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Title: MORPHOLOGICAL VARIATION, TAXONOMY AND HOST SPECIFICITY OF PHYTOPHTHORA MEGASPERMA

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Everett M. Hansen

Thirty-six isolates of Phytophthora megasperma Drechs. from 4 continents and 14 hosts were compared for variation in morphological and growth characteristics and to determine host range. Variation was identified between single spore isolates (SSI) from the same parent and between parent isolates. Variation from the parent by SSI was determined by comparing eight SSI from each parent, measured by one or both of the following methods: (1) the percentage of SSI that differed from their parent; and (2) the number of statistically different subgroups identified per nine cultures from each isolate. Comparisons were based on both qualitative and quantitative characteristics.

Some variation in SSI from the parent was observed in all characteristics. Diameter of oogonia and length/width ratios of sporangia were relatively stable characters (17 and 9% of SSI differed from parent) and few subgroups (38 and 57) were formed. Oogonia were produced in most (95%) colonies; of these most (96%) formed predominately paragynous antheridia. In contrast, length and width of sporangia of SSI often differed from parents (37 and 35%) and more subgroups were formed (90 and 80) for these characters. Few different SSI and subgroups were identified based on growth rate (9% and 24), colony morphology on clarified V-8 agar (13% and 10) and colony and hyphal morphology on corn

meal agar (11% and 21 and 7% and 17, respectively). A high correlation between the number of different SSI and subgroups was found for quantitative (r^2 = .98) characteristics. The number of differing SSI and the number of distinct subgroups were considered separate but not necessarily independent measures of variability in the population.

Variation among the 36 isolates was examined for evidence to support division of P. megasperma into varieties. All characteristics varied continuously over their ranges and multivariate analysis failed to identify groups corresponding to established varieties. Analysis did identify a relationship between isolate characteristics and host of origin. Revision of the taxonomic subgrouping of P. megasperma is suggested.

The amount of variation in morphological and growth characters was used to identify those most reliable for identification of isolates belonging to this species. Those considered reliable were diameter of oogonia, length/width ratio of sporangia, attachment of antheridia, production of oogonia (homothallism) and shape of sporangia. Large variation occurred in length and width values of sporangia and these characters were considered unreliable. Variation in growth rate, colony morphology on V-8 agar and colony and hyphal morphology on corn meal agar made these characteristics useful only during comparisons where isolates were from the same host and geographical location.

Host specificity reactions were compared in two separate tests using a total of 54 isolates, including the 36 isolates used for morphological comparisons. Isolates were inoculated to Douglas-fir, alfalfa, soybean, Noble fir, clover, cauliflower and broccoli. Isolates from soybean were pathogenic only to soybean. Alfalfa isolates were

differentiated into two groups: one group pathogenic only to alfalfa, and a second group intermediately pathogenic to both alfalfa and Douglas-fir. Isolates from Douglas-fir also formed two groups. One group was pathogenic only to Douglas-fir; the second was strongly pathogenic to Douglas-fir, soybean, and Noble fir. The latter 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 specialis* concept of *P. megasperma* is discussed.

Morphological Variation, Taxonomy, and Host Specificity of *Phytophthora megasperma*

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Redacted for privacy

Professor of Botany and Plant Pathology in Charge of Major

Redacted for privacy

Chairman of the Department of Botany and Plant Pathology

Redacted for privacy

Dean of Graduate School

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Typed by Dianne Webster for Philip B. Hamm

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MORPHOLOGICAL VARIATION, TAXONOMY, AND HOST SPECIFICITY OF *PHYTOPHTHORA MEGASPERMA*

GENERAL INTRODUCTION

Phytophthora megasperma Drechs. is an important plant pathogen which causes root rot on many diverse plant species. Damage due to this organism is most notable on alfalfa (*Medicago sativa* L.) (Erwin 1965), soybean (*Glycine max* (L.) Merr.) (Kaufmann and Gerdemann 1958; Hildebrand 1959) and to a lesser extent on arrowleaf clover (*Trifolium vesiculosum* Savi) (Pratt 1981), sugarcane (*Saccharum officinarum* L.) (van der Zwet and Forbes 1961), rose (*Rosa* spp.) (Nagai and Watambe 1978), and cauliflower (*Brassica oleracea* var. *botrytis*) (Tompkins et al. 1936).

Recently *P. megasperma* was identified as one of several *Phytophthora* spp. causing root rot on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in forest tree nurseries of the Pacific Northwest (Hansen et al. 1978; Hamm and Hansen 1981). Due to the importance of Douglas-fir to this area, a comprehensive project was initiated to determine the effects *Phytophthora* had on nursery seedling production and subsequent survival of trees on outplanted sites. During this work two groups of *P. megasperma* isolates were identified, differing in both morphological and growth characteristics (Hamm and Hansen 1980).

Disease development by soilborne *Phytophthora* spp. is favored where soils remain wet for prolonged periods; typically in low areas and sites with heavy soils. Zoospores (motile spores produced asexually) are released from sporangia (Figure 1) during wet periods and actively move toward host roots in response to chemotaxis. Once colonization of the host occurs, oospores can develop as a result of the sexual union between oogonia and antheridia. Oospores have thick walls to survive adverse

