The Survival and Activity of Phytophthora megasperma in Naturally Infested Soils

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ABSTRACT

Infective activity of *Phytophthora megasperma* in naturally infested soils was measured by a baiting technique using alfalfa seedlings. Activity decreased in three soils stored for 3-9 months at 25 C, but only in one soil stored at 4 C. Activity decreased slightly in soils stored at high moisture levels for 7 months, and it disappeared in soils stored at low moisture levels (equivalent to less than −40 bars tension). *P. megasperma* remained infective in 13 of 26 naturally infested soils stored 3.5 years at 25 C at undetermined moisture levels and in 24 of 46 soils stored 2.5 years.

Growth of alfalfa seedlings of susceptible cultivars in naturally infested soils resulted in greater increases in infective activity of the pathogen than did growth of seedlings of resistant cultivars. Significant decreases in infective activity from initial values occurred in the greenhouse in two soils not planted to alfalfa. When corn, oats, clover, soybeans, or peas were grown in samples of two soils for 3-6 months, decreases in infective activity were comparable to, or only slightly greater than, that which occurred in fallow soil. These results indicate that *P. megasperma* is not a strong saprophytic competitor in the soil.

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Although Phytophthora root rot, which is caused by *Phytophthora megasperma* Drechsler, has been recognized as an important soil-borne disease of alfalfa in North America for over twenty years (3, 5), little is known about the ecology of the causal organism in soil. Numerous reports have established that Phytophthora root rot is present in alfalfa throughout North America and Australia, and that it occurs most frequently in heavy soils, low-lying soils with poor drainage, and in irrigated fields (8, 17). However, little information is yet available to explain the occurrence and severity of disease in terms of the ability of the causal organism to survive in soils. The only recorded observations of active disease spread and inoculum increase in the field were made by Erwin (4), who described an instance of severe disease which appeared in a sandy soil within one year after virgin desert was farmed to alfalfa with irrigation.

Prior to 1970, the occurrence of *P. megasperma* in soils could be determined only by observations of disease symptoms in plants and/or isolations of the organism from diseased tissues. No methods were available for determining the presence of the pathogen in the soil in the absence of the host. In 1970, Marks and Mitchell (15) described a baiting technique using alfalfa seedlings by which *P. megasperma* could be detected in, and isolated from, naturally infested soils. They were able to detect the pathogen in low drainage areas of several fields, but not on contiguous well-drained slopes. Pratt and Mitchell (17) used this baiting technique to detect *P. megasperma* in soils of 48 of 109 low-lying fields throughout Wisconsin. They found that temperature and soil dilution affected baiting efficiency, and that planting naturally infested soils to alfalfa in the greenhouse greatly increased the infectivity, and hence the probability of detection, of *P. megasperma*. Hine et al. (10) used the baiting technique to detect *P. megasperma* in soils of alfalfa fields in Arizona.

The alfalfa-seedling bating technique may be used, not only to determine the presence of *P. megasperma,* but also to measure levels of infective activity of the pathogen in soils (15, 17). In this study, we have used the baiting technique to identify and evaluate factors which influence the survival and the levels of infective activity of *P. megasperma* in naturally infested soils.

MATERIALS AND METHODS.—Samples of naturally infested soils were obtained from several alfalfa fields in Wisconsin known to be infested with *P. megasperma* (17). All soil samples were stored in tightly sealed plastic bags at 25 C. To raise infective activity levels for storage and crop rotation experiments, seedlings of Vernal alfalfa, grown in steamed soil for 4-6 weeks at 20 or 24 C, were transplanted into the naturally infested soils in 15-cm diameter clay pots (six-to-eight seedlings/pot) and grown for 6-8 weeks at 24 C in the greenhouse with daily watering. Following growth of alfalfa, plants were removed and soil from all of the pots was mixed and sieved (2.4-mm openings, 8-mesh screen) prior to storage or use.

