Lack of Host Specificity Among Isolates of *Phytophthora megasperma*

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ABSTRACT

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Mahaleb cherry and Moapa 69 alfalfa seedlings were transplanted into pasteurized potting mix artificially infested with isolates of *Phytophthora megasperma* originally recovered from alfalfa, apple, apricot, cherry, Douglas fir, grape, juniper, kiwi, lilac, peach, pear, raspberry, soybean, or walnut and flooded for 48-hr periods at 2-wk intervals. After 17 wk at a soil temperature of 18–22 C, alfalfa and soybean isolates caused moderate root rot, a 40–62% decrease in root weight, and a 37–75% decrease in shoot weight on Mahaleb seedlings relative to uninoculated controls but no crown rot or plant death; all other isolates caused severe root rot, crown rot, and death of most Mahaleb seedlings. In the same experiment, isolates from fruit trees with small (29–34 μm) oogonia and high optimum (30 C) and maximum (36 C) growth temperatures were weakly to moderately virulent on alfalfa, as were isolates from soybean; isolates from fruit plants with large (38–43 μm) oogonia and low optimum (24 C) and maximum (30 C) growth temperatures and isolates from Douglas fir were moderately to highly virulent (23–95% decrease in root weight, 46–94% decrease in top weight relative to uninoculated controls). Alfalfa isolates were all highly virulent. Results were qualitatively similar in an experiment with soil temperatures of 18–29 C, but many isolates were less virulent than at the lower soil temperatures. Confirming this trend, a cherry isolate of *P. megasperma* caused 81–97% root rot on Mahaleb cherry when seedlings were grown in infested potting mix at constant soil temperatures of 10, 15, or 20 C but caused only negligible root rot at a constant soil temperature of 25 C. When Harosoy and Wayne soybeans were grown in potting mix infested with isolates of *P. megasperma* from the above hosts, those from soybean caused extensive root necrosis, whereas all other isolates caused varying degrees of minor root necrosis. Only soybean isolates caused expanding necrotic lesions when directly inoculated into hypocotyls of Wayne seedlings. Results from this study suggest that host-specific pathogenicity is not a common phenomenon among isolates of *P. megasperma*, although a given isolate may exhibit differential virulence against different hosts. The results further suggest that edaphic factors must be maintained to provide optimum conditions for disease development when assessing the pathogenicity of an isolate of *P. megasperma*, or its inherent virulence may be significantly underestimated.

Additional key words: *Glycine max*, *Medicago sativa*, *Prunus mahaleb*, soilborne disease, wet feet.

Phytophthora megasperma Drechsler sensu lato (5,17) is a diverse species encompassing a wide range of morphological and physiological types. Host specific isolates of *P. megasperma* have been reported from Douglas fir (4) and rose (14), and host specificity among isolates causing disease on alfalfa, soybean, and arrowleaf clover is considered so pronounced that the isolates have been designated formae speciales *medicaginis* Kuan and Erwin, *glycinea* Kuan and Erwin, and *trifolii* Pratt, respectively (9,15). Although host nonspecific isolates of *P. megasperma* are also recognized, including some that are pathogenic on alfalfa and Douglas fir (7), recent literature has generally emphasized the pathogenic specialization among isolates of this species.

P. megasperma is a pathogen with worldwide distribution (19) causing disease on a broad range of fruit and nut crops, vegetables, legumes, forest trees, and woody and herbaceous ornamentals (11). The extent of pathogenic specialization within *P. megasperma* is, therefore, an important consideration from a disease management perspective in addition to those primarily taxonomic (9) or ecological (7) in nature. For instance, common strategies designed to control disease through the reduction or exclusion of inoculum (e.g., sanitation or crop rotation) are fundamentally dependent on a knowledge of what potentially constitutes inoculum. That is, the potential for a particular isolate of *P. megasperma* to cause damaging levels of disease on more than one crop can directly influence the development of disease management practices for the crops in question.

Accordingly, this study was initiated to further examine the concept and extent of host specificity among isolates of *P. megasperma*. An abbreviated portion of this work has been published previously (20).

MATERIALS AND METHODS

Isolates. The 36 isolates of *P. megasperma* examined in this study are listed in Tables 1–3. The sources are Mircetich and Wilcox except for the following isolates: P 410 and P 1057 from alfalfa, P 405 from soybean, D. C. Erwin, University of California, Riverside; Alfalfa 1, Alfalfa 5, and AFI from alfalfa, R. L. Millar, Cornell University; C-17-2D, 304, and 306 from Douglas fir, P. B. Hamm, Oregon State University; 31-4-1 and 31-4-5 from soybean, S. D. Cohen, University of Maryland; and 32-1-5 and 32-1-7 from soybean, B. J. Castanho, Monsanto Corp., St. Louis, MO. The identity of all isolates was confirmed on the basis of colony morphology, cardinal growth temperatures, sporangium morphology, and the production of oospores in single culture with predominantly paragynous antheridia (17,18). Sporangium and oogonium dimensions were within the ranges established for the species (17,18).

