Morphological Differentiation of Host-Specialized Groups of *Phytophthora megasperma*

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ABSTRACT

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Pathogenicity, morphology, and growth of 54 isolates of *Phytophthora megasperma* from Douglas-fir, soybean, alfalfa, clover, and 10 other hosts were compared. Soybean and clover isolates were highly aggressive only on the host of origin. Douglas-fir and alfalfa isolates formed three pathogenicity groups: D1, aggressive on Douglas-fir; AL1, aggressive on alfalfa; and D2-AL2, weakly aggressive on both hosts. D1 isolates caused a severe reaction in soybean after hypocotyl inoculation, but caused only slight root rot after soil infestation. Isolates from nine other hosts did not cause disease on alfalfa, Douglas-fir, soybean, or clover; an isolate from noble fir was similar to D2-AL2 isolates. Isolates grouped by pathogenicity had similar morphology, growth at several temperatures, and sensitivity to the fungicide metalaxyl. No single morphological character, of the 10 compared, differentiated all host groups, but isolates D1, AL1, and D2-AL2 and those from soybean and clover were separated from each other by combinations of oogonium diameter, sporangium length, and temperature response. Most isolates from hosts other than Douglas-fir, soybean, clover, and alfalfa were similar in morphology and growth to D2-AL2 isolates. The host groups could not be assigned to *P. megasperma* var. *megasperma* or var. *sojae* because many isolates had oogonia of intermediate diameters.

Additional key words: *Phytophthora megasperma* var. *megasperma* and var. *sojae,* Ridomil.

Phytophthora megasperma Drechs. is an important but extremely diverse plant parasite. Several attempts have been made to define subspecific groups based on morphology or pathogenicity, but no comprehensive analysis of subgroups has been attempted. Waterhouse (18,19) and Newhook et al (13) recognized two varieties based on oogonium size. *P. megasperma* var. *sojae,* first created for soybean isolates (8), has oogonia with diameters averaging <40 μm and *P. megasperma* var. *megasperma,* corresponding to the original species description (3), has oogonia averaging >45 μm. Recent examinations of *P. megasperma* from many hosts showed that oogonium diameters vary widely within the population (7,10). Those of *P. megasperma* var. *megasperma* and var. *sojae* are at the extremes of this range, but the oogonia diameters of many isolates are intermediate.

Host specificity has been reported for isolates of *P. megasperma* from soybean, alfalfa, and clover and these have been designated formae speciales *glycinea, medicaginis,* and *trifolii,* respectively (10,14). *P. megasperma* isolates from Douglas-fir (6) and rose (12) also are reported to have a limited host range. Isolates from other hosts, however, apparently do not exhibit specific pathogenicity and both aggressive and nonaggressive isolates of *P. megasperma* have been recovered from Douglas-fir (6) and more recently from alfalfa in the Pacific Northwest. The observation that these strains could be separated by morphological characteristics led to the current study.

Our objective was to determine the extent to which distinctive morphology accompanies specialized pathogenicity in *P. megasperma* as a step toward a unified taxonomy of this species.

MATERIALS AND METHODS

Fifty-four isolates from 14 hosts were compared. Thirty-six of these, including 22 from Douglas-fir, alfalfa, soybean, and clover, and 14 from 10 other hosts are described elsewhere (7). Eighteen additional isolates from Douglas-fir, alfalfa, soybean, and clover are described in Table 1. Pacific Northwest isolates were identified as *P. megasperma* by their nonpapillate, ovoid to obpyriform sporangia that proliferate internally in liquid culture, and by smooth-walled oogonia, 30–60 μm in diameter, with mostly paragynous antheridia (7,13,18,19). Identity of other isolates was confirmed by the same characteristics.

Pathogenicity. Pathogenicity tests were made with healthy Douglas-fir, alfalfa, and clover seedlings transplanted to pasteurized soil amended with corn meal sand (CMS) inoculum (6) of *P. megasperma* and with soybean seedlings grown from seed in amended soil. At transplanting, CMS was mixed 1:16 with pasteurized clay-loam soil. Three pots were prepared for each isolate-host combination. Control seedlings were transplanted to

TABLE 1. Isolates of *Phytophthora megasperma*[a]

