *Rhizobium*.

Ross (21) reported the influence of *Endogone mycorrhiza* on *Phytophthora* root rot of soybeans, caused by the soilborne fungus *P. megasperma*. He reported an increase in plant death associated with the presence of *Endogone* sp. whereas no death occurred in the absence of *Endogone*.

In tests conducted in the greenhouse, on *Phytophthora* root rot of soybeans, Chou and Schmitthenner (5) indicated more plants were killed by *P. megasperma* var. *sojae* in sterile soil than when tested in combination with *Endogone mosseae* and *Rhizobium japonicum*. They indicated that these two organisms may have a suppressive effect on root rot severity such as that commonly attributed to soil microflora.

In our greenhouse studies with pasteurized field soil, more seedlings died when the seed was treated with *Rhizobium*. This indicates no disease protection from *Rhizobium*; on the contrary, a possible increase in disease might occur if these data can be extrapolated to the field. Sufficient nodulating bacteria were already present in the field soil and incidence of root rot was already high, so that additional *Rhizobium* added via the seed did not increase incidence of the disease. The soil was taken from an area where alfalfa had been grown for 8 consecutive years. Perhaps in another field not previously cropped to alfalfa or other closely related legumes and thus, low in *R. meliloti*, the use of *Rhizobium*-treated seed conceivably could increase *Phytophthora* root rot of alfalfa if *P. megasperma* were present. Fortunately *P. megasperma* is presently undetectable in such areas (F. A. Gray, unpublished).

Marks and Mitchell (18) concluded that large taproot lesions caused by *P. megasperma* on alfalfa consistently originate where lateral roots emerged. They point out that the phellem, which provides a barrier to infection, lacks continuity around the base of the emerging lateral roots and possibly the leakage of nutrients is a factor in the penetration and infection process. However, they did not indicate the presence or absence of *Rhizobium* nodules in their study. In our field studies, early root infections, caused by *P. megasperma*, were primarily associated with *Rhizobium* nodules. Initial root infections of both *P. megasperma* and *R. meliloti*, were initiated during the first few weeks (4-8) after planting. Thus, fungal infections, initially detected in nodules attached to young taproots, appear to progress from the infected nodule into the cortical root tissue, and to girdle the root at the point of nodule attachment. Leakage of root exudates, reported to occur during lateral root formation (18), could also take place during nodule formation. Exudates released during nodule formation may stimulate the germination of dormant oospores in the soil and/or attract nearby motile zoospores to the nodule surface.

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