Pathogenicity experiments. Pathogenicity of isolates of *P. megasperma* was determined on Mahaleb cherry (*Prunus mahaleb* L.), alfalfa (*Medicago sativa* L. 'Moapa 69'), and soybean (*Glycine max* (L.) Merr. 'Wayne' and 'Harosoy'). Pathogenicity on Mahaleb cherry was determined in two separate experiments by using the methods described previously (21). Briefly, this involves transplanting 8-wk-old seedlings into individual 1-L glazed crocks containing 20 cm^3 of vermiculite-oat-vegetable broth inoculum per 1,000 cm^3 of pasteurized potting mix (composed primarily of 2 volumes sand: 1 volume peat) and subjecting the seedlings to 48-hr periods of flooding (1 to 2 cm of free water standing on the soil surface) at 2-wk intervals beginning 14 days after transplanting. Pathogenicity and virulence were determined after 17-wk experimental periods on the basis of final root and shoot fresh weights, an estimate of the percent root mass visibly rotted, and the incidences of crown rot and seedling mortality. There were five replicate crocks per isolate in each experiment.

Pathogenicity on alfalfa was determined in the same experiments involving Mahaleb cherry, by using similar

procedures. Eight-week-old seedlings were transplanted into individual 1-L crocks containing artificially infested potting medium and flooded for 48-hr periods at 2-wk intervals beginning 14 days after transplanting, as described above. When flowering began approximately 8 wk after transplanting, the tops of all plants were harvested by cutting 2 to 3 cm above the crown, and the harvested weights were recorded. Top regrowth was harvested similarly 4 and 9 wk after the original cutting, then pathogenicity and relative virulence were assessed on the basis of cumulative fresh top weights, final fresh root weights, and a subjective root disease rating (0 = no apparent root rot; 1 = small black lesions on the tap and main lateral roots; 2 = larger lesions on the taproot with girdling of some laterals; 3 = extensive rotting of the taproot, moderate adventitious root system present; 4 = taproot rotted to just below the crown, extensive rotting of the adventitious root system, plant still alive; 5 = 100% root rot, plant dead). There were five replicate crocks per isolate in each experiment.

Pathogenicity on soybean was determined in two additional experiments. In the first, two techniques were used on young seedlings (first trifoliate leaves unfolding) of cultivar Wayne: 1) A small amount of mycelium grown on V-8 agar was inserted into slits made with a sterile scalpel on the seedling hypocotyl, the wound was sealed with petroleum jelly, and the presence of expanding necrotic lesions was recorded 2 wk later; and 2) seedlings were transplanted into individual 1-L crocks containing artificially infested potting mix as described for cherry and alfalfa, then flooded for 48- and 72-hr periods 6 and 20 days later, respectively. Pathogenicity was determined 5 days after the second flooding by washing the roots and rating disease on a 0–5 scale (0 = no apparent disease, 5 = dead). Five replicate seedlings per isolate were used for each technique. In the second experiment, five seeds of cultivar Wayne were planted into infested potting mix in each of five replicate 1-L crocks per isolate; five seeds of cultivar Harosoy were similarly planted into infested potting mix in each of five additional replicate crocks. All pots were flooded for a 48-hr period shortly after seedling emergence and for an additional 48-hr period 2 wk later, then total fresh root and top weights and a subjective root disease rating (0–5 scale) were determined on a per-pot basis.

All pathogenicity experiments were conducted in a greenhouse with supplemental lighting supplied as needed to provide a 15-hr day length.

TABLE 1. Pathogenicity and relative virulence of isolates of *Phytophthora megasperma* recovered from different hosts on Mahaleb cherry seedlings grown in infested potting mix for 17 wk in a greenhouse

