Host Specificity of *Phytophthora megasperma* from Douglas Fir, Soybean, and Alfalfa

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ABSTRACT

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A total of 28 isolates of *Phytophthora megasperma* from either Douglas fir, alfalfa, or soybean were tested for pathogenicity to Douglas fir, alfalfa, and soybean. Isolates from alfalfa and soybean were pathogenic only to their original hosts. One group of isolates from Douglas fir was strongly pathogenic to both Douglas fir and soybean; a second group was less pathogenic to Douglas fir and was not pathogenic to soybean. The first group from Douglas fir was virulent on seven soybean cultivars used to define races of *P. megasperma* f. sp. *glycinea* and on cultivar Tracy. Applicability of the formae speciales concept to *P. megasperma* is discussed.

Additional key words: Trifolium vesiculosum, Glycine max, Medicago sativa, Pseudotsuga menziesii.

Phytophthora megasperma Drechs. has been isolated from many hosts since its original description on hollyhock (3). However, recent investigations have involved primarily alfalfa and soybean, and host specificity of isolates from those crops was demonstrated. Hildebrand (7) recognized that isolates from soybean, which he designated *P. megasperma* var. *sojae*, differed from *P. megasperma* in having smaller oogonia. Hildebrand's isolates were not pathogenic to alfalfa or 47 other crop plant cultivars representing 28 species. Subsequently, host specificity has been demonstrated for isolates from alfalfa, soybean, and arrowleaf clover (10, 12; D. P. Maxwell and R. G. Pratt, *personal communication*). Kuan and Erwin (10) erected *formae speciales* to subdivide *P. megasperma* based on host specificity. Isolates from soybean

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and alfalfa were named *P. megasperma* f. sp. *glycinea*, and *P. megasperma* f. sp. *medicaginis*, respectively.

P. megasperma was one of several *Phytophthora* spp. causing root rot on Douglas fir in forest tree nurseries in the Pacific Northwest (5,6). The purpose of this report is to compare the pathogenicity and virulence of *P. megasperma* isolates from Douglas fir with isolates from other hosts reported to be host specific.

MATERIALS AND METHODS

Pathogenicity of 28 isolates of *P. megasperma* from three hosts was evaluated. Four isolates were from alfalfa, five from soybean (races 1 and 3), and 19 from Douglas fir. Isolates from Douglas fir had previously been identified by their nonpapillate, ovoid to obpyriform sporangia with internal proliferation in liquid culture, smooth-walled oogonia (39–59 μm diameter) and mostly paragynous antheridia (15,20).

Pathogenicity to Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) was determined by both stem and root inoculations. Seedlings grown for 8 mo in 250-cc plastic containers were inoculated in the main stem by cutting a 1.5 cm longitudinal slit to the cambium and placing a small quantity of mycelium from colonies grown in pea broth (18) between bark and xylem. Wounds were covered for 7 days with wet cotton wrapped with tape, then exposed. Three trees were inoculated with each isolate and randomized in the greenhouse. The length of the lesion was measured after 42 days. Average minimum and maximum temperatures were 21 and 28 C, respectively.

Root inoculations were accomplished by planting 2-yr-old Douglas-fir seedlings in infested soil. Inoculum was grown in cornmeal sand (CMS, 250 ml # 16 (1.6 mm diameter) quartz sand, 13 ml cornmeal, and 110 ml of distilled water autoclaved in 500 ml flasks) incubated 3 wk, then individual isolates were mixed 1:32 with a steamed, clay-loam soil. Single trees were transplanted into infested soil in 400-ml plastic tubes (three trees per isolate) and positions randomized on the greenhouse bench. Pots were watered daily to field capacity. At 8 wk, individual root systems were scored 0, 1, 2, 3, or 4 on the basis of root discoloration of 0–10, 11–25, 26–50, 51–75, and 76–100%, respectively.

Pathogenicity of *P. megasperma* isolates from Douglas fir, alfalfa, and soybean to soybean was compared by stem inoculations (11) of cultivar Harosoy. The Harosoy seeds were planted in 2.5 L pots, thinned to five plants per pot after 6 days and inoculated at 9 days. Small portions of oatmeal agar inoculum (11) or colonies grown for 7 days in pea broth at 20 C were inserted in a slit in the hypocotyl just below the cotyledons of each plant. Slits were covered with petrolatum. Fifteen plants were inoculated with each isolate. Slits on controls were cut and covered with petrolatum but not inoculated. The positions of the pots were randomized in the greenhouse and the plants were watered daily. Symptoms were recorded after 1, 2, and 8 days.