Isolate	Host	Location	Source
AL1-J1	Alfalfa	Mississippi	Pratt[b]
AL1-OA	Alfalfa	Mississippi	Pratt
AL2-3A	Alfalfa	Corvallis, OR	OSU[c]
AL2-3B	Alfalfa	Corvallis, OR	OSU
D1-304	Douglas-fir	Brownsville, OR	OSU
D1-306	Douglas-fir	Brownsville, OR	OSU
D1-284	Douglas-fir	Brownsville, OR	OSU
D2-C5	Douglas-fir	Brownsville, OR	OSU
D2-341	Douglas-fir	Brownsville, OR	OSU
D2-BIC	Douglas-fir	Brownsville, OR	OSU
D2-C6	Douglas-fir	Brownsville, OR	OSU
D2-307	Douglas-fir	Brownsville, OR	OSU
D2-Bt2	Douglas-fir	Brownsville, OR	OSU
D2-316	Douglas-fir	Brownsville, OR	OSU
SB-16	Soybean (Race 1)	Wisconsin	Grau[d]
SB-411	Soybean (Race 8)	Wisconsin	Grau
SB-36	Soybean (Race 7)	Wisconsin	Grau
SB-853	Soybean (Race 3)	Wisconsin	Grau

[a] Other isolates listed in Hamm and Hansen (7).
[b] R. G. Pratt, Mississippi State University, Mississippi.
[c] P. B. Hamm, Oregon State University.
[d] C. R. Grau, University of Wisconsin, Madison.

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soil amended with uninfested CMS. The pots were randomized on the greenhouse bench with a 15-hr photoperiod and watered to soil saturation daily. Average minimum and maximum greenhouse temperatures were 14 and 24 C, respectively. Disease ratings were averaged for each pot and the means for the three replicate pots were averaged for each host-isolate combination. All tests were repeated at least once. Root disease was rated by the proportion of dead roots: 0, 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%).

Bare-rooted, 2-yr-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were transplanted singly to CMS-amended soil in 400-ml plastic tubes. Disease was rated 12 wk after inoculation.

Alfalfa (*Medicago sativa* L. 'Vernal') and arrowleaf clover (*Trifolium vesiculosum* Savl. 'Meechee') seeds were germinated on moist filter paper then planted in pasteurized soil. Three seedlings were transplanted to each 600-ml container of CMS-amended soil. Root symptoms were rated after 8 wk. Oven-dry weights of all plants in a container were recorded.

Soybean (*Glycine max* (L.) 'Harosoy') seeds were planted directly into amended soil in 600-ml pots (Test 1), or in pasteurized soil layered over infested soil in 2,500-ml pots (Test 2). The seedlings were thinned to nine seedlings per pot. Root-disease ratings and oven-dry weights of whole plants were recorded in test 1 after 4 wk and in test 2 after 8 wk. Soybean hypocotyls were inoculated by inserting portions of mycelium from pea broth (17) colonies into longitudinal incisions about 0.5 cm long, midway between the cotyledons and soil. Inoculation points were covered with petrolatum. Nine-day-old soybean seedlings were inoculated and reactions were recorded after 9 days.

Morphology. Oogonium diameter and type of antheridia were assessed on 1-mo-old colonies on V-8 juice agar (15) incubated at room temperature. Largest, smallest, and average diameters and the percentage of oogonia with paragynous antheridia were recorded for each isolate. Sporangia were measured from colonies grown 7 days in pea broth at 20 C then rinsed and incubated overnight in soil extract water. Maximum, minimum, and average length and breadth, and length-to-breadth ratio of sporangia were recorded for each isolate.

Linear growth (millimeters per day) at maximum, minimum, and optimum temperatures was determined for each isolate grown in petri plates of corn meal agar 7 days at 5, 10, 20, 25, 30, and 35 C. Effects of the fungicide metalaxyl (Ridomil, Ciba-Geigy) on growth were also tested. Seitz-filtered metalaxyl was added to CMA after autoclaving to give a concentration of 1 mg active ingredient/liter, and 20 ml aliquots were dispensed into petri plates. Amended and unamended CMA plates were inoculated at the margin and incubated 5 days at room temperature.

Size measurements for isolates listed in Table 1 are the average of 40 observations. Growth rates are the means for two duplicate plates. Measurements for the 36 isolates from a parallel study of variation among single zoospore isolates (7) are the averages of 90 observations for each characteristic (10 from the parent isolate and from each of eight single-spore isolates of the parent). Growth means for these isolates represent 18 plates (two for the parent and for each of eight single-spore isolates).

Statistical analysis. Two multivariate analyses were used. A cluster analysis (Clus B) was used to join isolates with similar characteristics by minimizing the sum of squares around cluster means. It is an iterative method, terminating when no observation can be shifted to another group and the sum of squares reduced (11). A stepwise discriminant analysis was also used to orient host groups of isolates relative to one another and to determine which variables best discriminated the groups (2).