Morphological group	Isolate[d]	Host[e]	Experiment 1[a]				Experiment 2[b]			
			Fresh weight (g)[c]		Root rot (%)[c]	Crown rot incidence[f]	Fresh weight (g)[c]		Root rot (%)[c]	Crown rot incidence[f]
			Roots	Shoots			Roots	Shoots		
	Uninoculated control		20.4 A	28.1 A	5 F	0/5	12.4 A	26.1 A	4 G	0/5
AL-1[g,h]	P 1057	alfalfa	20.4 A	25.5 A	6 F	0/5	6.1 BC	9.7 DE	34 DEF	0/5
	9-2-7	alfalfa	18.1 A	23.0 BC	7 F	0/5	...[i]	...	...	...
	P 410	alfalfa	13.8 B	17.7 DE	17 EF	0/5	...	...	...	...
	Alfalfa 1	alfalfa	...	...	...	...	7.3 B	10.4 CD	34 DEF	0/5
	Alfalfa 5	alfalfa	...	...	...	...	5.6 C	6.8 EF	40 DE	0/5
	AFI	alfalfa	...	...	...	...	4.7 CD	6.5 EFG	43 D	0/5
SB[g,j]	P 405	soybean	18.7 A	27.4 A	5 F	0/5	...	...	...	...
	31-4-5	soybean	...	...	...	...	7.5 B	16.4 B	28 F	0/5
	31-4-1	soybean	...	...	...	...	7.0 B	13.3 BC	31 EF	0/5
High/small[k]	20-3-9	apple	12.6 BC	17.1 DE	40 D	0/5	...	...	...	...
	4-1-5	apricot	12.3 BC	18.4 CD	44 CD	0/5	1.9 FGH	1.8 H	90 B	3/5
	24-4-9	apple	9.4 CD	13.3 DEF	42 D	0/5	3.9 DE	5.8 FG	77 C	0/5
	24-1-9	cherry	8.7 D	12.7 EF	52 BC	0/5	1.0 GH	0.6 H	100 A	5/5
	CH261S-1	cherry	7.2 D	9.6 F	60 B	0/5	4.1 DE	6.3 EFG	80 C	1/5
Low/large[l,m]	CH275C-1	cherry	2.8 E	3.1 G	96 A	3/5	1.7 FGH	0.6 H	99 AB	4/5
	24-3-8	kiwi	2.6 E	2.6 G	91 A	3/5	1.8 FGH	0.6 H	100 A	5/5
	23-1-1	grape	2.3 E	1.8 G	92 A	2/5	1.4 GH	0.5 H	100 A	5/5
	27-1-5	walnut	2.0 E	2.2 G	93 A	2/5	1.1 GH	0.6 H	100 A	5/5
	15-1-9	pear	1.8 E	0.9 G	96 A	4/5	1.6 FGH	0.5 H	100 A	5/5
	16-4-8	juniper	1.3 E	1.6 G	96 A	4/5	1.8 FGH	0.5 H	100 A	5/5
	14-4-1	raspberry	1.3 E	0.7 G	98 A	4/5	2.0 FGH	0.7 H	98 AB	4/5
	29-3-10	apple	1.3 E	0.7 G	100 A	5/5	1.6 FGH	0.7 H	98 AB	4/5
	30-1-9	almond	1.1 E	0.5 G	100 A	5/5	1.8 FGH	0.4 H	100 A	5/5
	13-1-5	lilac	0.8 E	0.8 G	98 A	4/5	2.4 FG	1.5 H	97 AB	3/5
	29-4-1	apricot	0.7 E	0.6 G	100 A	5/5	1.7 FGH	0.6 H	98 AB	4/5
	22-2-3	peach	0.7 E	0.6 G	100 A	5/5	...	...	...	...
DF2[g,m]	C-17-2D	Douglas fir	...	...	...	...	2.9 EF	3.2 GH	80 C	2/5
DF1[g,n]	304	Douglas fir	...	...	...	...	1.4 GH	0.5 H	100 A	5/5
	306	Douglas fir	...	...	...	...	1.6 FGH	0.6 H	100 A	5/5

[a] Experiment conducted May–September. Soil temperature was 18–29 C.
[b] Experiment conducted October–February. Soil temperature was 18–22 C.
[c] Values represent mean of five replicates. Means within a column not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Baysian K-ratio LSD rule.
[d] Vermiculite-oat inoculum of the indicated isolate was incorporated into pasteurized potting mix at the rate of 20 cm^3 of inoculum: 1,000 cm^3 of pottting mix.
[e] Host from which originally isolated.
[f] All seedlings with crown rot died.
[g] *Sensu* Hansen and Hamm (7).
[h] Placed in the ALF subgroup by Hansen et al (6).
[i] ... = Isolate not examined in this experiment.
[j] Placed in the SOY subgroup by Hansen et al (6).
[k] High optimum and max growth temperatures, oogonia 29–34 μm (see text); placed in the AC subgroup by Hansen et al (6).
[l] Low optimum and max growth temperatures, oogonia 38–43 μm (see text).
[m] Placed in the BHR subgroup by Hansen et al (6).
[n] Placed in the DF subgroup by Hansen et al (6).

RESULTS

Isolate groups. Isolates of *P. megasperma* recovered from fruit plants and woody ornamentals separated into two distinct groups based on cardinal growth temperatures, colony morphology, and oogonium diameters. Isolates in the first group (designated low/large in Tables 1–3) were typical of those described previously on cherry (12) and walnut (13), i.e., they produced radiate colonies on Difco corn meal agar at 21 C; the optimum temperature for mycelial growth was 24 C, with poor growth at 30 C and no growth at 33 C (Fig. 1); and average oogonium diameters ranged from 38 to 43 μm when formed on clarified V-8 juice agar amended with β-sitosterol (12). Isolates in the second group (designated high/small in Tables 1–3) were distinguished by radial to rosette colonies formed on corn meal agar at 21 C; optimum mycelial growth at 30 C, with vigorous growth at 33 C and poor growth at 36 C (Fig. 1); and average oogonium diameters ranging from 29 to 34 μm. Both groups produced similar ovoid to obpyriform sporangia of equivalent dimensions. The cardinal growth temperatures and dimensions of the sporangia and oogonia of the alfalfa, soybean, and Douglas fir isolates were consistent with those reported by Hansen and Hamm for the AL-1, SB, D1, and D2 isolates from these same hosts (7).