Levels of infective activity of soils at one or more dilutions were measured by the alfalfa-seedling baiting technique (15, 17). Infested soils were diluted with a mixture of sand and loam (1:1, v/v) that had been steamed at 82.2 C (180 F) for 30 minutes and cooled. Ratios of 1:1, 1:4, and 1:16 were prepared in 474 cm^3 bottles using 90 cm^3 diluted soil per bottle and mixing on a roller mill for 30 minutes. Three replicate bottles were used for soil at each dilution. After being mixed, the contents of each bottle was distributed equally into three petri plates, and water was added to a depth of 3-4 mm above the soil surface. Seedlings of Vernal alfalfa, grown on moist filter paper in petri plates for 3-4 days at 28 C in alternate light and darkness, were injured to prevent further development by firmly pinching with tweezers at the tips of the radicles and below the cotyledons. Only seedlings with unfolded, green cotyledons were used. Six injured seedlings were evenly distributed over the soil in each flooded plate. After incubation of soil plates for 4 days at 22 C, the seedlings were removed and individually examined under a dissecting microscope (× 30) for Phytophthora sporangia. A seedling was scored positive if one or more sporangia were observed growing from it. One replicate value in any treatment consisted of the percent of seedlings scored positive if the 18 in the three plates of soil from each bottle. Sporangia did not develop on baits as a result of spread of *P. megasperma* from infected to noninfected baits during the 4-day incubation period.

To determine soil moisture content, 8-10 cm^3 aliquots from soils were weighed in aluminum foil dishes and reweighed after 24 hours of drying in an oven at 100 C. Moisture content values given for all soils are expressed as percentages of the oven-dried weights of the soils and are the means of three measurements of each soil. Moisture contents of soils at different water potentials were obtained from approximately 15-cm^3 aliquots of soil that had been flooded and then equilibrated to potentials of −0.1, −0.5, −1, −1 and −15 bars in ceramic pressure-plate extractors (Soil Moisture Equipment Co., Santa Barbara, California). Aliquots were weighed immediately after removal from extractors, and again after drying for 24 hours at 100 C. Three replicate moisture-content values were obtained for each soil at each of the five water potentials, and mean values were used to derive a moisture-characteristic curve for each soil at potentials of from −0.1 to −15 bars.

Statistical significance in all experiments with a completely random design was determined by analysis of variance. Significant differences were located by use of Duncan's multiple-range test (20).

RESULTS.—*Changes in infective activity of Phytophthora megasperma in soils planted to resistant and susceptible alfalfa.*—Seedlings of the susceptible cultivars Saranac and Vernal, and of the resistant lines MnP-B1 and MnP-D1 (9), grown 6 weeks in steamed sand:loam mixture (1:1, v/v) at 20 C, were washed free of soil, sorted into groups of comparable-sized plants, and

TABLE 1. The effect of growing resistant and susceptible cultivars of alfalfa on the infective activity of *Phytophthora megasperma* in naturally infested soils

Soil sample	Alfalfa cultivar[a]	Infective activity at soil dilution, replicates and mean[b]											
		1:1				1:4				1:16			
		A	B	C	mean	A	B	C	mean	A	B	C	mean
EC-2	Saranac	100	100	100	100 A	100	100	100	100 A	100	100	100	100 A
	Vernal	100	100	100	100 A	100	100	100	100 A	83	89	100	91 A
	MnP-B1	89	94	100	95 AB	55	66	100	74 B	17	33	61	37 B
	MnP-D1	50	72	100	74 B	35	50	61	49 C	0	0	11	4 B
G-2	Saranac	100	100	100	100 A	100	100	100	100 A	100	100	100	100 A
	Vernal	22	28	39	30 B	0	6	17	8 B	0	0	0	0 B
	MnP-B1	11	39	50	33 B	0	6	33	13 B	0	0	0	0 B
	MnP-D1	33	33	55	40 B	0	0	17	6 B	0	0	11	4 B
D-1	Saranac	100	100	100	100	100	100	100	100	100	100	100	100
	Vernal	11	100	100	70	0	100	100	67	0	100	100	67
	MnP-B1	11	100	100	70	0	100	100	67	0	100	100	67
	MnP-D1	6	6	28	13	0	6	6	4	0	0	6	2

[a]Saranac and Vernal are susceptible cultivars, MnP-B1 and MnP-D1 are resistant cultivars. Six-week-old seedlings of each cultivar transplanted into infested soil (6 seedlings/13-cm pot) and grown 8 weeks at 20 C.