RESULTS

Pathogenicity. Isolates from Douglas-fir and from alfalfa fell into two groups, distinguished by aggressiveness to the host of origin and by the reaction of soybean to Douglas-fir isolates and of Douglas-fir to alfalfa isolates (Table 2). One group of Douglas-fir isolates (D1) caused extensive necrosis of main roots on 2-yr-old Douglas-fir trees. The other less aggressive group (D2) was generally confined to the smaller lateral roots. D1 isolates induced severe, localized reactions often leading to stem collapse and mortality in hypocotyl-inoculated soybean, but D2 isolates caused only faint discoloration around inoculation points. D1 isolates caused slight, but significant, root disease and reduction in dry weight of soybeans planted in uniformly infested soil (Table 3). Results were similar when soybean roots grew through a layer of infested soil. D2 isolates caused no disease on soybeans in infested soil.

One group of alfalfa isolates (AL1) caused taproot necrosis on alfalfa, killing most plants and greatly reducing dry weights of survivors, but was not pathogenic on Douglas-fir. Other alfalfa isolates (AL2), in contrast, were usually confined to lateral roots of alfalfa and reduced dry weights less then AL1 isolates. AL2 isolates also caused some root rot on Douglas-fir (Table 2). Clover isolates were pathogenic only to clover and soybean isolates only to

TABLE 2. Pathogenicity of *Phytophthora megasperma* isolates from Douglas-fir, alfalfa, clover, and soybean to those hosts

	Disease rating[y]				Hypocotyl inoculation
	Douglas-fir	Alfalfa	Clover	Soybean	(% mortality)
Isolate					
D1-B3A	2.7	0.0	0.1	0.2	75
D1-B217	3.0	0.0	0.3	0.4	75
D1-345	3.3	0.6	1.0	0.2	40
D2-520	1.3	0.5	0.1	0.0	0
D2-C17	2.0	0.0	0.1	0.1	0
D2-336	1.0	0.0	0.0	0.0	0
AL1-S1	0.3	3.7	0.1	0.1	0
AL1-PC3	1.3	4.0	0.4	0.6	0
AL1-M1	0.3	4.0	0.7	0.0	0
AL2-P1	1.7	1.2	1.0	0.0	0
AL2-3B	1.0	1.7	0.8	0.0	0
AL2-3A	1.3	0.7	0.4	0.1	7
CL-102	0.0	0.0	3.4	0.1	0
CL-105	0.3	0.2	3.6	0.0	0
SB-411	0.0	0.0	0.0	2.4	53
SB-908	0.3	0.0	0.0	2.2	100
Control	0.0	0.2	0.0	0.0	0
Isolate groups					
Douglas-fir Group 1	3.0 a[z]	0.2 c	0.5 b	0.3 b	60 b
Douglas-fir Group 2	1.5 b	0.2 c	0.1 c	0.0 c	0 c
Alfalfa Group 1	0.3 c	3.9 a	0.4 b	0.2 b	0 c
Alfalfa Group 2	1.3 b	1.2 b	0.7 b	0.0 c	2 c
Clover	0.2 c	0.1 c	3.5 a	0.0 c	0 c
Soybean	0.2 c	0.0 c	0.0 c	2.3 a	73 a
Control	0.0 c	0.2 c	0.0 c	0.0 c	0 c

[y] Disease rating: 0, 1, 2, 3, and 4 = 0, 1–25, 26–50, 51–75, and 76–100% root death, respectively.

[z] Ratings in a column followed by different letters are significantly different ($P = 0.01$), according to Duncan's new multiple range test.

TABLE 3. Average dry weight developed by alfalfa, clover, and soybean plants following inoculation with *Phytophthora megasperma*

Isolate groups	Host		
	Alfalfa	Clover	Soybean
Douglas-fir D1	0.34 a[z]	0.76 b	4.9 b
Douglas-fir D2	0.39 a	0.91 ab	5.6 a
Alfalfa AL1	0.13 b	0.84 ab	5.3 a
Alfalfa AL2	0.24 c	0.87 ab	5.2 a
Clover	0.42 a	0.23 c	5.1 a
Soybean	0.38 a	0.65 b	3.4 c
Control	0.49 a	1.05 a	5.1 a

[z] Values represent the average weight in grams of three plants per pot for alfalfa and clover, and nine plants per pot for soybean. Ratings in a column followed by different letters are significantly different ($P = 0.01$), according to Duncan's new multiple range test.

soybean. Of the 14 isolates from 10 additional hosts, only JU-K9 from *Juniperus* sp. and D2-NF from Noble fir caused significant root rot on Douglas-fir (comparable to that caused by D2 isolates) and none caused root disease on alfalfa, clover, or soybean.