Pathogenicity and relative virulence of isolates of *P. megasperma*. In the first experiment with Mahaleb cherry

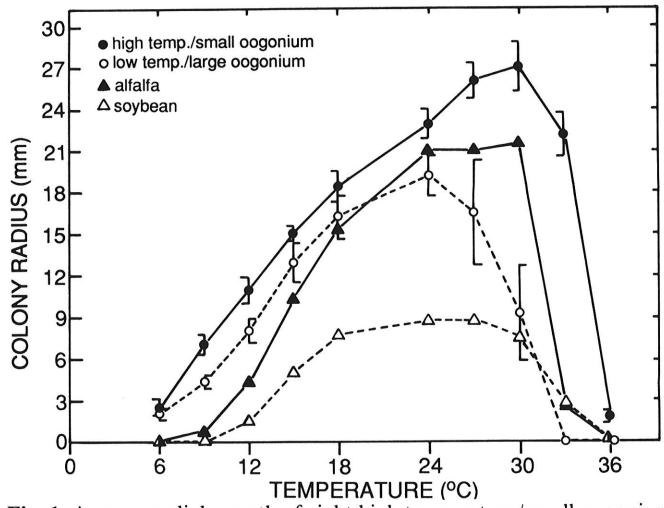

Fig. 1. Average radial growth of eight high temperature/small oogonium (29–34 μm) fruit tree isolates (●) and 11 low temperature/large oogonium (39–43 μm) fruit and woody ornamental plant isolates (o) of *Phytophthora megasperma* after 4 days on corn meal agar at different temperatures. Average radial growth of four alfalfa (▲) and six soybean (△) isolates of *P. megasperma* is shown for comparison. Vertical bars denote one standard deviation.

TABLE 2. Pathogenicity and relative virulence of isolates of *Phytophthora megasperma* from different hosts on Moapa 69 alfalfa grown in infested potting mix

Morphological group	Isolate[d]	Host[e]	Experiment 1[a] Fresh weight (g)[c] Roots	Experiment 1[a] Fresh weight (g)[c] Tops	Experiment 1[a] Root disease index[c]	Experiment 2[b] Fresh weight (g)[c] Roots	Experiment 2[b] Fresh weight (g)[c] Tops	Experiment 2[b] Root disease index[c]
	Uninoculated control		31.5 A	129.6 A	0.0 G	9.5 A	70.6 A	0.0 G
High/small[f]	24-1-9	cherry	26.8 AB	105.7 BC	1.4 DEF	8.1 ABC	51.5 B	1.0 F
	4-1-5	apricot	23.3 BC	114.0 AB	1.0 F	8.8 AB	52.4 B	0.8 FG
	24-4-9	apple	21.2 C	90.1 CDE	1.8 DE	5.7 D–G	48.5 BC	1.6 EF
	20-3-9	apple	18.6 CD	101.7 BCD	1.0 F	...[g]	...	...
	CH261S-1	cherry	13.5 E	87.3 DEF	1.0 F	6.3 C–F	49.0 BC	1.6 EF
SB[h,i]	P 405	soybean	18.7 CD	107.3 BC	1.0 F	...	...	...
	31-4-1	soybean	...	...	...	6.5 B–E	54.8 B	1.0 F
	31-4-5	soybean	...	...	...	5.9 C–G	45.3 BCD	1.0 F
Low/large[j,k]	24-3-8	kiwi	14.3 DE	71.9 FG	1.4 DEF	0.9 JKL	6.9 HIJ	4.2 AB
	13-1-5	lilac	13.1 EF	80.3 EFG	1.4 DEF	1.3 JKL	11.4 G–J	4.2 AB
	16-4-8	juniper	12.3 EFG	73.1 EFG	1.4 DEF	2.9 HIJ	19.3 FGH	3.2 CD
	27-1-5	walnut	11.2 E–H	73.5 EFG	1.6 DEF	1.2 JKL	4.2 IJ	4.4 A
	15-1-9	pear	10.2 E–I	73.0 EFG	1.4 DEF	1.2 JKL	7.8 G–J	4.6 A
	29-4-1	apricot	8.4 F–J	51.0 HI	1.6 DEF	3.9 GHI	25.7 EF	3.0 CD
	23-1-1	grape	7.7 G–K	65.5 GH	1.6 DEF	4.2 FGH	20.0 FG	2.4 DE
	29-3-10	apple	7.4 G–K	43.8 I	2.0 CD	2.4 H–K	14.8 F–I	3.4 BC
	22-2-3	peach	7.3 G–L	51.8 HI	1.4 DEF	...	...	...
	CH275C-1	cherry	6.3 H–M	49.7 HI	1.2 EF	1.3 JKL	9.7 G–J	4.2 AB
	30-1-9	almond	5.9 I–M	39.0 I	1.4 DEF	0.9 JKL	5.2 IJ	4.4 A
	14-4-1	raspberry	5.8 I–M	47.1 I	2.6 C	1.6 I–L	5.2 IJ	4.4 A
AL-1[h,l]	P 1057	alfalfa	3.8 J–M	10.4 J	3.8 B	0.2 KL	0.8 J	5.0 A
	9-2-7	alfalfa	2.8 KLM	8.0 J	4.2 AB	...	...	...
	P 410	alfalfa	1.9 M	6.6 J	4.4 A	...	...	...
	AFI	alfalfa	...	...	...	0.3 KL	0.5 J	4.8 A
	Alfalfa 5	alfalfa	...	...	...	0.1 L	0.4 J	5.0 A
	Alfalfa 1	alfalfa	...	...	...	0.1 L	0.5 J	5.0 A
DF1[h,m]	306	Douglas fir	...	...	...	1.8 I–L	9.8 G–J	4.4 A
	304	Douglas fir	...	...	...	7.4 A–D	33.5 DE	1.4 F
DF2[h,k]	C-17-2D-2	Douglas fir	...	...	...	4.5 E–H	38.4 CDE	1.6 EF