[b]Diluent soil was a steamed mixture of sand and loam (1:1, v/v). Replicate values = percentages of 18 seedling baits on which *Phytophthora* sporangia were observed. Replicates of EC-2 and G-2 soils are samples of single pots; replicates of D-1 soils each represent data for different pots. Means not followed by the same letters within a column for each soil are significantly different, $P = 0.05$, as determined by Duncan's multiple-range test.

TABLE 2. The effect of temperature during storage on the infective activity of *Phytophthora megasperma* in naturally infested soils

Soil sample	Temp (C)	Storage time (months)	Infective activity at soil dilution of[a] 1:1	1:4	1:16
C-1		0	100 A[b]	100 A	91 AB
	4	3	100 A	100 A	81 ABC
		6	100 A	100 A	68 CD
		9	100 A	85 A	72 BC
	25	3	100 A	94 A	100 A
		6	100 A	98 A	50 DE
		9	100 A	94 A	41 E
K-3		0	100 A	85 A	41 AB
	4	3	100 A	92 A	26 B
		6	100 A	94 A	50 A
		9	94 A	70 AB	35 AB
	25	3	100 A	56 AB	18 BC
		6	65 B	48 AB	17 BC
		9	56 B	22 B	2 C
W-2		0	100 A	87 AB	79 A
	4	3	100 A	100 A	44 ABC
		6	100 A	98 AB	61 AB
		9	100 A	89 AB	68 AB
	25	3	100 A	100 A	46 ABC
		6	100 A	78 B	26 BC
		9	56 B	24 C	13 C

[a]Diluent soil a steamed mixture of sand and loam (1:1, v/v).
[b]Values = mean percentages of three replicates of 18 seedling baits on which *Phytophthora* sporangia were observed. Values not followed by the same letters within a column for each soil are significantly different, $P = 0.05$, as determined by Duncan's multiple-range test.

transplanted into 13-cm diameter pots (six seedings per pot) of three naturally infested soils with little infective activity. For soils EC-2 and G-2, one pot of each soil was planted with seedlings of each cultivar; for soil D-1, three pots were planted with seedlings of each cultivar. All pots were maintained at 20 C and watered daily for 8 weeks. Soils were then harvested, sieved, and assayed for infective activity of *P. megasperma* at the three dilutions (Table 1). For the EC-2 and G-2 soils, three replicate assays were made for each pot of infested soil planted to each cultivar. For the D-1 soil, one assay was made of soil at each dilution for each pot. Analyses of variance were performed with data from the EC-2 and G-2 soils at each dilution.

Differences in infective activity resulting from growth of different cultivars were significant ($P = 0.05$) in both the EC-2 and G-2 soils (Table 1). With the G-2 soil, infective activity following the growth of Saranac plants was greater than that following growth of Vernal, MnP-B1, and MnP-D1 plants at each dilution. There were no significant differences between the effects of the latter cultivars on the infective activity. In the EC-2 soil, infective activity values following growth of plants of both Vernal and Saranac were similar at all dilutions and different from values determined following growth of plants of the resistant cultivars at the 1:4 and 1:16 dilutions.

With the D-1 soil, only growth of Saranac plants resulted in high infective activity values in all pots. Growth of plants of Vernal and MnP-B1 resulted in high infective activity in two of three pots, while growth of plants of MnP-D1 did not result in high levels in any pot (Table 1).