Morphology and growth. Groups of isolates defined initially by pathogenicity were similar in morphological characteristics, growth at several temperatures, and sensitivity to metalaxyl (Table 4). It was not possible to separate isolate groups completely by size or shape of sporangia or oogonia, but lengths of sporangia and diameters of oogonia in combination did separate them (Fig. 1). Douglas-fir and alfalfa isolates each formed two distinct groups; AL1 and D1 isolates had small oogonia and short sporangia relative to AL2 and D2 isolates. Mean oogonium diameters of D2 isolates were particularly variable, ranging from 41.5 to 58.5 μm. Soybean isolates had small oogonia and long sporangia (41 and 66 μm, respectively); clover isolates had larger oogonia (45 μm) and the shortest sporangia of those measured (44 μm). One D2 isolate fell within the range of the soybean isolates. Isolates from other hosts were not plotted on Fig. 1, but fell within the range of the D2-AL2 isolates.

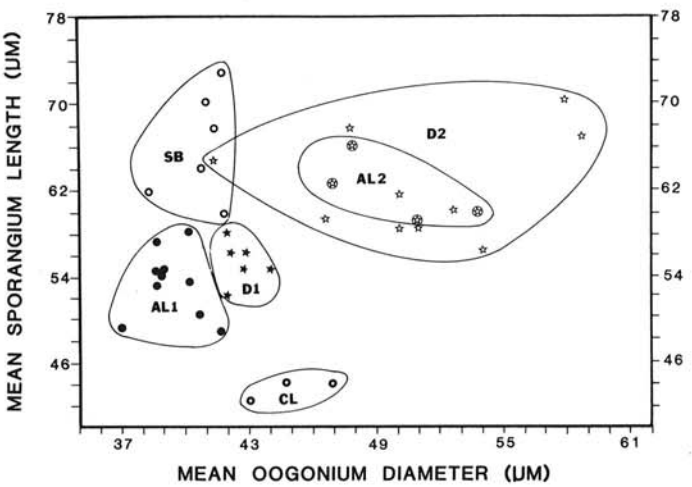

Fig. 1. Separation of isolates of *Phytophthora megasperma* host groups by using oogonium diameter and sporangium length. Hosts are alfalfa (AL1, AL2), clover (CL), Douglas-fir (D1, D2), and soybean (SB). Values are averages of 90 observations for each characteristic for each isolate.

Isolates could also be distinguished by the temperature-growth relationship (Table 4). D1 and AL1 isolates had the highest optima (27 and 26 C). D1 isolates grew faster than others at all temperatures above 5 C. At 5 C, D2 and AL2 isolates grew most rapidly. Isolates from other hosts responded to temperature like D2-AL2 isolates, except for rose isolates that did not grow at 5 C.

Isolate groups also differed in response to the fungicide metalaxyl. Soybean, clover, and alfalfa AL1 isolates were completely inhibited by 1 mg of metalaxyl per liter. Inhibition of Douglas-fir isolates (D1 and D2) and alfalfa AL2 isolates ranged from 44 to 67%. Isolates from other hosts, except rose, also grew on the amended medium (Table 4).

Host groups. Cluster analyses were generated for the 40 isolates from alfalfa, clover (CL), soybean (SB), and Douglas-fir and for those plus the 14 isolates from additional hosts. Variables were maximum, minimum, and average diameters of oogonia, maximum, minimum, and average lengths and breadths of sporangia, length-to-breadth ratio of sporangia, optimum temperature, and growth rate at the optimum temperature, at 5 C and at 35 C. All 14 variables were given equal weight in the analysis. The Clus B program divided the isolates first into two clusters then sequentially into 3, 4, 5, 6, 7, 8, and 9 clusters. When the 40 AL1, AL2, D1, D2, SB, and CL isolates were analyzed as a group, the first division separated D2, AL2, and SB isolates from the rest. The second division (three clusters) separated SB isolates, and the third, CL isolates. D1 and AL1 isolates were separated in the sixth division (seven clusters). AL2 and D2 isolates generally stayed together, although individual isolates became grouped with other clusters. After five clusters, host groups were split, but new combinations crossing host lines were not formed (Table 5).

Addition of isolates from other hosts did not change the tendency for isolates from a host to cluster. The first division grouped D2, AL2, and SB isolates in a cluster with most of the additional isolates. At the fourth division, the AL1, D1, and CL isolates formed one cluster and the four apple and rose isolates formed another new cluster. At eight clusters, AL1, D1, and CL isolates were separated; AL2 and D2 isolates remained together, although mixed in two groups.