[a] Experiment conducted May–September. Soil temperature was 18–29 C.
[b] Experiment conducted October–February. Soil temperature was 18–22 C.
[c] Values represent means of five replicates. Means within a column not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Baysian K-ratio LSD rule.
[d] Vermiculite-oat inoculum of the indicated isolate was incorporated into pasteurized potting mix at the rate of 20 cm^3 of inoculum: 1,000 cm^3 of potting mix.
[e] Host from which originally isolated.
[f] High optimum and max growth temperatures, oogonia 29–34 μm (see text); placed in the AC subgroup by Hansen et al (6).
[g] ... = Isolate not examined in this experiment.
[h] *Sensu* Hansen and Hamm (7).
[i] Placed in the SOY subgroup by Hansen et al (6).
[j] Low optimum and max growth temperatures, oogonia 38–43 μm (see text).
[k] Placed in the BHR subgroup by Hansen et al (6).
[l] Placed in the ALF subgroup by Hansen et al (6).
[m] Placed in the DF subgroup by Hansen et al (6).

(conducted May–September, soil temperature 18–29 C), the lone soybean isolate was nonpathogenic, the alfalfa isolates were nonpathogenic to weakly virulent, the high temperature/small oogonium fruit tree isolates were moderately virulent (40–60% root rot, 28–65% decrease in root weight relative to the uninoculated controls, but no crown rot or plant death), and the low temperature/large oogonium isolates from fruit plants and woody ornamentals were all highly virulent (91–100% root rot and a high incidence of crown rot and seedling mortality), regardless of host of origin (Table 1). In the second experiment (conducted October–February, soil temperature 18–22 C), all isolates tested were pathogenic on Mahaleb seedlings. The low temperature/large oogonium isolates from fruit plants and woody ornamentals and the Douglas fir isolates were all highly virulent (80–100% root rot and a high incidence of crown rot and seedling death); the high temperature/small oogonium isolates from fruit trees were more variable, ranging from moderately to highly virulent (77–100% root rot, 0–100% incidence of crown rot and death); and isolates from soybean and alfalfa were moderately virulent, causing a 40–62% decrease in root weight and a 37–75% decrease in shoot weight relative to uninoculated controls but no crown rot (Table 1). *P. megasperma* was reisolated from diseased roots exposed to every isolate except those originally obtained from soybean, with a modified PVP medium (12).

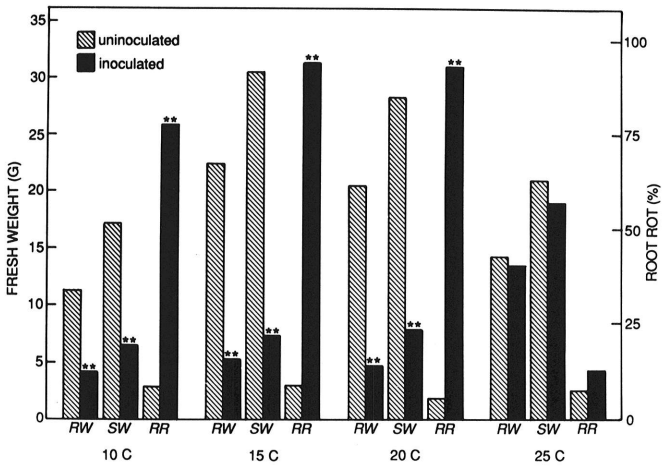

Fig. 2. Effect of soil temperature on the severity of root rot of Mahaleb cherry caused by *Phytophthora megasperma*. Mahaleb seedlings were grown for 3 mo in infested and uninfested potting mix maintained at constant soil temperatures of 10, 15, 20, and 25 C, and flooded for 48 hr periods at 2-wk intervals. Disease severity was assessed on the basis of final fresh root weights (RW), fresh shoot weights (SW), and an estimate of the percent root mass visibly rotted (RR). Mean values for inoculated seedlings denoted (**) are significantly different ($P = 0.01$) from the uninoculated check treatment according to the one-sided Student's t test.

Similar differences in virulence to alfalfa were noted among isolates and between experiments. In the first experiment (May–September, soil temperature 18–29 C), the soybean isolate and the high temperature/small oogonium fruit tree isolates were weakly to moderately virulent (15–57% decrease in root weight, 12–33% decrease in top weight, root disease index 1.0–1.8), the low temperature/large oogonium fruit and ornamental plant isolates were moderately virulent (55–82% decrease in root weight, 45–70% decrease in top weight, root disease index 1.2-2.6), and the alfalfa isolates were highly virulent (88–94% decrease in root weight, 92–95% decrease in shoot weight, root disease index 3.8–4.4) (Table 2). In the second experiment (October–February, soil temperature 18–22 C), the soybean isolates and the high temperature/small oogonium fruit isolates were again weakly to moderately virulent (7–40% decrease in root weight, 22–36%

TABLE 3. Pathogenicity and relative virulence of isolates of *Phytophthora megasperma* from different hosts on Harosoy soybeans planted in infested potting mix