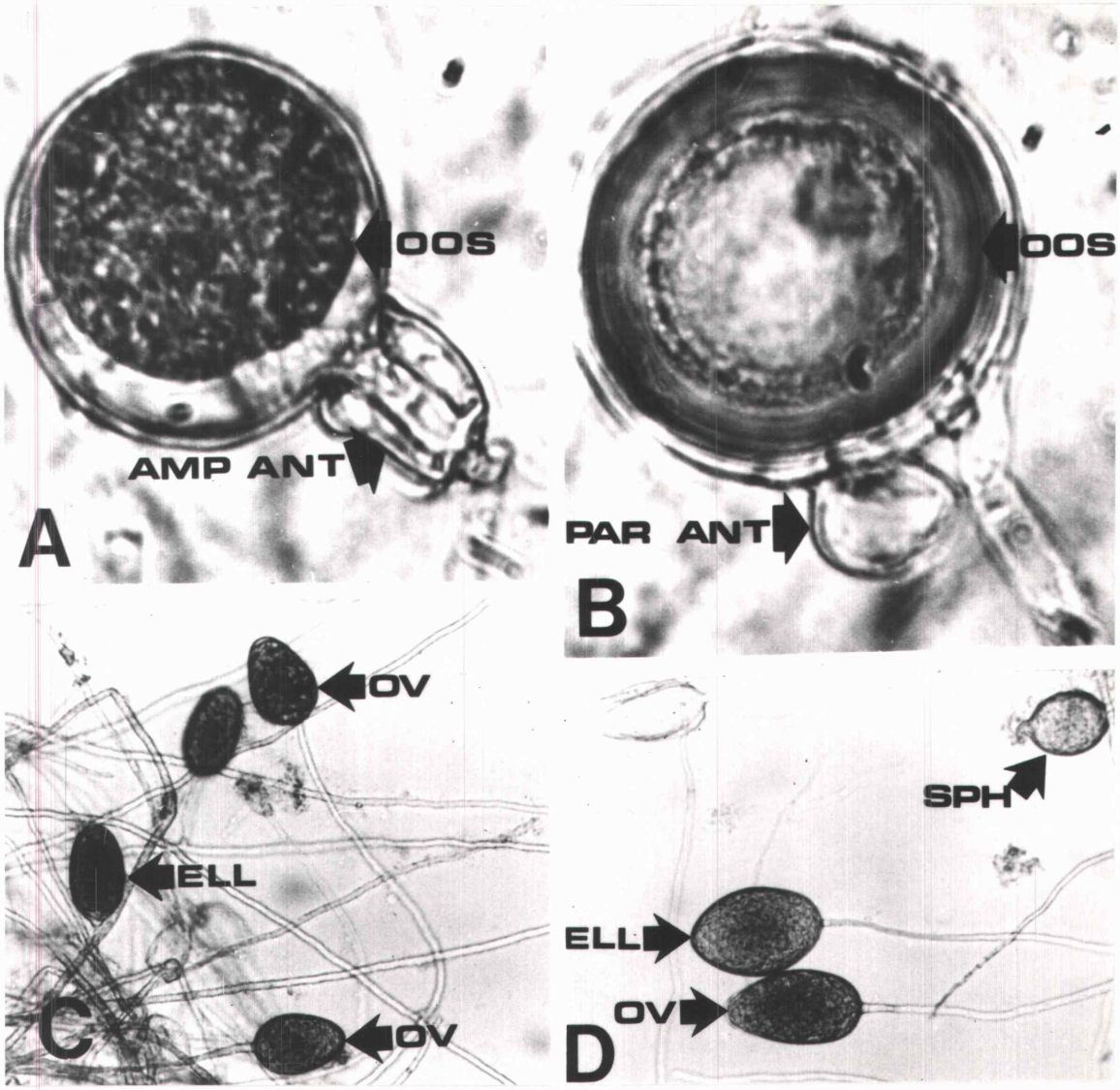

Figure 1. Oogonial and sporangial characteristics of Phytophthora megasperma. A-B. Oogonia containing oospores (oos) with amphigynous (amp ant) and paragynous (par ant) antheridia. C-D. Sporangia with ovoid (ov), ellipsoid (ell) and spherical (sph) shapes. Note the variation between sporangia of similar shapes.

conditions. During favorable periods oospores germinate, producing either hyphae which infect nearby roots or sporangia which liberate zoospores.

Oogonial diameter, oogonial production (homothallic versus heterothallic), antheridial type (paragynous versus amphigynous) and size and shape of sporangia are the characteristics used most often for species determinations. Phytophthora megasperma characteristically has non-papillate, ovoid to obpyriform sporangia ($\bar{x}$ 55 X 35 µm), and large oogonia (30-60 µm diameter) which form readily in single strain culture (homothallic). Antheridia are predominantly paragynous (Waterhouse 1963, 1970; Newhook et al. 1979).

Currently, P. megasperma is subdivided in two ways. Some workers identify two varieties based on the diameter of oogonia produced. Others separate isolates by their ability to cause diseases of specific hosts (formae speciales). Differentiation within P. megasperma by these methods has caused confusion during isolate identification.

The distinction of subgroups within P. megasperma and recovery of two groups of isolates from Douglas-fir emphasizes the variation present in the species, although the mechanism causing variation is unclear. One possible source of variation, however, may be zoospores. Colonies arising from zoospores were often presumed to be genetically identical to parent colonies, but Stamps (1953) found variation in colony morphology, growth rate, hyphal morphology, and size, shape, and frequency of reproductive organs between single zoospore isolates (SSI) of P. cactorum. These results were confirmed for colony morphology and growth rate by Buddenhaggen (1958) and MacIntyre and Elliott (1974). Others have reported variation in SSI in other Phytophthora spp. (Apple 1957;

Wallin 1957; Shaw and Elliott 1961; Caten and Jink 1968; Shepherd and Pratt 1974). No attempt has been made to quantify the extent of variation due to SSI in P. megasperma or any other Phytophthora spp.

Variation among SSI may well be responsible for the taxonomic controversy that has existed in P. megasperma for some time. Drechsler (1931) first identified this organism causing root rot of hollyhock, and described large oogonia ($\bar{x}$ 47 µm) as the most diagnostic characteristic. Tompkins et al. (1936) amended this description to include isolates with smaller oogonia (>30 µm) found on cauliflower. Subsequently, subgroupings based on morphological, physiological and pathological characteristics were suggested. Kaufmann and Gerdemann (1958) reported a Phytophthora spp. on soybean with oogonia within the range described by Tompkins et al. ($\bar{x}$ 37 µm) but chose to establish a new species, P. sojae. Later, Hildebrand (1959) reported small spored isolates ($\bar{x}$ 36 µm), including P. sojae, to be host specific to soybean and established P. megasperma var. sojae. Waterhouse (1963; 1970) and Newhook et al. (1978) agreed with a varietal subgrouping but based their distinction on oogonial size; isolates with small oogonia (<45 µm) were placed in P. megasperma var. sojae and those with large oogonia (>45 µm) in P. megasperma var. megasperma. Confusion has occurred due to these classification systems because: (1) isolates with intermediate oogonia diameters have been reported (Kuan and Erwin 1980), and (2) isolates with small oogonia have been reported from hosts besides soybean (van der Zwet and Forbes 1961; Erwin 1963; Pratt 1981).

Kuan and Erwin (1980), who questioned varietal designations based on oogonial size, reported that isolates from alfalfa and soybean were pathogenic only to those hosts. They established P. megaperma f. sp.

medicaginis and *P. megaperma* f. sp. *glycinea*, respectively. Others have reported host specificity of *P. megasperma* to rose (Nagai and Watanche 1978) and recently to arrowleaf clover (Pratt 1981). Whether *P. megasperma* isolates from Douglas-fir were host specific was unknown.

Objectives of this research were: (1) identify the extent to which variation occurs between SSI of *P. megasperma*; (2) identify specific morphological and physiological characteristics most useful for taxonomic considerations in this species; (3) determine whether *P. megasperma* should be separated into subgroups based on diameter of oogonia or some other characteristic; and (4) determine whether the *formae speciales* concept is applicable to the two groups of *P. megasperma* from Douglas-fir.

This thesis has been divided into three chapters, each with its own Introduction, Materials and Methods, Results and Discussion sections. A comprehensive summary of the most important results and their ramifications to the taxonomy of *P. megasperma* is included after Chapter III.

CHAPTER I

MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION AMONG SINGLE SPORE ISOLATES OF PHYTOPHTHORA MEGASPERMA

INTRODUCTION

Phytophthora megasperma is an important plant pathogen, described originally by Drechsler (1931) with distinct morphological and physiological characteristics. Since then, however, isolates have been reported that differed substantially from Drechsler's description, particularly in spore size. Subsequent workers have suggested various approaches to the taxonomy of this group of isolates, including: (1) supporting Drechsler's original species concept with expanded size limits (Tompkins et al. 1936; Erwin 1965); (2) erecting new taxa splitting the isolates into two species (Kaufmann and Gerdemann 1958) or varieties (Hildebrand 1959), or (3) ignoring morphological characteristics and emphasizing host specific reactions (forma specialis) (Kuan and Erwin 1980; Pratt 1981). No attempt has been made to define the variation possible within isolates, however. The differences that have been found among isolates of P. megasperma might be no more than the variation found among single zoospore isolates (SSI). Zoospores, produced asexually, are often persumed to maintain parental characteristics. Stamps (1953), however, found that colony morphology, growth rate, hyphal morphology, and the size, shape, and frequency of reproductive organs differed among SSI of P. cactorum. These results were confirmed for colony morphology and growth rate by Buddenhaggen (1958) and MacIntyre and Elliott (1974), and expanded to include streptomycin resistance (Shaw and Elliott 1961). Variation between SSI has also been

reported in other Phytophthora spp. Variation in cultural and pathological characteristics was reported in P. parasitica var. nicotianae (Apple 1957), others reported variation in growth rate by P. cinnamomi (Shepherd and Pratt 1974) and growth rate, pathogenicity, production of sporangia and colony morphology by P. infestans (Wallin 1957). Variation among SSI in growth rate and pathogenicity has been described in P. megasperma (Hilty and Schmitthenner 1962).

Apparently deviation from the parent in growth rate and pathogenicity commonly occurs among SSI of many Phytophthora spp. However, variation in other characteristics, especially those used in taxonomic comparison, has generally not been considered. Only Stamps (1953) looked at morphological characteristics but few SSI were observed and no attempt was made to determine the range of variation of individual characters. Recently (Chapter II) isolates belonging to P. megasperma were identified with a wider range of morphological and growth characteristics than originally described. The present work was initiated to determine: (1) the frequency and magnitude of variation occurring between parent isolates and SSI of P. megasperma; and (2) which characteristics exhibit the least amount of variation between single spore and parent isolates.