The differential cultivars described by Laviolette and Athow (11) and cultivar Tracy were inoculated with three isolates from Douglas fir and single isolates of races 1 and 3, *P. megasperma* f. sp. *glycinea*. Six to 13 plants of each cultivar (depending on availability) were inoculated as described above and placed in a controlled temperature chamber (25–27 C) on a 12-hr night-day cycle. Mortality was recorded 6 days after inoculation.

Seeds of alfalfa (*Medicago sativa* L. 'Vernal') were germinated

TABLE 1. Reciprocal pathogenicity of *Phytophthora megasperma* isolates from Douglas fir, Vernal alfalfa, and Harosoy soybean

Host of origin and isolate no.		Source	Douglas fir		Alfalfa	Soybean[x]		
			Lesion growth (mm/day)[x]	Root rating[y]	Root rating[y]	Wilted (%)	Mortality (%)	
						1 day	2 days	9 days
Douglas fir — Group 1[z]								
B3A		Oregon	1.7	4.0	1.7	60	0	47
304		Oregon	2.0	0.3	1.4	73	0	73
B2-17		Oregon	0.7	3.7	1.1	67	0	73
306		Oregon	1.3	3.7	1.8	73	0	53
284		Oregon	2.7	3.7	1.3	40	0	67
345		Oregon	1.9	3.0	2.0	93	0	73
Douglas fir — Group 2[z]								
C-17-2N-5		Oregon	0.4	0.0	1.8	0	0	0
341		Oregon	0.3	1.7	1.6	0	0	0
C-17-2D-2		Oregon	0.3	2.0	1.4	0	0	0
BIC		Oregon	0.3	0.0	0.9	0	0	0
C-17-2N-4		Oregon	0.4	0.0	1.8	0	0	0
307		Oregon	0.3	0.0	2.3	0	0	0
336		Washington	0.4	2.7	1.8	0	0	0
260		Oregon	0.3	0.0	1.3	0	0	0
B+2		Oregon	0.3	0.7	1.3	0	0	0
316		Oregon	0.4	0.0	1.7	0	0	0
520		Oregon	0.3	0.3	1.1	0	0	0
337		Oregon	0.3	0.3	1.5	0	0	0
C-17-2N-6		Oregon	0.3	0.3	1.3	0	0	0
Soybean								
36	Race 1	C. Grau—WI	0.2	0.1	1.4	<1	0	67
908	Race 1	C. Grau—WI	0.2	1.3	1.6	<1	33	87
16	Race 3	C. Grau—WI	0.3	0.0	2.3	0	13	73
411	Race 3	C. Grau—WI	0.1	0.0	1.3	20	20	67
909	Race 3	C. Grau—WI	0.2	0.7	1.4	<1	27	100
Alfalfa								
5b		D. Maxwell—WI	0.2	0.0	2.5	0	0	0
DA		D. Maxwell—WI	0.2	1.0	3.0	0	0	0
Jefferson 1A		R. Pratt—MS	0.3	0.3	3.4	0	0	0
Ozaukee-2A		R. Pratt—MS	0.3	0.7	2.2	0	0	0
Control			0	0.3	.3	0	0	0

[x] Stem inoculation.

[y] Seedlings transplanted to infested soil. Douglas-fir root disease ratings: 0,1,2,3,4,=0–10, 11–25, 26–50, 51–75, and 76–100%, respectively. Alfalfa root disease ratings 0, 1, 2, 3, 4, = <1, 1–25, 26–50, 51–75, and 76–100%, respectively.

[z] One of two groups of *P. megasperma* isolated from Douglas fir: Group 1, pathogenic to both Douglas fir and soybean; Group 2, pathogenic only to Douglas fir.

on moist filter paper for 48–72 hr and planted in 400-ml wax-lined soft drink cups with bottom perforations (five seedlings per cup). Plants were transplanted to infested clay-loam soil after 5–6 wk. Inoculum was prepared as for the Douglas-fir root test. Three seedlings for each isolate were transplanted to each of three 540-ml cups of infested soil. Controls were transplanted similarly using sterile CMS. Pots were arranged randomly in the greenhouse and watered daily to field capacity. Average minimum and maximum daily temperatures during this test were 20 and 28 C. After 4 wk, top and root symptoms were rated and fresh weight of plants from each pot was obtained. Top symptoms were rated 1 (healthy), 2 (stunted and chlorotic), or 3 (severely wilted or dead). Root symptoms were scored 0, 1, 2, 3, or 4 for < 1, 1–25, 26–50, 51–75, or 76–100% root discoloration, respectively.