Morphological group	Isolate[a]	Host[b]	Fresh wt roots (g)[c]	Fresh wt tops (g)[c]	Disease index[c]
	Uninoculated control		18.7 A	19.7 A	0.0 L
High/small[d]	CH270S-1	cherry	18.4 A	19.2 AB	0.8 IJK
	CH261S-1	cherry	18.1 A	19.6 A	0.6 JK
	24-4-9	apple	17.9 AB	19.5 A	0.4 KL
	20-1-3	apple	16.3 BC	17.5 BCD	0.6 JK
	25-1-3	cherry	15.7 CDE	19.4 A	1.0 HIJ
	12-4-3	apple	14.9 C–F	20.4 A	1.0 HIJ
	24-1-9[d]	cherry	13.5 FGH	18.9 ABC	1.0 HIJ
	4-1-5[d]	apricot	12.4 GHI	15.4 FG	1.6 EFG
AL-1[e,f]	Alfalfa 1	alfalfa	15.9 CD	16.5 DEF	1.6 EFG
	AFI	alfalfa	13.4 FGH	15.6 EFG	2.6 BC
	P 1057	alfalfa	12.6 GHI	16.6 DEF	2.0 DE
Low/large[g,h]	13-1-5	lilac	14.9 C–F	20.1 A	1.4 FGH
	15-1-9	pear	14.8 C–F	18.9 ABC	1.8 DEF
	24-3-8	kiwi	14.7 C–F	18.7 ABC	1.8 DEF
	CH275C-1	cherry	13.8 FGH	15.7 EFG	1.0 HIJ
	14-4-1	raspberry	13.4 FGH	17.3 CDE	2.0 DE
	23-1-1	grape	13.1 FGH	16.3 DEF	1.4 FGH
	30-1-9	almond	11.1 I	15.9 DEF	1.8 DEF
DF2[e,h]	C-17-2D-2	Douglas fir	13.9 E–H	19.0 ABC	0.8 IJK
DF1[e,i]	304	Douglas fir	14.1 D–F	17.4 BCD	2.2 CD
	306	Douglas fir	12.3 HI	14.0 G	2.8 B
SB[e,j]	31-4-5	soybean	2.5 J	4.5 H	4.8 A
	32-1-5	soybean	1.5 J	2.1 I	4.8 A
	32-1-7	soybean	1.5 J	2.2 I	4.8 A

[a] Vermiculite-oat inoculum of the indicated isolate was incorporated into pasteurized potting mix at the rate of 20 cm^3 of inoculum: 1,000 cm^3 of potting mix.
[b] Host from which originally recovered.
[c] Values represent the mean of five replicate pots, with five seeds planted per pot. Means within a column not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Baysian K-ratio LSD rule.
[d] High optimum and max growth temperatures, oogonia 29–34 μm (see text); placed in the AC subgroup by Hansen et al (6).
[e] *Sensu* Hansen and Hamm (7).
[f] Placed in the ALF subgroup by Hansen et al (6).
[g] Low optimum and max growth temperatures, oogonia 38–43 μm (see text).
[h] Placed in the BHR subgroup by Hansen et al (6).
[i] Placed in the DF subgroup by Hansen et al (6).
[j] Placed in the SOY subgroup by Hansen et al (6).

decrease in shoot weight, root disease index 0.8–1.6), the Douglas fir and the low temperature/large oogonium fruit and ornamental plant isolates were moderately to highly virulent (23–95% decrease in root weight, 46–94% decrease in top weight, root disease index 1.4–4.6), and each alfalfa isolate was highly virulent, causing nearly uniform plant mortality (Table 2). *P. megasperma* was reisolated from diseased roots exposed to every isolate, with a modified PVP medium (12).

In the first pathogenicity experiment on soybean, all four soybean isolates caused expanding necrotic lesions when directly inoculated into seedling (cultivar Wayne) hypocotyls. In contrast, no other isolate of *P. megasperma* caused lesions when similarly inoculated, including: three from alfalfa, four from Douglas fir (two DF 1 and two DF 2 types [4,7]), eight low temperature/large oogonium fruit isolates, and seven high temperature/small oogonium fruit isolates. Similarly, when seedlings were transplanted into infested potting mix and flooded, the soybean isolates caused extensive root necrosis (disease index = 4.2–4.8 on a 0–5 scale), whereas all other isolates caused only minor root necrosis (disease index = 0.6–1.6). Similar results were obtained when seeds of cultivar Wayne or Harosoy were planted directly into infested potting mix and flooded after emergence (Table 3). The soil temperature ranged between 18 and 22 C in both experiments.