Effect of temperature on infective activity of Phtophthora megasperma in soils during storage.—To determine whether temperature during storage affected changes in infective activity of *P. megasperma* in soils, samples of three soils with high levels of activity (following growth of alfalfa) were assayed at the beginning of the experiment (the initial infective activity), and after storage in sealed plastic bags for 3, 6, and 9 months at 4 C and 25 C. Moisture contents of soils determined at each sampling time showed that soil moisture levels remained constant in all soils at both temperatures for 9 months.

Infective activity declined significantly ($P = 0.05$) in all soils during storage at 25 C, but not at 4 C as measured at one or more dilution levels (Table 2). With one exception (C-1 at 1:16 dilution after 6 months), no values significantly lower than initial values were recorded for any soil at 4 C. No significant declines in infective activity were detected at any dilution level after storage for 3 months at 25 C in any soil, but values lower than initial values were recorded after storage for 6 or 9 months in all soils.

Effect of soil moisture on infective activity of Phytophthora megasperma in soils during storage.—To

TABLE 3. The infective activity of *Phytophthora megasperma* in naturally infested soils stored at 25 C for seven months at various moisture contents

Soil sample	Soil moisture[a] (%)	Infective activity[b]
D-1	17.3	76
	15.0	87
	10.9	89
	5.6	0
	3.0	0
Marshfield	12.4	100
	10.4	91
	6.9	0
	4.4	0
Mauston	10.1	87
	5.8	37
	5.3	0
	2.1	0
Winnebago	15.7	41
	9.6	44
	6.5	15
	2.8	0
	1.9	0

[a]Matric water potentials of all soils at all moisture contents are less than −15 bars except for D-1 at 17.3% (−11 bars) and Winnebago at 15.7% (−8 bars).
[b]Infective activity measured at a 1:1 (v/v) dilution of soils with a steamed mixture of sand and loam (1:1, v/v). Final activity expressed as percent of initial activity.

TABLE 4. The infective activity of *Phytophthora megasperma* in naturally infested soils after fallowing and after growth of different crops

Soil sample	Treatment[a]	Infective activity at soil dilution of[b] 1:1	1:4	1:16
Mauston	alfalfa	100 A[c]	98 A	113 A
	fallow	72 A	35 C	6 B
	clover	85 A	39 C	15 B
	soybeans	83 A	59 B	4 B
	peas	63 A	19 C	15 B
	oats	81 A	37 C	4 B
	corn	78 A	31 C	17 B
Winnebago	alfalfa	106 A	139 A	158 A
	fallow	88 AB	37 B	25 B
	clover	33 C	39 B	21 B
	soybeans	47 C	39 B	4 B
	peas	59 BC	45 B	33 B
	oats	47 C	53 B	33 B
	corn	45 C	32 B	13 B

[a]Soils maintained in greenhouse at 25 C for six months with daily watering. Tops of soybeans, peas, oats and corn removed after three months. Alfalfa and clover cut back every 4 weeks.

[b]Diluent soil was a steamed mixture of sand and loam (1:1, v/v).

[c]Final activity expressed as percent of initial activity. Values not followed by the same letter within a column for each soil are significantly different, $P = 0.05$, as determined by Duncan's multiple-range test.

determine whether changes in infective activity of *P. megasperma* in soils during storage were related to differences in soil moisture content, samples of four soils with high initial activity (following growth of alfalfa) were assayed before and after 7 months storage at different moisture levels. Initial activities of the soils were measured at the 1:1 dilution. Six samples of each soil were spread thinly on a lab bench and dried for 0, 0.5, 1, 2, 4, or 48 hours. Samples were then sealed in plastic bags and stored at 25 C. After 7 months, moisture content and infective activity at the 1:1 dilution were determined for all samples. Water potentials equivalent to measured moisture contents were determined from soil moisture characteristic curves obtained from pressure-plate extractors (Table 3).

High levels of infective activity were maintained in samples of three of the four soils during the storage at the highest moisture levels. In the fourth soil (Winnebago), less than 50% of initial activity was maintained in the three samples with the highest moisture content (Table 3).