A multivariate least squares discriminant analysis was used to test the similarity of the 40 isolates within the host groups D1, D2, AL1, AL2, SB, and CL. The 14 measured variables were compressed to two canonical variables and the isolates plotted on a two-dimensional scattergram. The AL2 and D2 isolates grouped together; other isolates were clearly separated by host of origin. The discriminant analysis was repeated adding the 14 isolates from

TABLE 4. Morphology and growth of *Phytophthora megasperma* isolates from Douglas-fir, alfalfa, soybean, clover, and other hosts

Hosts	Isolates Code	Isolates (No.)	Oogonium diameter (μm)	Sporangium length (μm)	Sporangium ratio	Optimum temperature (C)	Linear growth (mm/day) 5 C	Linear growth (mm/day) 35 C	Inhibition by metalaxyl (%)
Douglas-fir	D1	(6)[y]	42.5 ± 1.0[z]	55.5 ± 1.9	1.5 ± 0.1	27.1 ± 2.5	0.5 ± 0.2	1.1 ± 0.5	76 ± 2.9
Alfalfa	AL1	(10)	39.3 ± 1.3	53.3 ± 2.9	1.4 ± 0.1	26.0 ± 2.1	0.2 ± 0.1	0.5 ± 0.1	99 ± 2.2
Soybean	SB	(6)	40.8 ± 1.3	53.3 ± 2.9	1.6 ± 0.1	20.0 ± 0.0	0.1 ± 0.2	0.6 ± 0.5	100 ± 0.0
Clover	CL	(3)	44.9 ± 1.8	43.9 ± 1.2	1.4 ± 0.1	22.5 ± 2.5	0.2 ± 0.1	0.0 ± 0.0	100 ± 0.0
Douglas-fir	D2	(11)	51.5 ± 5.1	63.1 ± 4.6	1.5 ± 0.1	21.6 ± 2.0	0.9 ± 0.4	0.0 ± 0.0	44 ± 8.9
Alfalfa	AL2	(4)	50.0 ± 3.0	62.6 ± 3.0	1.5 ± 0.1	22.5 ± 2.0	1.3 ± 0.1	0.0 ± 0.0	44 ± 1.8
Rose	Ro-PA		44.1	62.0	1.3	25.0	0.0	0.0	100
	Ro-PB		38.9	68.2	1.6	25.0	0.0	0.0	100
Apple	Ap-T14		42.4	64.2	1.6	22.5	1.0	0.0	63
	Ap-T47		43.4	57.1	1.5	20.0	0.7	0.0	42
Grape soil	Gr-K10		40.4	61.6	1.4	20.0	1.7	0.0	28
	Gr-K11		51.8	66.1	1.5	20.0	1.6	0.0	79
Juniper	Ju-K9		42.6	62.0	1.4	22.5	1.8	0.0	42
Cherry	CH-K1		47.4	61.7	1.5	25.0	1.3	0.1	63
Pear	Pe-K8		51.4	60.5	1.5	25.0	1.5	0.0	28
Poplar	Po-T28		49.2	62.7	1.6	20.0	1.0	0.0	41
Brassica	Br-T56		43.3	65.5	1.6	20.0	1.0	0.0	47
Type	Ty-T32		42.8	66.8	1.6	20.0	0.0	0.0	...

[y] Number of isolates for morphology and temperature measurements. Metalaxyl test based on three, eight, two, three, three, and two isolates for D1, AL1, SB, CL, D2, and AL2, respectively.
[z] Mean and standard deviation.

other hosts, each treated as a separate group. On the resulting scattergram (Fig. 2), most isolates from other hosts appeared among or adjacent to the D2-AL2 isolates. Rose isolates (Ro-PA and Ro-PB) appeared between groups SB, AL1, and D1.

Stepwise discriminant analysis identified growth at optimum temperature, oogonium diameter, and sporangium length as the three most significant variables for separation of groups. Length-to-breadth ratio of sporangia was least significant. F values showing dissimilarity between main host groups were significant ($P = 0.01$) (Table 6).