Significance of differences between disease scores were determined by using Duncan's new multiple range test (17).

RESULTS

Pathogenicity to Douglas fir. Isolates from Douglas fir formed two distinct groups based on their pathogenicity to Douglas fir and soybean. Six isolates from Douglas fir (DF Group 1) were pathogenic to both Douglas fir and soybean. Thirteen isolates (DF Group 2) caused damage only on Douglas fir (Tables 1 and 2).

DF Group 1 isolates caused extensive root rot and large lesions on Douglas-fir seedlings. Reddish brown subcortical lesions on the tap root extended above the soil line on most trees. Ten of 13 DF Group 2 isolates caused little or no discoloration of tap roots. Lateral roots were discolored only near apical areas when inoculated with alfalfa, soybean, or DF Group 2 isolates. Similarly, lengths of lesions caused by alfalfa, soybean, and DF Group 2 isolates were significantly smaller than those of DF Group 1 isolates.

Pathogenicity to soybean. A preliminary experiment showed that inoculum grown on oatmeal agar or pea broth was equally effective in killing 9-day-old Harosoy seedlings. Thus, in subsequent tests, inoculum grown on pea broth was used.

Isolates of DF Group 1 caused extensive wilting of 'Harosoy' soybeans 24 hr after inoculation (Tables 1 and 2). Other isolates caused few symptoms during this period. Mortality was first observed at 48 hr on plants inoculated with soybean isolates and at 96 hr on plants inoculated with DF Group 1 isolates. After 9 days, mortality caused by soybean and DF Group 1 isolates was 79 and 64%, respectively.

Pathogenicity to soybean race differentials. As predicted by Laviolette and Athow (11), isolate 908 (race 1) was avirulent to all cultivars except Harosoy, and isolate 909 (race 3) was avirulent except to Harosoy and Harosoy 63 (Table 3). Group 1 DF isolates 304 and 306 were pathogenic to all soybean cultivars tested, including Tracy. A single isolate of DF Group 2 (341) was avirulent to all soybean differentials and Tracy. No mortality occurred in the controls.

Pathogenicity to alfalfa. Alfalfa isolates caused significantly more stunting, wilting, and taproot necrosis on alfalfa than did isolates from other hosts (Tables 1 and 2). Top symptoms caused by Douglas-fir and soybean isolates were similar and usually not severe. Controls had no top symptoms. Average fresh weights of alfalfa plants inoculated with isolates from Douglas fir and soybean were greater than the average fresh weight of plants inoculated with alfalfa isolates. Fresh weight of control plants was greater than each of the other treatments.

DISCUSSION

Results of this study confirm the occurrence of host specificity among isolates of *P. megasperma* and support the grouping of isolates into *formae speciales* (10; D. P. Maxwell and R. G. Pratt, *personal communication*). Seemingly, this contrasts with earlier reports where *P. megasperma* isolates cause damage on more than one plant species (1,7–9,13,14). These reports were based on damping-off of seedlings, or stem or fruit inoculations were made without collaborating evidence from root inoculations or disease in the field. If interpretation of host range is limited to cases of clearly established pathogenicity, however, then these early reports also support host specificity.

The term host specificity has been used to imply pathogenicity by *P. megasperma* isolates showing limited or highly selective pathogenicity. By this method, host specificity may involve two or several hosts as used for *Fusarium* (16). Results of Nagai et al (14), where rose isolates killed root-inoculated hollyhock as well as those reported here on Douglas fir, indicate dual pathogenicity for these host-specific *P. megasperma* isolates. In our tests, isolates from alfalfa and soybean were pathogenic only to their original hosts while DF Group 1 isolates were strongly pathogenic both to Douglas fir and soybean, though not alfalfa. This indicates that some plant species may be common hosts for populations of *P. megasperma* which differ in pathogenic specialization or that host ranges of different formae speciales may overlap.