Thirty-five isolates were obtained from around the world; eight SSI were obtained from each parent isolate. Morphological structures and growth rate were measured for each isolate of each isolate set. Variation was assessed by determining the number of SSI and subgroups significantly differing from the parent isolates for each character.

MATERIALS AND METHODS

Thirty-five isolates of P. megasperma were obtained from four continents and fourteen hosts (Table 1). These isolates were identical to those used during taxonomic comparisons (Chapter II) and to isolates used for host specific determinations (Chapter III).

Single zoospore isolates were obtained by growing parent isolates in pea broth for 7-9 days, washing the mycelial mat with sterile distilled water and incubating overnight in autoclaved soil extract water. Zoospores that had been released were then removed by pipette and diluted in warm (40°C) corn meal agar containing 20 PPM pimaricin (CMP). The agar containing zoospores was poured into petri plates and incubated 1-2 days until identifiable colonies were formed. Eight separate zoospore colonies were obtained from each isolate. Colonies were randomly picked though occasionally when a difference in colony morphology was detected, these colonies were selected for further tests. No attempt was made to identify multinucleate zoospores.

Morphological structures were measured and growth rate determined for each culture belonging to each isolate set (Parent and 8 SSI). Mean values for morphological structures were based on 10 randomly selected mature structures (observed at 900X) and 2 replications per isolate for growth determinations. Inference to the population was made using this information. The lack of production of structures was also recorded. Colony and hyphal morphological characteristics were treated separately as described below.

Diameter of oogonia and oospores as well as the type of antheridia, production of oogonia, and wall thickness of oogonia were from colonies grown 1 mo on clarified V-8 agar at 20°C. Types of antheridia were

Table 1. Sources of isolates of Phytophthora megasperma.

Isolate	Host	Location	Source
W1	Alfalfa	Washington	Peaders[1]
S1	Alfalfa	Salem, Oregon	OSU[2]
P1	Alfalfa	Corvallis, Oregon	OSU
M1	Alfalfa	Medford, Oregon	OSU
PC3	Alfalfa	Princeton, Oregon	OSU
PC5	Alfalfa	Klamath Falls, Ore.	OSU
S2	Alfalfa	Salem, Oregon	OSU
P3	Alfalfa	Corvallis, Oregon	OSU
5b	Alfalfa	Wisconsin	Maxwell[6]
DA	Alfalfa	Wisconsin	Maxwell
K2	Almond	Red Bluff, Calif.	Mircetich[7]
K3	Almond	Red Bluff, Calif.	Mircetich
K10	Grape Soil	Napa Co., Calif.	Mircetich
K11	Grape Soil	Napa Co., Calif.	Mircetich
B3A	Douglas-fir	Brownsville, Ore.	OSU
B217	Douglas-fir	Brownsville, Ore.	OSU
345	Douglas-fir	Brownsville, Ore.	OSU
336	Douglas-fir	Toledo, Wash.	OSU
C-17-2D2	Douglas-fir	Elkton, Oregon	OSU
520	Douglas-fir	Brownsville, Ore.	OSU
908	Soybean (Race 1)	Wisconsin	Grau[8]
909	Soybean (Race 2)	Wisconsin	Grau
105	Clover	Mississippi	Pratt[9]
117	Clover	Mississippi	Pratt
102	Clover	Mississippi	Pratt
T14	Apple	New Zealand	IMI[3] 144023
T47	Apple	New Zealand	IMI[3] 147131
PA	Rose	Japan	Nagai[10]
PB	Rose	Japan	Nagai
K1	Cherry	Stockton, Calif.	Mircetich
K8	Pear	Walnut Grove, Calif.	Mircetich
K9	Juniper	Davis, Calif.	Mircetich
T32[5]	Hollyhock (type)	Washington, D.C.	IMI[3] 32035
T56	Brassica sp.	United Kingdom	IMI[3] 56348
T28	Populus robusta	United States	ATCC[4] 28765
NF1	Noble fir	Corvallis, Ore.	OSU

[1] R. N. Peaders, I.A.R.E.C., Prosser, Washington.

[2] OSU = P. B. Hamm, Oregon State University.

[3] IMI = Imperial Mycological Institute, Kew, Surrey, England.

[4] ATCC = American Type Culture Collection, Rockville, Maryland.

[5] Isolate not used for comparisons in Chapter I because zoospores were not produced.

[6] D. P. Maxwell, Univ. of Wisconsin, Madison.

Table 1 continued.

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[7]S. Mircetich, USDA, Univ. of California, Davis.

[8]C. R. Grau, Univ. of Wisconsin, Madison.

[9]R. G. Pratt, USDA, Mississippi State University, Mississippi

[10]Y. Nagai, Laboratory of Plant Pathology, Chiba, Japan.

classified: 0 = 100% paragynous; 1 = 76-99% paragynous; 2 = 51-75% paragynous; 3 = 50-75% amphigynous; 4 = 76-100% amphigynous. Oogonia production ratings were based on number visible at 100X in a randomly selected field of view (1,2,3,4,5 = 0, <5, 5-25, 25-50,>50, respectively). Those with a 1 rating usually contained oogonia (except as noted), but these were found only by scanning the entire colony.

Sporangia were produced in 7 day old pea broth (Trione 1959) colonies rinsed with distilled water and incubated overnight in soil water (equal volume of soil and water left over night and then filtered). Width and length of sporangia were measured and length to width ratios were calculated.

Growth rates (mm/day) were measured from CMP plates containing 20 ml of medium. Two replications of each SSI of each isolate set were incubated for 7 days at 20°C and the colony diameter was measured.

Colony morphology characteristics were observed on two media. Clarified V-8 agar plates, used previously for oogonia observations, were compared for overall colony morphology (aerialness, colony pattern, etc.). CMP plates, used previously for growth studies, were also used to compare overall colony morphology and to compare hyphal morphology and branching patterns. Each isolate set was compared separately. SSI that appeared different from the parent were tallied. In addition, the nine isolates of each set were divided into subgroups that appeared similar. The number of unique subgroups was tallied for each set.

Tukey's multiple range test (Steel and Torrie 1960) was used to compare isolate means for diameter and wall thickness of oogonia, width and length of sporangia, length to width ratio of sporangia, and colony growth at 20°C. By this method the number of SSI that were statistically

different ($p = 0.05$) from the parent isolate and the number of different subgroups (>1) in each isolate set could be identified. The least variable of these characteristics could be identified. A subgroup was established when a mean or group of means were identified as being different. The average number of different SSI and subgroups in the 35 isolate sets was calculated for each character.

A regression analysis was used to determine if a relationship occurred between: (1) the number of different SSI and the number of subgroups that were identified and; (2) the average difference between the largest and smallest mean for each character for the 35 isolate sets and the corresponding number of different SSI and subgroups observed for that character.

RESULTS

Oogonia Characteristics

Oogonia and Oospore: Maximum and minimum mean values for diameter of oogonia and oospores among the nine isolates of each set (parent and eight SSI) are listed by host in Table 2. The difference between largest and smallest isolates in each set was between 2 and 13 µm for both structures ($\bar{x} = 6.5$, SD = 2.9 and $\bar{x} = 6.0$, SD = 2.6 for diameter of oogonia and oospores, respectively). Tukey's multiple range test identified 17% of all SSI as significantly different (Table 3) from their parent isolate by diameter of oogonia and 38 different subgroups were formed. Generally SSI that differed significantly in diameter of oogonia, also differed in diameter of oospores.