Effect of soil temperature on disease development. To examine the effect of soil temperature on disease development suggested by results from the cherry/alfalfa pathogenicity experiments, we transplanted 8-wk-old Mahaleb seedlings into 1-L glazed crocks filled with potting mix infested with a low temperature/large oogonium isolate (# 5-4-5) of *P. megasperma* obtained from cherry. The soil was maintained at a constant temperature of 10, 15, 20, or 25 C for the duration of the 3-mo experimental period by placing the crocks in thermostatically controlled water baths immediately after transplanting, and plants were flooded for 48-hr periods at 2-wk intervals beginning 2 wk after transplanting. Drainage was accomplished after flooding or irrigation periods by means of a vacuum pump attached to a glass tube, which was inserted at the time of transplanting into a layer of coarse gravel that collected excess water at the bottom of each crock. At the end of the experiment, seedlings grown in potting mix infested with *P. megasperma* at soil temperatures of 10, 15, and 20 C had 81–97% root rot and significantly ($P = 0.01$) less root and shoot fresh weights than the uninoculated controls. In contrast, seedlings grown in infested potting mix with a constant soil temperature of 25 C had negligible root rot, and root and shoot fresh weights were not significantly ($P = 0.05$) different from uninoculated controls (Fig. 2). Subsequent laboratory studies showed that sporangia of this isolate of *P. megasperma* germinated indirectly in flooded soil at 10, 15, and 20 C, but only germinated directly at 25 C.

DISCUSSION

The results of this study suggest that host specificity is not a common phenomenon among isolates of *P. megasperma*, although a given isolate may exhibit differential virulence against various hosts. For instance, alfalfa isolates were consistently the most aggressive against alfalfa in our tests, yet on this host merely represented one end of an overlapping continuum of virulence ranging from mild to extreme (Table 2). Similarly, isolates of *P. megasperma* from all hosts were pathogenic on Mahaleb cherry, although those from alfalfa and soybean were less virulent than isolates from other hosts (Table 1).

Our results suggest that isolates of *P. megasperma* from Douglas fir, deciduous fruit and nut trees, and woody ornamentals are potentially much more virulent on alfalfa than previous reports have indicated (4,7,9). However, the severity of disease caused by *P. megasperma* can be strongly influenced by subtle changes in edaphic factors, and it is possible that previous studies provided suboptimal conditions for infection and disease development by nonalfalfa isolates. For instance, high soil temperatures can clearly reduce or prevent the development of disease caused by some isolates of *P. megasperma* (Fig. 2), probably because these temperatures are not conducive for the release of zoospores from sporangia (16; authors, *unpublished*). Isolate # 5-4-5 would likely be judged nonpathogenic on Mahaleb cherry in an experiment where the soil temperature was consistently 25 C or higher but would be judged highly virulent if the soil temperature were maintained at 15–20 C (Fig. 2). Similarly, many nonalfalfa isolates were only weakly to moderately virulent on alfalfa when the soil temperature ranged between 18 and 29 C in our Experiment 1 but were moderately to highly virulent at the lower soil temperatures of 18–22 C in Experiment 2 (Table 2).

Soil water matric potential (ψ_m) is another factor that exerts a major influence on the incidence and severity of disease caused by *P. megasperma*. For example, our previous studies (22,23) have shown that isolate CH 275 C-1 is highly virulent on Mahaleb cherry under a soil water regime that includes regular 48-hr flooding ($\psi_m = 0$) periods but causes relatively little disease if flooding periods are restricted to 0–24 hr duration. It is possible that previous studies may have overestimated the degree of pathogenic specialization among isolates of *P. megasperma* by failing to regulate ψ_m altogether (4,7) or by attempting to "flood" by placing test pots in water-filled saucers (9). In the latter instance, soil ψ_m in the test pots would be maintained at zero only at or below the level of water in the saucer; at a height 10 cm above this level (i.e., in the probable vicinity of the plant crown), soil ψ_m would be −10 mbar, which is distinctly suboptimal for infection of both Mahaleb cherry (22) and alfalfa (9) by *P. megasperma*. Clearly, it is important that edaphic factors be maintained to provide optimum conditions for disease development when assessing the pathogenicity of an isolate of *P. megasperma*, or its inherent virulence may be significantly underestimated.

This appears to be the first report in which the pathogenicity of high temperature/small oogonia isolates of *P. megasperma* from fruit trees has been examined. The relative lack of virulence of these isolates on alfalfa and soybean in compârison with Mahaleb cherry further strengthens the argument that oogonium size, per se, is an unreliable indicator of the host preference of isolates of *P. megasperma* (6). It is interesting to note that these same isolates, which we differentiated from other fruit plant isolates on the basis of cultural and morphological characters, were independently separated from other isolates on the basis of electrophoretic patterns of total proteins in a recent study by Hansen et al, i.e., as belonging to their "AC" group (6). Further research will be required to determine whether these isolates represent a product of selection in fruit orchards as hypothesized by the aforementioned authors (6), or perhaps represent a poorly defined group of isolates of *P. megasperma* with small oogonia that are capable of attacking a wider variety of crops, including cauliflower (17) and asparagus (3).

Only soybean isolates of *P. megasperma* were highly virulent to soybeans in our tests, confirming the general conclusions of previous workers (4,7,9,15). However, unlike previous investigators (2,4), we did not obtain a hypersensitive response in soybean hypocotyls after direct inoculation with "DF 1"-type isolates from Douglas fir, although this may be due to the limited number of hypocotyl inoculations performed in the present study or to methodological differences among workers. Whereas our results may support the concept of designating soybean isolates of *P. megasperma* as forma specialis *glycinea* (9), they are equally consistent with Kaufmann and Gerdemann's original contention (8) that the causal agent of root and stem rot of soybean is actually a distinct species, i.e., *P. sojae*. Hansen and Hamm found that isolates of *P. megasperma* from several "host-specialized" groups, including those highly virulent to soybean, were morphologically distinct but opined that the differences were not sufficient to warrant the erection of separate species at that time (7). However, subsequent work by Hansen et al (6) provides further evidence that isolates from soybean that are currently designated as *P. megasperma* might more appropriately be placed within a separate taxon.