DISCUSSION

This work confirms, expands, and integrates previous reports of the pathogenicity (6,10,12,14), distinctive morphology (5,7,8,10), and sensitivity to metalxyl (9) of many isolates of *P. megasperma.* Isolates were separated into distinctive groups based on pathogenicity, morphology, and growth rate. The groups generally corresponded to host of origin, except for those from alfalfa and Douglas-fir, which formed three groups: those aggressive to alfalfa (AL1) or to Douglas-fir (D1) and those weakly pathogenic to both hosts (D2-AL2). Twelve of 14 isolates from additional hosts were similar to D2-AL2 isolates; rose isolates were similar in morphology and growth to aggressive isolates from alfalfa (AL1) and soybean. Although pathogenicity was the only single characteristic that reliably separated the isolate groups, they were separated by combined morphological and growth characters as well. The groups were confirmed by three independent forms of analysis. When isolates were plotted by oogonium and sporangium size (Fig. 1), host groups were evident but only clover isolates were separated clearly from the rest. Multivariate comparisons made by using the clustering program without prior presumption of isolate relationships generally grouped isolates by host of origin. Eighteen new isolates from alfalfa, Douglas-fir, and soybean (Table 1) corresponded with 22 previously collected isolates from those hosts (Table 4). Stepwise discriminant analysis confirmed the uniqueness of host groups suggested by the previous tests (Table 6). The addition of isolates from other hosts did not appreciably alter results of either cluster or discriminant analysis.

Host specificity is a useful concept in plant pathology, but it must be interpreted carefully. "Host specific" isolates of *P. megasperma* from alfalfa, clover, soybean, and Douglas-fir were aggressive, often lethal, on their respective hosts of origin, but caused slight but significant disease on other hosts as well. Furthermore, some isolates from alfalfa and Douglas-fir (AL2-D2) were not highly aggressive on any host tested, although they did cause significant disease both in greenhouse and field situations. Forma specialis designation based on host specificity should be confined to isolates that demonstrate aggressive pathogenic behavior and not automatically extended to all isolates recovered from a host. For example, AL2 isolates were recovered from symptomatic alfalfa plants in western Oregon, but do not represent *P. megasperma* f. sp. *medicaginis.* Damage in the fields was slight, although plants were chlorotic, and isolates did not kill alfalfa in inoculation tests. AL1 isolates, on the other hand, are typical of *P. megasperma* f. sp. *medicaginis.* They were associated with severe disease in the field and caused taproot necrosis, often lethal, after inoculation. This important difference in behavior was not noted in previous work (6) because Oregon isolates were not included.

Slight, but significant, nonspecific pathogenicity was also exhibited by the isolates typical of *P. megasperma* f. sp. *medicaginis* (AL1), f. sp. *glycinea*, and f. sp. *trifolii* (Tables 2 and 3). Root necrosis caused by alfalfa isolates on clover, for example, was confined to root tips in these tests, in contrast to the progressive, often lethal necrosis induced by the isolates typical of *P. megasperma* f. sp. *trifolii* when inoculated to clover.

D1 isolates were previously described by us as pathogenic to soybean as well as Douglas-fir, based on hypocotyl inoculation of soybean (6). The strong reaction of soybean to stem inoculation, often leading to mortality, was confirmed in the present study, but these isolates induced only minor root disease on soybean after soil inoculation. Isolates causing mortality in hypocotyl inoculation are