Types of Antheridia: The range of variation in attachment of antheridia (Figure 1) is listed in Table 2. Eight sets included SSI that were

Table 2. Range of mean values of oogonial characteristics from 35 isolates of Phytophthora megasperma.[1]

Host and Isolate	Oogonia Diameters[2]	Antheridia Types[3]	Oogonia Production[4]	Oogonia Wall Thicknesses	Oospore Diameters
Alfalfa					
W1	40-45(45)	0-2(2)	2-5(2)	1.2-1.9(1.2)	35-39(39)
S1	36-42(40)	0-3(3)	1 (1)	1.2-2.3(2.3)	29-36(31)
P1	44-50(47)	0-1(0)	2-4(3)	1.2-2.1(1.3)	93-47(41)
M1	34-39(39)	0-4(2)	3-5(5)	0.9-1.2(1.0)	30-33(32)
PC3	36-40(39)	0-2(1)	1 (1)	0.9-1.8(1.0)	29-34(32)
PC5	32-43(40)	0-4(4)	1 (1)	0.9-2.5(0.9)	24-37(34)
S2	38-41(39)	0-2(0)	1-3(2)	1.0-1.5(1.2)	32-35(33)
P3	44-52(45)	0 (0)	3-5(3)	1.0-1.9(1.2)	39-46(40)
5b	38-42(38)	0-3(3)	1-2(1)	0.9-2.6(0.9)	31-36(31)
DA	38-43(43)	0-2(0)	1-2(1)	0.9-1.4(1.1)	29-36(36)
Almond					
k2	45-50(50)	0 (0)	2-3(3)	1.3-1.6(1.1)	38-44(43)
k3	38-44(38)	0-1(0)	2-4(4)	1.1-1.4(1.2)	32-34(32)
Grape Soil					
k10	50-55(50)	0-1(0)	3-5(3)	1.1-1.7(1.1)	45-47(45)
k11	38-44(41)	0-1(0)	3-4(4)	1.0-1.4(1.2)	33-38(35)
Douglas-fir					
B3A	39-47(47)	0-1(0)	4-5(5)	0.9-1.5(1.4)	32-39(39)
B217	41-46(43)	0-1(1)	1-5(1)	1.2-1.4(1.2)	35-40(35)
345	41-47(44)	0-1(0)	2-5(4)	1.0-1.6(1.5)	35-40(38)
336	38-51(45)	0-2(0)	1-3(2)	0.9-1.5(1.2)	32-46(39)
C-17-2D2	54-59(59)	0 (0)	2-3(3)	1.0-1.6(1.6)	47-51(51)
520	46-55(53)	0-1(1)	2-3(3)	0.9-1.5(1.2)	42-49(45)

Table 2 continued.

Host and Isolate	Oogonia Diameters[2]	Antheridia Types[3]	Oogonia Production[4]	Oogonia Wall Thicknesses	Oospore Diameters
Soybean					
908	40-44(43)	0-1(0)	4-5(5)	1.1-1.7(1.1)	35-37(36)
909	40-46(40)	0-1(0)	4-5(4)	1.2-1.8(1.2)	34-38(34)
Clover					
105	37-50(41)	0-2(1)	2-3(2)	1.0-1.4(1.0)	25-39(33)
117	40-48(48)	0-2(0)	1-2(1)	1.0-1.4(1.2)	35-39(39)
102	41-49(41)	0-1(0)	1-3(3)	1.1-2.0(1.1)	33-43(33)
Apple					
T14	40-46(44)	0 (0)	2-3(2)	1.0-1.4(1.1)	33-39(37)
T47	41-49(49)	0-1(0)	1-3(2)	1.0-1.5(1.3)	33-41(41)
Rose					
PA	37-42(42)	0-2(0)	1-5 (1)	0.9-1.1(1.1)	32-38(32)
PB	44[5]	1[5]	4[5]	1.0[5]	40[5]
Cherry					
K1	45-50(45)	0 (0)	3-4(4)	1.2-1.5(1.3)	38-43(38)
Pear					
K8	44-57(51)	0 (0)	3-4(3)	0.9-1.4(1.2)	39-48(46)
Juniper					
K9	40-47(42)	0 (0)	3-4(3)	1.0-1.6(1.2)	34-40(36)

Table 2 continued.

Host and Isolate	Oogonia Diameters[2]	Antheridia Types[3]	Oogonia Production[4]	Oogonia Wall Thicknesses	Oospore Diameters
Brassica					
T56	36-47(44)	0-1(0)	1-2(2)	1.0-1.3(1.3)	36-40(37)
Poplar					
T28	49-51(50)	0-1(0)	1-2(2)	1.2-1.3(1.2)	38-45(39)
Noble fir					
NF1	49-51(49)	0 (0)	2-5(2)	1.1-1.9(1.2)	41-45(43)
Overall Mean Range	6.5(SD=2.9)	1.1(SD=.73)	2.5(SD=.7)	0.62(SD=.45)	6.0(SD=2.6)

[1]Values represent range of means from 9 cultures (parent and 8 single zoospore isolates) from each isolate. Each mean represents 10 observations of typical, mature oogonia. Values in microns.

[2]Values in brackets identify parent isolate value.

[3]Antheridia type classified: 0 = 100% paragynous; 1 = 71-99% paragynous; 2 = 51-75% paragynous; 3 = >51-75% amphigynous; 4 = >75% amphigynous.

[4]Oogonia production ratings = 1,2,3,4,5 for 0, <5, 5-25, 25-50, and >50, respectively, clearly visible in single field of view at 125X.

[5]Oogonia were produced by only one single spore isolate.

Table 3. The number of SSI differing from the parent cultures and the number of subgroups per isolate set for morphological and growth characteristics.

Host and Isolate	Oogonia Diameters	Oogonia Wall Thicknesses	Sporangia Lengths	Sporangia Widths	Sporangia Ratios	Growth Rate 20°C
Alfalfa						
W1	6,1[1]	1,1	6,5	5,4	2,2	0,0
S1	0,2	0,1	4,4	3,2	0,1	0,0
P1	1,1	1,1	4,4	3,2	0,2	0,0
M1	1,1	0,1	2,2	4,2	0,2	1,1
PC3	0,0	0,0	5,2	0,1	3,2	0,0
PC5	0,0	2,2	0,1	2,3	3,2	0,0
S2	0,0	0,1	5,2	5,2	1,2	0,0
P3	3,2	1,1	5,4	4,3	0,1	0,0
5b	0,0	3,2	0,0	2,2	2,2	0,1
DA	0,1	0,1	4,5	6,4	3,2	0,1
Almond						
k2	1,1	0,0	0,1	1,2	1,2	1,1
k3	0,0	0,0	0,1	5,2	4,2	2,1
Grape Soil						
k10	0,0	0,0	6,3	2,1	0,0	0,0
k11	0,1	0,1	2,3	8,3	5,1	1,2
Douglas-fir						
B3A	2,2	0,0	7,2	4,3	2,3	0,0
B217	0,0	0,0	4,3	4,3	0,1	0,0
345	0,0	3,1	3,4	3,3	0,0	1,1
336	4,2	0,1	1,1	1,1	2,2	2,1

Table 3 continued.

Host and Isolate	Oogonia Diameters	Oogonia Wall Thicknesses	Sporangia Lengths	Sporangia Widths	Sporangia Ratios	Growth Rate 20°C
Douglas-fir continued						
C-17-2D2	3,1	1,2	4,3	1,3	1,2	0,0
520	1,2	0,2	8,4	3,4	7,1	7,2
Soybean						
908	0,1	3,2	2,1	0,1	1,1	0,0
909	1,2	2,2	2,2	0,1	0,1	4,1
Clover						
105	1,3	0,0	3,4	3,2	5,3	0,1
117	1,1	1,1	4,3	3,3	4,2	0,1
102	6,1	2,2	5,3	2,1	1,3	0,0
Apple						
T14	1,3	0,0	2,3	3,3	1,2	2,2
T47	3,2	0,1	1,2	2,2	0,2	0,2
Rose						
PA	2	2	2,3	3,4	1,1	0,0
PB	2	2	3,3	3,3	3,0	0,0
Cherry						
K1	1,1	0,0	2,2	2,4	1,1	0,0

Table 3 continued.