Infective activity disappeared abruptly from samples of two soils (D-1 and Marshfield) at the lowest moisture levels. Less striking decreases in infective activity with lowered moisture content occurred in the other two soils (Winnebago and Mauston). For each soil, the lowest moisture level at which some infective activity was retained during storage was determined (by extrapolation of the moisture characteristic curves) to be a water potential of less than −40 bars.

Effects of long-term storage on survival of Phytophthora megasperma in soils.—Seventy-two samples of soils from alfalfa fields in Wisconsin, formerly rated positive for the presence of *P. megasperma* (17), were retested after 2.5 - 3.5 years storage in plastic bags at 25 C. Six 3-week-old Vernal seedlings were transplanted into one 10-cm pot of soil of each sample and grown for 6 weeks at 20 C. Soils were harvested, sieved, and assayed for the presence of *P. megasperma* with the seedling baiting technique using three plates of undiluted soil per sample. Samples were rated positive if the organism was detected on any of the 18 seedlings in the three plates (17).

Thirteen of 26 samples stored 3.5 years and 24 of 46 stored 2.5 years were rated positive. In most soil samples rated positive after planting to alfalfa, all seedlings in the three assay plates were heavily infected by *P. megasperma.*

Effect of crop rotations on changes in infective activity of Phytophthora megasperma in soils in the greenhouse.—To determine whether the growth of nonhost crops in infested soil would affect changes in infective activity of *P. megasperma,* levels of activity in two soils were determined before and after growth and decay of roots of five nonhost crops. Initial levels were measured following growth of alfalfa in the soils. Three 15-cm diameter pots of each soil were then planted to each of four nonhost species [peas (*Pisum sativum* L.), soybeans (*Glycine max* (L.) Merr.), clover (*Trifolium pratense* L.), and oats (*Avena sativa* L.)], and three 20-cm pots were planted to corn (*Zea mays* L.). Six-week-old Vernal alfalfa seedlings were transplanted into three additional 15-cm pots of each soil, and three pots were not planted to any crop. All pots were maintained at 25 C and watered daily. After 2-3 weeks, peas and soybeans were thinned to six plants per pot, clover to ten plants, oats to twelve plants, and corn to five plants. Tops of clover and alfalfa were cut back every 4 weeks; growth of other plants was unimpeded for 3 months, when tops of all plants were cut back. Pots were watered daily for an additional 3 months to promote decay of all plants except alfalfa and clover, which remained alive. Soils from the three pots of each crop were removed from the pots, thoroughly mixed, and sieved prior to measurement of *P. megasperma* activity.

The high infective activity levels recorded at the beginning of the experiment were maintained or increased in both soils after 6 months when there was continued growth of alfalfa (Table 4). All other treatments resulted in significant decreases in infective activity from initial values at one or more dilutions.

Evidence for a depression in infective activity of *P. megasperma* over that in fallow soil following growth of a nonhost crop was observed only in the Winnebago soil. Activity levels in soil samples planted to corn, oats, clover, and soybeans, measured at the 1:1 dilution, were significantly lower ($P = 0.05$) than the activity in fallow soil. This effect was not observed at the 1:4 and 1:16 dilutions.

In the Mauston soil, infective activity following growth of soybeans, as measured at the 1:4 dilution, was greater than that in samples left fallow or planted to any other crop except alfalfa. This is an anomalous effect and was not observed at the 1:16 dilution (Table 4).

DISCUSSION.—The infective activity of *P. megasperma* in naturally infested soils increased in the presence of alfalfa under conditions favorable for plant

growth and disease development. Activity decreased in soils both during storage and in the greenhouse in the absence of alfalfa. Decreases in infective activity during storage were affected by temperature and soil moisture content.