The diversity among isolates of *P. megasperma* was first noted 50 yr ago (17), and the best systematic approach to recognizing this variability is still a matter of debate (1,2,6). Recent attempts to

subdivide the taxon into formae speciales (9,15) have been supported on the basis of improved communication relative to the isolates in question (2). However, our results strongly support the cautions already raised that these designations not be applied widely (1) or extended automatically to all isolates recovered from a particular host (7), lest an inappropriate implication be communicated; host specific pathogenicity among isolates of *P. megasperma* appears to be the exception rather than the norm. Alternatively, differential virulence or tendencies towards host preference among diverse groups of isolates currently designated *P. megasperma* may provide useful clues in constructing a revised taxonomic system for this heterogeneous species, based on recognizable morphological and physiological (6,7) differences among such groups.

LITERATURE CITED

1. Brasier, C. M. 1983. Problems and prospects in *Phytophthora* research. Pages 351-364 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN. 392 pp.
2. Erwin, D. C. 1983. Variability within and among species of *Phytophthora*. Pages 149-165 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN. 392 pp.
3. Falloon, P. G., Mullen, R. J., Benson, B. L., and Grogan, R. G. 1985. Control of Phytophthora rot with metalaxyl in established asparagus. Plant Dis. 69:921-923.
4. Hamm, P. B., and Hansen, E. M. 1981. Host specificity of *Phytophthora megasperma* from Douglas fir, soybean, and alfalfa. Phytopathology 71:65-68.
5. Hamm, P. B., and Hansen, E. M. 1982. Single-spore isolate variation: The effect on varietal designation in *Phytophthora megasperma*. Can. J. Bot. 60:2931-2938.
6. Hansen, E. M., Brasier, C. M., Shaw, D. S., and Hamm, P. B. 1986. The taxonomic structure of *Phytophthora megasperma*: Evidence for emerging biological species groups. Trans. Br. Mycol. Soc. 87:557-574.
7. Hansen, E. M., and Hamm, P. B. 1983. Morphological differentiation of host-specialized groups of *Phytophthora megasperma*. Phytopathology 73:129-134.
8. Kaufmann, M. J., and Gerdemannn, J. W. 1958. Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology 48:201-208.
9. Kuan, T.-L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
10. Kuan, T.-L., and Erwin, D. C. 1982. Effect of soil matric potential on Phytophthora root rot of alfalfa. Phytopathology 72:543-548.
11. MacFarlane, H. H., compiler. 1968. Plant Host-Pathogen Index to Volumes 1–40, Review of Applied Mycology. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 820 pp.
12. Mircetich, S. M., and Matheron, M. E. 1976. Phytophthora root and crown rot of cherry trees. Phytopathology 66:549-558.
13. Mircetich, S. M., and Matheron, M. E. 1983. Phytophthora root and crown rot of walnut trees. Phytopathology 73:1481-1488.
14. Nagai, Y., Takeuchi, T., and Watanabe, T. 1978. A stem blight of rose caused by *Phytophthora megasperma*. Phytopathology 68:684-688.
15. Pratt, R. G. 1981. Morphology, pathogenicity, and host range of *Phytophthora megasperma*, *P. erythroseptica*, and *P. parasitica* from arrowleaf clover. Phytopathology 71:276-282.
16. Sugar, D. 1977. The development of sporangia of *Phytophthora cambivora*, *P. megasperma*, and *P. drechsleri* and severity of root and crown rot in *Prunus mahaleb* as influenced by soil matric potential. M. S. thesis. University of California, Davis. 56 pp.
17. Tompkins, C. M., Tucker, C. M., and Gardner, M. W. 1936. Phytophthora root rot of cauliflower. J. Agric. Res. 53:685-692.
18. Waterhouse, G. M. 1963. Key to the species of *Phytophthora* De Bary. Commonw. Mycol. Inst. Mycol. Pap. 92. 22 pp.
19. Waterhouse, G. M., and Waterston, J. M. 1966. C. M. I. Descriptions of Pathogenic Fungi and Bacteria. No. 115. Commonw. Mycol. Inst., Kew, Surrey, England. 2 pp.
20. Wilcox, W. F., and Mircetich, S. M. 1982. Host non-specificity of *Phytophthora megasperma* isolates recovered from 14 different plant species. (Abstr.) Phytopathology 72:995.
21. Wilcox, W. F., and Mircetich, S. M. 1985. Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75:221-226.
22. Wilcox, W. F., and Mircetich, S. M. 1985. Influence of soil water matric potential on the development of Phytophthora root and crown rots of cherry. Phytopathology 75:648-653.
23. Wilcox, W. F., and Mircetich, S. M., 1985. Effects of flooding duration on the development of Phytophthora root and crown rots of cherry. Phytopathology 75:1451-1455.