Host and Isolate	Oogonia Diameters	Oogonia Wall Thicknesses	Sporangia Lengths	Sporangia Widths	Sporangia Ratios	Growth Rate 20°C
Pear						
K8	1,1	0,1	3,4	2,2	3,2	0,1
Juniper						
K9	0,1	1,2	1,0	3,0	5,2	0,0
Brassica						
T56	2,3	0,0	1,1	4,1	1,1	2,2
Poplar						
T28	3,1	0,0	-[3],3	-[3],1	-[3],2	1,3
Noble fir						
NFL	0,1	1,1	1,2	2,2	4,2	0,0
Overall Different[4]	17,38	8,30	37,90	35,80	23,57	9,24

[1]The first value refers to number of single spore isolates (out of 8) significantly different from the parent culture. The second value represents the number of subgroups (>1) identified significantly different among the nine cultures of each isolate (parent and 8 single spore isolates). Statistical analysis based on Tukey's multiple range test p = 0.05.

[2]Structures were not formed by either parent or SSI.

[3]Parent isolates failed to form sporangia.

[4]The first value identifies percentage of SSI that differed from the parent. The second value refers to the total number of subgroups formed.

completely paragynous (rating = 0) and others that were nearly all amphigynous (rating = 4). Most (95%) formed >51% paragynous antheridia ($\bar{x}$ rating = 1.1, SD = .73). Overall, 64% of SSI were entirely paragynous while <1% were 75-100% amphigynous. Some SSI from all 35 isolates produced entirely paragynous antheridia.

Production of Oogonia: Variation among SSI in production of oogonia is shown in Table 2. Production varied from none to 20-50 (ratings 0-4) per field of view. Most isolates formed many oogonia ($\bar{x}$ rating = 2.5, SD = 0.7). Oogonia were not produced by 15 SSI (one each from WI, K2, S20, T56, T14, four from PB, and six from PA).

Wall Thickness of Oogonia: Minimum and maximum mean wall thickness of oogonia are listed in Table 2 for the 35 isolates. The range between thickest and thinnest walls among the 9 isolates of a set varied from 0.2-1.7 µm ($\bar{x}$ = .62, SD = .45) overall. Eight percent of the SSI differed significantly from the parent isolate and 30 different statistical subgroups were formed by this characteristic (Table 3).

Sporangia Characteristics

Length of Sporangia: Maximum and minimum mean values for sporangia are listed in Table 4. The maximum difference in length of sporangia between isolates of a set ranged from 7-34 µm ($\bar{x}$ = 18, SD = 4.6). Tukey's multiple range test identified 37% of the SSI as significantly different from the parent isolate by this characteristic; 90 different statistical subgroups were formed (Table 3).

Width of Sporangia: Mean widths of sporangia differed by 5 to 20 µm ($\bar{x}$ = 11.3, SD = 3.7) among the isolates of the 35 sets (Table 4).

Table 4. Range of mean values of sporangial characteristics from 35 isolates of Phytophthora megasperma.[1]

Host and Isolate	Sporangia Lengths	Sporangia Widths	Sporangia Ratio (length/width)
Alfalfa			
W1	39-64(56)[2]	29-38(38)	1.1-1.8:1
S1	47-65(47)	34-43(34)	1.2-1.8:1
P1	56-68(58)	40-48(41)	1.2-1.8:1
M1	44-63(45)	30-44(31)	1.1-1.6:1
PC3	53-61(61)	39-44(43)	1.1-1.8:1
PC5	50-58(53)	37-44(43)	1.1-1.6:1
S2	47-66(47)	34-43(34)	1.1-1.9:1
P3	61-73(61)	41-52(41)	1.3-1.8:1
5b	54-61(57)	36-45(40)	1.2-2.1:1
DA	39-64(54)	28-44(42)	1.1-1.7:1
Almond			
k2	59-70(66)	39-48(46)	1.1-2.0:1
k3	64-72(64)	41-50(41)	1.2-1.8:1
Grape Soil			
k10	54-73(54)	36-51(36)	1.3-1.8:1
k11	57-68(63)	40-51(51)	1.2-1.7:1
Douglas-fir			
B3A	45-59(59)	31-40(38)	1.2-1.9:1
B217	50-61(64)	31-41(40)	1.3-1.9:1
345	45-64(60)	32-40(39)	1.3-2.0:1
336	48-66(66)	35-48(43)	1.1-2.3:1
B-17-2D2	53-72(70)	39-46(44)	1.2-1.8:1
520	43-77(77)	32-47(41)	1.1-2.6:1
Soybean			
908	64-81(64)	40-47(43)	1.2-2.3:1
909	59-75(64)	37-42(38)	1.3-2.3:1
Clover			
105	33-52(42)	23-34(33)	1.1-2.0:1
117	34-53(50)	26-40(34)	1.1-1.7:1
102	36-54(50)	24-30(29)	1.1-2.1:1
Apple			
T14	51-78(61)	33-49(41)	1.2-3.6
T47	48-65(60)	35-44(41)	1.2-1.9

Table 4 continued.

Host and Isolate	Sporangia Lengths	Sporangia Widths	Sporangia Ratio (length/width)
Rose			
PB	51-83(60)	32-53(38)	1.1-2.1
PA	55-72(55)	40-60(43)	1.1-1.8
Cherry			
K1	54-70(60)	36-48(40)	1.2-1.8
Pear			
K8	51-74(58)	36-49(42)	1.1-1.8
Juniper			
K9	56-71(62)	39-50(50)	1.2-2.2
Brassica			
T56	59-83(61)	34-47(41)	1.2-3.3
Poplar			
T28	51-76(60)	35-42(41)	1.2-2.8
Noble fir			
NF1	55-70(61)	38-48(47)	1.2-1.7
Overall Mean Range	18.5(SD=4.6)	11.3(SD=3.7)	0.96 (SD=0.5)

[1]Values represent range of means obtained from nine cultures (parent and 8 single zoospore isolates) from each isolate. Each mean represents 10 observations from typical, mature sporangia. All values in microns.

[2]Values in brackets identify parent isolate values.

Tukey's test indicated that 35% of the SSI were significantly different from the parents and 80 significantly different subgroups were formed. Generally, SSI that differed significantly from parent isolates in width of sporangia also differed in length of sporangia.

Maximum differences in mean values for length/width ratio of sporangia ranged from 0.5 to 2.4:1 ($\bar{x}$ difference = 0.96:1, SD = 0.46). The overall number of SSI significantly different from the parent isolate and the number of significantly different groups were 23 and 57, respectively. Sporangia were not produced by 5 SSI (1 each K2, T47, PB and 2 in 5b).

Growth Characteristics

Growth Rate: Maximum and minimum mean values for growth in CMP are listed in Table 5. The mean difference in growth rate at 20°C between fastest and slowest isolates ranged from 0.3-2.9 mm/day ($\bar{x}$ = 1.7 mm, SD = 0.3) among the sets. Overall 9% of SSI differed significantly from their parent and 24 different subgroups were formed. Generally parent isolates grew slower than SSI.

Colony and Hyphal Morphology: Colony and hyphal morphology comparisons are listed in Table 6. None to as many as seven SSI were identified as being different from the parent when colonies were compared on clarified V-8 agar. Overall, 13% of the SSI were observed to be different when compared, and 10 subgroups were formed.

The number of SSI different from their parent by colony morphology on CMP ranged as high as 8. Overall 8% of the SSI were different from the parent, and few subgroups were identified (17). Likewise 11% of the SSI were different based on hyphal morphology, 21 different subgroups were formed.

Table 5. Range of mean values for colony growth (mm/day)[1] at 20°C of 35 isolates of *Phytophthora megasperma*.