Planting soils with seedlings of one or both susceptible alfalfa cultivars resulted in greater increases in infective activity than planting with seedlings of one or both resistant cultivars. However, variance in results between soils in different pots planted to seedlings of the same cultivar was very high. Growth of seedlings of Vernal and MnP-B1 in the D-1 soil was accompanied by great increases in infective activity in two of three pots, but little or no increase occurred in the remaining pots with each cultivar. These results are typical of soils with low levels of infective activity, and they indicate that the failure to detect *P. megasperma* with alfalfa seedling baits in soil samples following growth of alfalfa cannot be taken to preclude the presence of the pathogen. The results further suggest that the distribution of *P. megasperma* in soils of alfalfa fields in Wisconsin, as determined previously (17), may have been underestimated because in some samples activity may not have been raised sufficiently for detection by planting to alfalfa for 8 weeks.

The effects of resistant and susceptible alfalfa cultivars on the activity of *P. megasperma* in soil are similar to reported effects of different tobacco cultivars on populations of *P. parasitica* var. *nicotianae* in soil. Flower and Hendrix (6, 7) reported that populations of this pathogen increase rapidly in the soil in the presence of susceptible plants, but slowly with resistant plants. They concluded that propagule population levels of *P. parasitica* var. *nicotianae* are determined by the amount and rate of pathogenesis. We also suggest that for Phytophthora root rot of alfalfa, high levels of infective activity in soil reflect high propagule levels of the pathogen; that such levels are produced only in disease situations; and that any factors which affect pathogenesis will also affect infective activity levels of *P. megasperma* in soils.

The rate of decline in infective activity of *P. megasperma* in stored soils over time is affected by temperature. High levels of activity were maintained at 4 C over 9 months, but not at 25 C, under similar and constant soil moisture conditions. An opposite effect of temperature was reported for *P. parasitica;* chlamydospores survived well in soil at 16-34 C, but survival was much reduced at temperatures below 10 C (21).

Infective activity of *P. megasperma* declined slightly in soils stored for 7 months at high moisture levels (near −15 bars water potential), but it disappeared from soils stored at low moisture levels (less than −40 bars). Similarly, survival of inoculum of *P. parasitica* (12, 21), *P. cinnamomi* (24), and *P. palmivora* (22) is reported to be poor or nonexistent in dry soils. Species of *Pythium,* in contrast, may survive well for several months to many years in dry soils (13, 18, 19, 21). We did not determine whether *P. megasperma* could again be recovered by planting dried soils to alfalfa to raise infective activity prior to testing with seedling baits. Populations of *P. parasitica* were reported to increase in soil remoistened after drying (12).

Although infective activity of *P. megasperma* decreased in all soils not maintained in alfalfa in the crop rotation experiment, in only one of two soils was the depression in activity following growth and decay of roots of nonhost plants significantly greater than that which occurred in fallow soil. These results suggest that crop rotations will not provide a practical means for control of Phytophthora root rot of alfalfa in Wisconsin or other midwestern states. The main crops which could be grown in rotation with alfalfa (corn, oats, soybeans, clover) did not uniformly or greatly depress infective activity of *P. megasperma* in the two soils studied here. To be useful in disease control, crop rotations would have to result in great decreases in infective activity in soil because even low levels are rapidly increased in the presence of susceptible alfalfa.

Crop rotations have seldom been reported to be useful in the control of other *Phytophthora* spp. in soil. Short-term rotations with agronomic crops did not significantly depress soil population levels of *P. parasitica* var. *nicotianae* (1, 7, 16). However, weeds were more effective than corn in rotations with tobacco in causing less disease by this pathogen (2). Growth of *Citrus* spp. in infested soil for one year rendered *P. cinnamomi* undetectable, but growth of macadamia (also a nonhost) did not (24).

The fact that high levels of infective activity of *P. megasperma* in soil are not maintained in the presence of growing and decaying roots of nonhost crops suggests that this organism has a narrow host range, and that it is not a strong competitive saprophyte. Similarly, studies with *P. cinnamomi* (14, 24), *P. erythroseptica* (23) and *P. parasitica* (11) have indicated that these species also cannot maintain high inoculum levels by colonization of nonhost material in soil.

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