The DF Group 1 isolates of *P. megasperma* from Douglas fir could be considered a new race of *P. megasperma* f. sp. *glycinea* due to their pathogenicity to soybean (Tables 1 and 2). However, none of the soybean isolates exhibited similar pathogenicity to Douglas fir. Also, the DF Group 1 isolates appear to be virulent to seven cultivars of soybean which are used to differentiate races of *P. megasperma* f. sp. *glycinea*, and also to the cultivar Tracy reported to be resistant to races 1 through 9 (11). This suggests that DF Group 1 isolates have a different physiological basis for pathogenicity to soybean than do isolates of *P. megasperma* f. sp. *glycinea*.

The second group of isolates from Douglas fir, DF Group 2, were also host specific. Lesion size produced by DF Group 2 isolates was significantly greater on Douglas fir than lesions produced by isolates from the leguminous hosts. DF Group 2 isolates were avirulent to both soybean, and alfalfa. However, DF Group 2 isolates did not cause significantly greater root disease of Douglas fir than isolates from soybean, or alfalfa. Low root disease ratings of DF Group 2 isolates on Douglas fir probably resulted from the

TABLE 2. Differences in pathogenicity of *Phytophthora megasperma* isolates to Douglas fir, Vernal alfalfa, and Harosoy soybean

Host of origin	Douglas fir		Alfalfa	Soybean[w]		
	Lesion growth (mm/day)[w]	Mean root rating[x]	Mean Root rating[x]	Wilted (%)	Mortality (%)	
				1 day	2 days	9 days
Douglas Fir—Group 1[y]	1.70 d[z]	3.10 c	1.6 b	68 c	0 a	64 b
Douglas Fir—Group 2[y]	0.34 c	0.61 ab	1.5 b	0 a	0 a	0 a
Soybean	0.21 b	0.55 a	1.6 b	4 b	19 b	79 c
Alfalfa	0.25 b	0.50 a	2.8 c	0 a	0 a	0 a
Control	0.0 a	0.30 a	0.3 a	0 a	0 a	0 a

[w] Stem inoculation.

[x] Seedlings transplanted to infested soil. Douglas-fir root discoloration ratings 0,1,2,3,4=0–10, 11–25, 26–50, 51–75, and 76–100%, respectively. Alfalfa root discoloration ratings 0, 1, 2, 3, 4 = < 1, 1–25, 26–50, 51–75, and 76–100%, respectively.

[y] One of two groups of *P. megasperma* isolated from Douglas fir: Group 1, pathogenic to both Douglas fir and soybean; Group 2, pathogenic only to Douglas fir.

[z] Means with common letter not significantly different ($P = 0.05$) based on Duncan's new multiple range test.

TABLE 3. Percent mortality of soybean differential cultivars when inoculated in the hypocotyl with isolates of *Phytophthora megasperma* from Douglas fir and soybean[y]

	Isolate					
	Soybean		DF Group 1		DF Group 2	
Cultivar	908 Race 1	909 Race 3	304	306	341	Control
Harosoy	92	85	75	100	0	0
Sanga	0	0	50	75	0	0
Harosoy 63	10	100	60	100	0	0
Mack	0	0	75	100	0	0
Altona	0	17	50	83	0	0
PI 103.091	0	0	33	75	0	0
PI 869.972-1[z]	0	0	100	75	0	0
Tracy	0	0	25	67	0	0

[y] Six plants inoculated per isolate for Altona, 10 for Harosoy 63, and 13 for all other cultivars.
[z] Used in place of PI 171.442.

short test period and emphasis of the rating system on tap root lesions rather than rootlet necrosis. Similar isolates are prevalent in tree nurseries in the Pacific Northwest (5,6) where conditions are favorable for disease development for long periods.

Stem inoculations of the soybean race differential cultivars produced an unexpected range of symptoms. Previous workers (11) have classed soybean cultivars as resistant or susceptible to particular races based on mortality, implying a uniform reaction. However, we found degrees of susceptibility ranging from large necrotic areas near the area inoculated to complete plant mortality. Resistant plants showed little or no discoloration. These minor variations might be caused by a variable inoculum size or mixed seed lots, or may reflect variation in aggressiveness between isolates of a given race. Survival of all soybean plants inoculated with alfalfa, clover, and DF Group 2 isolates, as well as control plants, suggests that mechanical injury was not the cause.