Host and Isolate	Range of Mean Values
Alfalfa	
W1	2.9-3.7(3.1)[2]
S1	4.4-5.5(4.4)
P1	4.1-4.5(4.1)
M1	2.4-2.9(2.9)
PC3	3.1-3.6(3.3)
PC5	3.4-4.4(3.6)
S2	2.9-4.0(3.4)
P3	3.7-4.5(3.7)
5b	2.1-3.8(2.9)
DA	4.0-4.9(4.9)
Almond	
k2	2.9-4.3(4.1)
k3	4.1-4.7(4.1)
Grape Soil	
k9	4.4-5.4(4.7)
k10	4.1-5.2(4.8)
Douglas-fir	
B3A	6.4-7.1(6.5)
B217	6.4-7.0(6.5)
345	5.9-7.1(5.9)
336	3.1-4.7(3.1)
C-17-2D2	4.7-5.3(4.9)
520	2.9-5.8(4.0)
Soybean	
908	2.5-3.4(2.5)
909	2.5-3.5(2.5)
Clover	
105	2.6-4.9(3.1)
117	2.2-4.4(3.1)
102	2.8-4.1(3.4)
Apple	
T14	2.4-4.4(4.1)
T47	2.7-4.9(3.9)

Table 5 continued.

Host and Isolate	Range of Mean Values
Rose	
PE	2.4-3.9(3.2)
PA	4.1-4.7(4.6)
Cherry	
K1	3.3-4.6(3.7
Pear	
K8	3.6-5.2(4.0)
Juniper	
K9	3.6-5.0(3.7)
Brassica	
T56	4.1-5.0(4.1)
Poplar	
T28	3.4-5.1(4.4)
Noble fir	
NF1	4.2-45.(4.2)
Overall Mean Range	1.7(SD = .3)

[1]Values represent range of means obtained from nine cultures (parent and 8 single spore isolates) from each isolate. Each mean represents 2 plates/culture.

[2]Values in brackets identify parent isolate.

Table 6. The number of SSI differing from the parent cultures and the number of subgroups per isolate set for colony and hyphal morphology.[1]

Host and Isolate	V-8 agar colony morphology	CMA hyphal morphology	CMA colony morphology
Alfalfa			
W1	0,0[2,3]	1,1[2,3]	1,1[2,3]
S1	0,0	0,0	0,0
P1	0,0	0,0	0,0
M1	0,0	0,0	0,0
PC3	0,0	0,0	0,0
PC5	0,0	0,0	0,0
S2	0,0	0,0	0,0
P3	0,0	0,0	0,0
5b	0,0	1,1	0,0
DA	0,0	1,1	0,0
Almond			
k2	0,0	4,3	4,4
k3	0,0	0,0	1,2
Grape Soil			
k10	3,1	0,0	0,0
k11	0,0	0,0	0,0
Douglas-fir			
B3A	2,1	0,0	0,0
B217	6,1	0,0	0,0
345	2,1	1,0	1,0
336	5,1	0,0	0,0
C-17-2D2	0,0	0,0	0,0
520	0,0	2,2	1,1
Soybean			
908	4,1	0,0	0,0
909	1,0	0,0	0,0
Clover			
105	0,0	3,3	3,3
117	0,0	1,1	1,1
102	0,0	8,1	0,0
Apple			
T14	0,0	0,0	0,0
T47	2,1	8,4	8,5

Table 6 continued.

Host and Isolate	V-8 agar colony morphology	CMA hyphal morphology	CMA colony morphology
Rose			
PB	0,0	0,0	0,0
PA	7,1	0,0	0,0
Cherry			
K1	1,1	0,0	0,0
Pear			
K8	1,0	0,0	0,0
Juniper			
K9	1,1	1,1	1,1
Brassica			
T56	0,0	0,0	0,0
Poplar			
T28	0,0	3,3	0,0
Noble fir			
NFL	0,0	0,0	0,0
Overall Difference[4]	13%,10	11%,21	8%,17

[1]Numbers refer to classification group. Colonies from each isolate set (parent and 8 SSI) were given similar numbers when colonies could not be differentiated based on colony or hyphal morphology. Colony morphology compared aerialness and pattern. Hyphal morphology compared hyphal branching.

[2]Identifies number of single spore isolates that differed from their parent isolate.

[3]Identifies total number of different subgroups (>1) among the nine cultures of each isolate.

[4]The first value identifies percentage of SSI that differed from the parent. The second value refers to the total number of subgroups formed.

A highly significant correlation was found between the mean number of SSI statistically different from their parents and the mean number of subgroups that occurred in the isolate sets (r^2 = .98) for morphological characteristics and growth rate. The range between largest and smallest or fastest and slowest isolates of a set was also correlated with the number of statistically different SSI and the number of subgroups, (r^2 = .73 and .72, respectively).

DISCUSSION

Variation between SSI and the parent isolate was observed in all characteristics in each isolate set. Generally, fewer SSI differed from the parent cultures in diameter of oogonia, wall thickness of oogonia, length/width ratio of sporangia, and growth rate (17, 8, 23, 9%, respectively). In contrast, more SSI differed from their parent by width or length of sporangia (35 and 37%, respectively). Similar results were obtained when the number of different subgroups was used as the criterion for variability (38, 30, 57 and 24, respectively), for diameter of oogonia, wall thickness of oogonia, width/length ratio of sporangia and growth rate. More subgroups were identified when sporangia widths and lengths (80 and 90, respectively) were examined.

There was relatively little variation among SSI in the qualitative characteristics assessed on the two media. The average proportions of SSI varying from the parent based on colony morphology on V-8 agar and colony and hyphal morphology on CMP were small (13, 11 and 8%, respectively) as were the number of subgroups formed (10, 21 and 17, respectively).

These comparisons (number of differing SSI, and number of subgroups)

can be considered separate though not necessarily independent methods to identify the amount of variation that can be expected within the population of P. megasperma. This conclusion is supported by the high correlation between the number of different SSI and subgroups for morphological characteristics and growth rate means (r^2 = .98). Likewise, a comparative number of different SSI and subgroups were found for colony and hyphal morphology comparisons.

The variation reported here is not unlike that previously reported for other Phytophthora spp. Unfortunately, figures for direct comparisons could not be calculated from that information. However, when Shepherd and Pratt (1974) compared growth rate of 9 parent isolates to one SSI of each parent, SSI were always significantly different. Stamps (1953) reported that 1 of 4 SSI of P. cactorum was statistically different from the parent in diameter of oogonia, length and length/width ratio of sporangia. Buddenhaggen (1958) reported that half of 100 SSI from one parent isolate of P. cactorum differed from the parent in growth rate based on colony size. Variation has also been found in pathological reactions. Caten (1970) reported that as many as 45% of the SSI of P. infestans he tested had different reactions based on aggressiveness to potatoes. In another report 8 of 9 SSI of P. infestans caused differential pathological reactions (Wallin 1951). Finally, Hilty and Schmitthenner (1962) found 2 of 94 SSI of P. megasperma different from their parent based on their pathogenicity to soybean. In addition they found that SSI from a smaller sample could be differentiated by their reaction to 2 susceptible and 2 resistant soybean varieties. Obviously variation among SSI does occur in many characteristics. Work cited above was not intended to

quantify variation in a population, so in most cases few parent isolates were used for comparison. Since parent isolates differ in the amount of variation among their single spore progeny (Table 3), the variation reported here from 35 parent isolates is considered closer to the actual amount of variation that occurs between zoospores of P. megasperma.

The characteristics examined here have been assigned varying degrees of importance for the identification of P. megasperma isolates. Characteristics that are uniform among the population of P. megasperma but distinguish it from other species should be given the most weight during taxonomic comparisons. This work identifies diameter of oogonia (and diameter of oospores since they are highly correlated, $r^2 = .98$, Chapter II) and width to length ratio of sporangia as good taxonomic characters. This conclusion is based on the low number of SSI different from the parent, and the low number of different subgroups found for these characters (Table 3). Two additional characteristics are also useful for taxonomic comparisons. Even though oogonia were produced in varying quantities among isolate sets (Table 2) most SSI (95%) formed oogonia and were, therefore, homothallic. Similarly, the type of anthridia was also consistent (96% of those that formed oogonia produced >51% paragynous antheridia). Isolates not forming oogonia or predominantly paragynous antheridia are still considered P. megasperma based on their conformation to the other characteristics used to define the species.