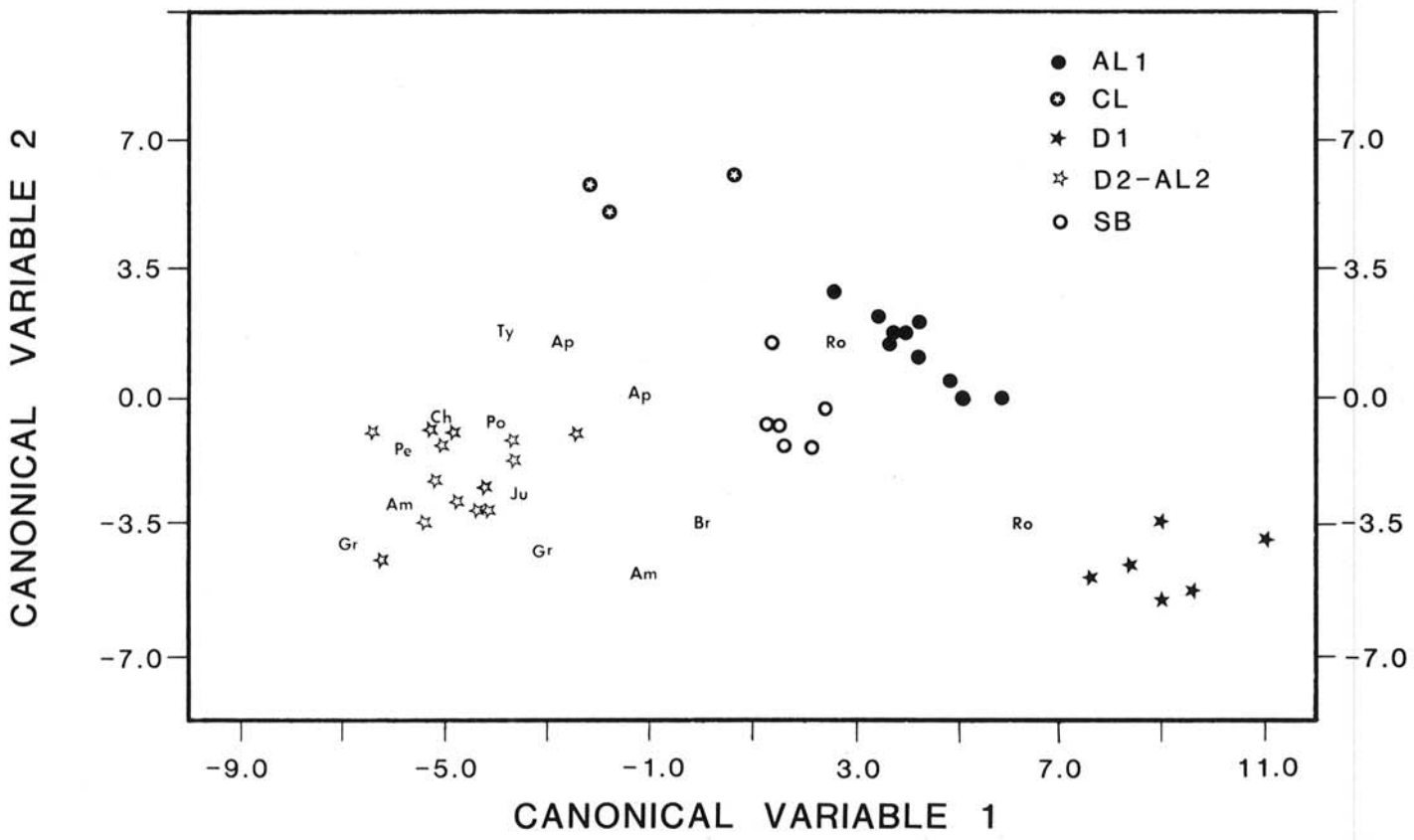

Fig. 2. Separation of main host groups of isolates of *Phytophthora megasperma* and additional isolates from other hosts by compression of 14 morphological and growth characteristics into two canonical variables plotted on a two-dimensional scattergram. Hosts are identified in Table 4.

TABLE 5. Sequential clustering of 40 *Phytophthora megasperma* isolates based on multivariate dissimilarity values calculated for 10 morphological and four growth variables. Isolates from Douglas-fir (D), alfalfa (AL), soybean (SB), and clover (CL)

Number of clusters				
2	3	4	5	7
AL1-J1	AL1-J1	AL1-J1	AL1-J1	AL1-J1
AL1-OA	AL1-PC5	AL1-PC5	AL1-PC5	AL1-PC5
AL1-PC5	AL1-S2	AL1-S2	AL1-S2	AL1-S2
AL1-S2	AL1-5b	AL1-5b	AL1-5b	AL1-5b
AL1-5b	AL1-S1	AL1-S1	AL1-S1	AL1-S1
AL1-S1	AL1-M1	AL1-M1	AL1-M1	AL1-M1
AL1-M1	AL1-PC3	AL1-PC3	AL1-PC3	AL1-PC3
AL1-PC3	AL1-W1	AL1-W1	AL1-W1	AL1-W1
AL1-W1	AL1-DA	AL1-DA	AL1-DA	AL1-DA
AL1-DA	CL-105	D1-B217	D1-B217	AL1-OA
CL-105	CL-102	D1-B3A	D1-B3A	D1-304
CL-102	Cl-117	D1-345	D1-345	
CL-117	D1-306	D1-306	D1-306	AL2-3A
D1-306	D1-284	D1-284	D1-284	AL2-3B
D1-284	D1-304	D1-304	D1-304	D2-C6
D1-304	D1-B3A			D2-520
D1-B3A	D1-B217	AL2-P3	AL2-P3	D2-307
D1-B217	D1-345	AL2-P1	AL2-P1	D2-336
D1-345		AL2-3A	AL2-3A	D2-C17
	AL2-P3	AL2-3B	AL2-3B	D2-Nf1
AL2-P3	AL2-P1	D2-C5	D2-C5	D2-Bt2
AL2-P1	AL2-3A	D2-341	D2-341	
AL2-3A	AL2-3B	D2-C6	D2-C6	D2-341
AL2-3B	D2-C5	D2-307	D2-307	D2-BIC
D2-C5	D2-341	D2-Bt2	D2-Bt2	AL2-P3
D2-341	D2-C6	D2-316	D2-316	AL2-P1
D2-C6	D2-307	D2-520	D2-520	SB-411
D2-307	D2-336	D2-336	D2-336	
D2-BIC	D2-316	D2-C17	D2-C17	CL-105
D2-316	D2-520	D2-Nf1	D2-Nf1	CL-117
D2-520	D2-Bt2			Cl-102
D2-336	D2-C17	SB-16	SB-16	
D2-C17	D2-Nf1	SB-411	SB-411	SB-16
D2-Bt2		SB-36	SB-36	SB-909
D2-Nf1	SB-16	SB-853	AL1-OA	SB-36
SB-16	SB-411	SB-909	D2-BIC	SB-908
SB-411	SB-36	SB-908		SB-853
SB-36	SB-853	D2-BIC	CL-105	
SB-853	SB-909	AL1-OA	CL-117	D2-C5
SB-909	SB-908		CL-102	D2-316
SB-908	D2-BIC	CL-105		
	All-OA	CL-117	SB-853	D1-B217
		CL-102	SB-908	D1-345
			SB-909	D1-B3A
				D1-284
				D1-306