Host specificity of *P. megasperma* and Hildebrand's unfortunate choice of the varietal name *"sojae"* for small-spored isolates have created taxonomic problems. Although the Latin description for the variety is based on morphological characters, subsequent workers have emphasized host specificity (2,4,10,19) and have not used the varietal name for isolates from other hosts. Use of formae speciales without varietal designation emphasizes pathogenic differences between isolates of *P. megasperma* and leaves morphological questions unanswered.

The dual pathogenicity of DF Group 1 isolates to soybean and Douglas fir was unexpected. These hosts, though unrelated phylogenetically and biogeographically, share susceptibility to this group of *P. megasperma* isolates. DF Group 1 isolates might be considered a distinct formae speciales, but designation is delayed pending further host range studies. DF Group 2 isolates also exhibited host specificity, but pathogenicity to Douglas fir was less conclusively demonstrated. This condition emphasizes that the broadest possible range of isolates and hosts should be used to establish taxonomic grouping based on morphology or host specificity.

LITERATURE CITED

1. BHELWA, B. H. 1962. Seed decay, seedling blight, and root rot of *Cicer arietinum* caused by *Phytophthora cryptogea*. M.S. Thesis, Ohio State University, Columbus. 94 pp.
2. BOESEWINKEL, H. J. 1974. *Phytophthora* on asparagus in New Zealand. Plant Dis. Rep. 58:525-529.
3. DRECHSLER, C. 1931. A crown rot of hollyhocks caused by *Phytophthora megasperma* n. sp. J. Wash. Acad. Sci. 21:513-526.
4. ERWIN, D. C. 1965. Reclassification of the causal agent of root rot of alfalfa from *Phytophthora cryptogea* to *P. megasperma*. Phytopathology 55:1139-1143.
5. HANSEN, E. M., P. B. HAMM, A. J. JULIS, and L. F. ROTH. 1979. Isolation, incidence and management of *Phytophthora* in forest tree nurseries in the Pacific Northwest. Plant Dis. Rep. 63:607-611.
6. HANSEN, E. M., L. F. ROTH, P. B. HAMM, and A. J. JULIS. 1980. Survival, spread and pathogenicity of *Phytophthora* spp. on Douglas-fir trees planted on forest sites. Phytopathology 70:422-425.
7. HILDEBRAND, A. A. 1959. A root and stalk rot of soybean caused by *Phytophthora megasperma* Drechsler var. *sojae*, var. nov. Can. J. Bot. 37:927-957.
8. JOHNSON, H. W., and B. L. KEELING. 1969. Pathogenicity of *Phytophthora megasperma* isolated from subterranean clover roots. Phytopathology 59:1279-1283.
9. JONES, J. P., and H. W. JOHNSON. 1969. Lupine, a new host for *Phytophthora megasperma* var. *sojae*. Phytopathology 59:504-507.
10. KUAN, T.-L., and D. C. ERWIN. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
11. LAVIOLETTE, F. A., and K. L. ATHOW. 1977. Three new physiologic races of *Phytophthora megasperma* var. *sojae*. Phytopathology 67:267-268.
12. MATSUMOTO, N., and T. ARAKI. 1978. Alfalfa root rot caused by *Phytophthora megasperma* Drechsler in Japan. Ann. Phytopathol. Soc. Jpn. 44:214-217.
13. MORGAN, F. L. 1964. Infection of cranesbill by the soybean *Phytophthora*. Plant Dis. Rep. 48:140-141.
14. NAGAI, Y., T. TAKEUCK, and T. WATANABE. 1978. A stem blight of rose caused by *Phytophthora megasperma*. Phytopathology 68:684-688.
15. NEWHOOK, F. J., G. M. WATERHOUSE, and D. J. STAMPS. 1979. Tabular key to the species of *Phytophthora* de Bary. Commonw. Mycol. Inst., Mycol. Pap. 143. 20 pp.
16. SNYDER, W. C., and H. N. HANSEN, 1940. The species concept in *Fusarium*. Am. J. Bot. 27:64-67.
17. STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York. 481 pp.
18. TRIONE, E. J. 1959. The pathology of *Phytophthora lateralis* on native *Chamaecyparis lawsoniana*. Phytopathology 49:306-310.
19. Van der ZWET, T., and L. L. FORBES. 1961. *Phytophthora megasperma*, the principal cause of seed-piece rot of sugarcane in Louisiana. Phytopathology 51:634-640.
20. WATERHOUSE, G. M. 1963. Key to the species of *Phytophthora* de Bary. Commonw. Mycol. Inst., Mycol. Pap. 92. 22 pp.