In contrast, SSI varied greatly in length and width of sporangia (37 and 35% and 90 and 80, respectively for the number of different SSI and number of subgroups formed) although their shape (length/width ratio) was constant. Even though wall thickness of oogonia, growth rate, colony pattern and hyphal morphology were stable characters among

the SSI of a parent, they are considered to be of little taxonomic value. Wall thickness of oogonia does not separate P. megasperma from other Phytophthora species. Large variations in colony pattern and hyphal morphology were found between isolate sets of P. megasperma (Chapter II). Hilty and Schmitthenner (1962) and Buddenhaggen (1958) came to a similar conclusion because of variation in these characteristics. Differences in culture morphology with age have also been reported (Stamps 1953). These characteristics are similar for isolates originating from the same location or host, however (Chapter II), and could be useful for comparing unknown isolates to those of known geographical and host origins.

Variation among zoospore progeny of P. megasperma may partially explain the appearance of root rot of Douglas-fir caused by this species in forest tree nurseries of the Pacific Northwest. Many of these nurseries were established where agricultural crops had been grown for decades. Since P. megasperma is a common agricultural pathogen, it is conceivable that these fungi were present before nursery establishment and had or developed the ability to attack a new host. A large host range capability is supported by the recovery of P. megasperma weakly pathogenic to Douglas-fir from alfalfa in fields not unlike those used for conifer seedling production (Chapter III). In addition, some isolates from Douglas-fir are also pathogenic to soybean (Hamm and Hansen 1981; Chapter III). P. megasperma may also have the ability to change host range as evidenced by the susceptibility of once resistant cultivars of soybean to new races of P. megasperma (Hilty and Schmitthenner 1959) or the differential reactions of SSI from the same parent of

P. infestans to potato (Wallin 1957). In contrast, Caten (1970) found no change in virulence among SSI of P. infestans and concluded that SSI were not important for development of new physiologic races.

Variation such as that reported here has important implications for the identification of isolates within P. megasperma. Kaufmann and Gerdemann (1958), Hildebrand (1959), Waterhouse (1963, 1970), and Newhook et al. (1979) have proposed subspecific taxa based on diameter of oogonia and host specificity. The variation in diameter of oogonia seen among SSI of a single parent bridges the reported distinction between varieties, however. The original distinctions were based on several isolates from a narrow geographical or host range which can be misleading. We found some SSI did not produce oogonia, or sporangia or either (SSI of PA) and one parent isolate did not form oogonia though its SSI did. Also, the number of SSI that were significantly different among individual isolate sets ranged from 0 to 8, depending on the characteristic, and would have given misleading information if one or more of these characteristics had been used for taxonomic purposes. Since population variation can be high between parents and their SSI, a large number of representative isolates (including SSI from each) should be used for defining taxa.

The biological mechanism(s) necessary for changes of the magnitude seen to occur among spores developed asexually has not been identified. Galendo (1965), Caten and Jinks (1968), and Caten (1971) suggested that variation is due to cytoplasmic factors; Leach and Rich (1969) suggested heterokaryoses and a parasexual mechanism; Romero and Erwin (1969) and Gallegly (1976) suggested a sexual mechanism, and Shepherd and Pratt (1973) implicate both heterokaryotic and cytoplasmic

mechanisms. Determining the mechanism causing variation among SSI was beyond the scope of this report. Identification of the mechanism for variation would be important for future taxonomic and disease control work.

CHAPTER II

VARIATION WITHIN PHYTOPHTHORA MEGASPERMA FROM 14 HOSTS AND THE EFFECT ON THE TAXONOMY OF THIS SPECIES

INTRODUCTION

Phytophthora megasperma Drechs. is an important but variable plant pathogen. Drechsler (1931) originally described isolates of this species with large oogonia ($\bar{\mu}$ = 47 µm), separating them from smaller spored (24-30 µm) isolates of P. cactorum. Tompkins et al. (1936) later amended the description of P. megasperma to include smaller oogonia (>30 µm). Since then subgroups have been described based on morphological, physiological, or pathological characteristics. Kaufmann and Gerdemann (1958) reported a Phytophthora spp. on soybean having oogonia within the range reported by Tompkins et al. (1936) for P. megasperma ($\bar{\mu}$ = 36.9 µm), but chose to establish a new species, P. sojae. Later, Hildebrand (1959) reported similar small spored isolates ($\bar{\mu}$ = 36.3 µm) to be host specific to soybean and erected P. megasperma var. sojae. Waterhouse (1963; 1970) and later Newhook et al. (1979) agreed with a varietal division but based their distinction on size of oogonia rather than host specificity. By this method isolates with small oogonia (<45 µm) were placed in P. megasperma var. sojae and those with larger oogonia (>45 µm) in P. megasperma var. megasperma. Confusion has occurred because: (1) isolates with small oogonia have been reported from other hosts (Erwin 1965; Irwin 1974), and (2) isolates with intermediate diameters of oogonia have been reported (Kuan and Erwin 1980). In addition, Kuan and Erwin (1980) reported host specialization of isolates from alfalfa and soybean and suggested subgroupings of

P. megasperma based on this characteristic. Others have reported specificity of isolates to other hosts (Nagai et al. 1978; Hamm and Hansen 1981; Pratt 1981).

The renewed use of host specialization to differentiate isolates of P. megasperma has left unanswered the morphological questions concerning varietal separation. The present work was initiated to determine: (1) if subgroups of P. megasperma exist that can be separated by diameter of oogonia or some other morphological characteristic; (2) what characters are most reliable for taxonomic considerations; and (3) whether Drechslers' description of P. megasperma should be revised to widen the range limits.

To accomplish this project P. megasperma isolates from 15 hosts and 4 continents were obtained. Oregon State University isolates were identified by their nonpapillate, ovoid to obpyriform sporangia (55 X 35 μm) with internal proliferation in liquid culture, and smooth walled oogonia, 30-60 μm in diameter, with mostly paragynous antheridia (Waterhouse 1963; 1970; Newhook et al. 1979). Identification of isolates originating from outside the Pacific Northwest was confirmed based on the same characteristics. Nine cultures, the original and 8 single zoospore subcultures were examined for each isolate. Single zoospore subcultures were included to better quantify isolate variation (Hilty and Schmitthenner 1962; Chapter I).

MATERIALS AND METHODS

Morphological and growth characteristics were compared between thirty-six isolates of P. megasperma (Table 1). Some isolates had previously been identified as large (oogonia >45 μm) or small spored

(<45 μm) types (Kuan and Erwin 1980; Hamm and Hansen 1981). Isolates were identical to those used for zoospore variability investigations (Chapter I) and for host specificity determinations (Chapter III).

Nine cultures, the original and 8 single zoospore isolates (SSI) obtained as previously described (Chapter I) were used collectively as isolate representatives. Range and mean values for 24 characteristics of oogonia, oospores, sporangia and growth rate were measured or calculated; colony morphology characteristics were compared for similarities. Characteristics were also compared by host of origin for isolates from alfalfa, rose, Douglas-fir, grape soil, almond, apple, clover and soybean where more than one isolate per host was available. Microscopic strucutres (10/culture, therefore 90/isolate) were randomly located and measured at 900X. Specific characters and the methods used to obtain them are described below.