not necessarily aggressive under more natural conditions.

The groups of similar isolates identified here cannot be assigned to the varieties of *P. megasperma* described by Waterhouse (18,19) or Newhook et al (13). Seven isolates in our study had oogonia averaging $<40\ \mu m$, 20 averaged $>45\ \mu m$, and 25 were intermediate. Splitting isolates arbitrarily by oogonium size divides isolate groups that are otherwise similar. The first division in the multivariate cluster analysis formed a relatively large-spored group ($\bar{x}$ diameter = 51 μm) and a small-spored group ($\bar{x}$ = 41 μm), but the diameter ranges of the isolates overlapped and soybean isolates similar to those forming the original *P. megasperma* var. *sojae* (8) were grouped with the larger-spored isolates. Another possible division is to separate the less aggressive D2-AL2 isolates. These have, for the most part, larger oogonia than the aggressive isolates. Sansome and Brasier (16) showed differences in chromosome number between several large-spored and small-spored isolates of *P. megasperma*. Po-28 was similar to the D2-AL2 group in our tests; the same isolate was determined by Sansome and Brasier (16) to be tetraploid. It would be interesting to learn whether this difference correlates with spore size, host specificity, or with the host groups defined here.

TABLE 6. Matrices for descriminant analysis of isolate groups of *Phytophthora megasperma* based on the three most significant variables and on all 14 growth and morphological variables

3 Variables[a] Degrees of freedom = 3, 33				
	AL1	D2-AL2	SB	CL
D2-AL2	47.09[b]			
SB	20.57	17.35		
CL	19.74	25.73	28.66	
D1	38.77	98.70	66.42	65.16
14 Variables Degrees of freedom = 14, 22				
	AL1	D2-AL2	SB	CL
D2-AL2	25.33[b]			
SB	6.19	12.23		
CL	6.25	9.20	7.75	
D1	11.33	38.12	13.13	18.96

[a] Oogonium diameter, sporangium length, and growth at optimum temperature.

[b] *F*-values in matrices are highly significant ($P = 0.01$) between each isolate group.

Isolates of *P. megasperma* present in Douglas-fir nurseries are presumed to have originated in agricultural crops previously grown on the sites (4). The similarities in pathogenicity, morphology, and growth between AL2 and D2 isolates tend to confirm this. Similarly, isolates D1 and AL1 have many common characteristics, although they can be separated by morphology, growth, and pathogenicity.

The *P. palmivora* "complex" was described for many years as a morphologically variable assemblage of tropical heterothallic isolates from cacao, rubber, and other hosts. Recent critical evaluations, however, recognized morphological forms, later elevated to species (1), with distinctive host and geographic limits. The recognition of morphologically distinct groups of *P. megasperma* from different hosts suggests a similar situation although the differences between groups are not sufficient to warrant separate species. It seems reasonable to hypothesize that morphological differentiation among *Phytophthora* species is a consequence of the isolation of populations by agricultural practice and host specialization. Since neither provides perfect isolation (as evidenced by movement of pathogens in agricultural commerce and by the dual pathogenicity described here), it is not surprising that many intermediate isolates are found. It is premature to give taxonomic rank to these fluid populations.

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