Oogonia and Oospores

Characteristics of mature oogonia and oospores were observed on clarified V-8 agar colonies grown for 1 month at 20°C. Diameters of 10 oogonia and oospores and oogonial wall thicknesses were measured for each culture and averaged for the nine cultures of each isolate. Maximum, minimum and average sizes were determined for each isolate. Type of antheridia and production of oogonia (presence or absence and number) were also determined. Types of antheridia were classified: 0 = 100% paragynous; 1 = 76-99% paragynous; 2 = 51-75% paragynous; 3 = 50-75% amphigynous; 4 = 76-100% amphigynous. Production of oogonia, based on number of oogonia plainly visible in one randomly picked field of view (100X) was rated 1, 2, 3, 4, 5 for 0, 5, 6-20, 21-50, and >50 oogonia, respectively.

Sporangia

Sporangia were produced in pea broth colonies (Trione 1974) grown 7 days at 20°C prior to washing with soil extract water. Widths and lengths were measured for typical mature sporangia (10/culture). Maximum, minimum, average length and width values and length/width ratios for each isolate were calculated for the 90 random observations. Shapes of sporangia were recorded and rated by the most numerous kind produced. Shapes of sporangia (Figure 1) were recorded: 1, 2, 3, 4, 5, 6, 7 for spherical, ovoid, ellipsoid, ovoid-obpyriform, obturbinate, obovoid and irregular, respectively. Shapes were compared to those of plate 1 of Newhook et al. (1979).

Colony Morphology

Observations were made on clarified V-8 agar colonies previously used for oogonia observations. Comparisons were based on: (1) amount of aerial hyphae (1 = appressed, 2 = slight, 3 = fluffy); (2) petal or star shaped pattern (1 = none, 2 = faint, 3 = distinct); and (3) colony margin appearance (1 = even or slightly undulating, 2 = rough or irregular).

Growth Characteristics

Maximum, minimum, and optimum temperatures for growth (mm/day) were determined. Agar blocks (.5 cm^3) from actively growing corn meal agar colonies were transferred (2/colony, therefore 18/isolate) to individual corn meal agar plates. Colonies were placed for 7 days (11 days at 5°) in incubators at 5, 10, 15, 20, 25, 30 and 35°C. Means were based on 18 plates.

Statistical Analysis

Two multivariate analyses were used. A cluster analysis was used to join isolates with similar characteristics based on smallest dissimilarity values (Lancer and Williams 1967). Characteristics used for analysis included those listed in Tables 7-11 except type of antheridia and colony morphology information. Growth rate data from Table 11 were converted to more useful information (growth at 5° and 35°C, and optimum temperature and degrees of optimum temperature for growth). A stepwise discriminate analysis was used to examine the orientation of the clusters relative to one another. This analysis generated a value for each isolate based on the reduction of the number of variables to a single point on an axis which maximized the ratio of the among group sum of squares to the within group of squares. The program then computed an F value for the dissimilarity between the clusters (Cooley and Lohies 1971). Correlation coefficients were simultaneously calculated.

RESULTS

Oogonia

Mean diameters of the 36 isolates ranged from 37 to 55 µm, averaging 40.1 µm. Individual oogonia ranged from 21 to 64 µm (Table 7). Maximum diameters for each isolate were generally above 45 µm and minimum diameters were below 45 µm. All isolates formed predominately paragynous antheridia, although some alfalfa isolates (S1, S2, PC5, and M1) produced up to 50% amphigynous antheridia. Most isolates formed some amphigynous antheridia. Production of oogonia ranged from infrequent to numerous, although most

Table 7. Oogonial characteristics by host of origin for 36 isolates of Phytophthora megasperma.[1]

Host and Isolate	Range	Mean	Antheridia[2]	Production[3]	Wall Thickness
Alfalfa					
W1	34.7-50.0	40.8	1	2.9	1.6
S1	26.7-48.1	39.3	2	1.0	1.7
P1	36.5-53.4	47.1	1	2.9	1.4
M1	31.2-45.4	37.1	2	4.4	1.0
PC3	24.2-50.4	38.6	1	1.2	1.4
PC5	27.0-56.1	38.8	2	1.2	1.5
S2	31.2-46.3	39.0	2	1.3	1.1
P3	36.5-56.1	48.2	0	3.8	1.3
5b	31.2-50.7	40.0	1	1.3	1.7
DA	33.8-47.2	40.7	1	1.4	1.2
Mean		41.1		2.1	1.4
Almond					
K2	46.3-53.4	47.6	1	2.8	1.4
K3	30.3-44.5	39.9	1	3.1	1.2
Mean		43.8		2.9	1.3
Grape Soil					
K10	31.2-51.6	51.8	0	3.7	1.3
K11	43.6-62.3	40.4	1	3.3	1.3
Mean		46.1		3.5	1.3
Douglas-fir					
B3A	21.3-55.2	42.6	1	4.6	1.2
B217	31.2-52.5	42.7	1	3.4	1.3
345	31.2-57.9	44.1	1	3.2	1.3
336	33.8-56.1	46.6	1	2.2	1.3

Table 7 continued.

Host and Isolate	Range	Mean	Antheridia[2]	Production[3]	Wall Thickness
Douglas-fir continued					
C-17-2D2	48.1-62.3	55.3	0	2.9	1.3
520	38.3-60.5	51.0	1	2.8	1.2
Mean		47.0		3.2	1.3
Soybean					
908	32.9-49.0	41.7	1	4.2	1.4
909	34.7-49.0	41.5	1	4.1	1.4
Mean		41.6		4.1	1.4
Clover					
105	32.0-57.9	43.2	1	2.4	1.2
117	35.6-57.9	44.8	1	1.3	1.1
102	33.8-58.7	46.8	1	2.1	1.4
Mean		44.9		1.9	1.6
Apple					
T14	34.7-50.7	40.5	0	2.3	1.2
T47	35.6-53.4	43.3	1	2.3	1.2
Mean		42.0		2.3	1.2
Rose					
PA	36.6-49.0	38.9	1	1.0	1.0
PB	38.3-49.0	44.1	1	1.0	1.0
Mean		41.5		1.0	1.0

Table 7 continued.

Host and Isolate	Range	Mean	Antheridia[2]	Production[3]	Wall Thickness
Mixture					
K1	35.6-55.2	47.4	0	3.3	1.3
K8	43.6-64.1	51.4	0	3.3	1.3
K9	31.2-51.6	42.6	0	3.7	1.3
T32[4]	32.4-50.0	42.7	1	1.0	1.1
T56	32.9-53.4	43.3	1	1.7	1.2
T28	38.3-57.9	49.2	1	1.4	1.3
NF1	48.1-57.0	49.8	0	3.0	1.4
Grand Mean		40.1		2.6	1.4
Overall Range	21.3-64.1		0-2	1.0-4.6	1.0-1.7

[1]Values based on 90 observations/isolate from clarified V-8 agar grown 1 mo. at 20°C.

[2]Antheridia ratings: 0 = 100% paragynous; 1 = 76-99% paragynous; 2 = 51-75% paragynous; 3 = 50-75% amphigynous; 4 = 76-100% amphignous.

[3]Oogonia production ratings 1,2,3,4,5 = 0, <5, 6-20, 21-50, >50, respectively, visible in field of view at 100X.

[4]SSI of this isolate could not be obtained so values are means of ten observations of parent culture.

isolates formed oogonia readily. Wall thicknesses of oogonia were from 1.0-1.7 µm, averaging 1.4 µm.

Diameter of oogonia from alfalfa, apple, soybean and rose were nearly identical (41-42 µm), as were isolates from almond, grape soil, Douglas-fir, and clover (44-47 µm). Average diameter of isolates K9, B3A, B217, 345, 105, 117, 102, T56, T47 and 909 were below 45 µm. One or more SSI from each produced oogonia whose diameters averaged 45 µm.

Oogonia were produced in all isolates. Production of oogonia was generally lower for alfalfa (mean rating 2.1), rose (mean rating 1.0), apple (mean rating 2.3) and clover (mean rating 1.9) and high for Douglas-fir (mean rating 3.2), grape soil (mean rating 3.5), almond (mean rating 2.9) and soybean (mean rating 4.1). Wall thickness of oogonia was uniform regardless of host of origin.

Oospores

Diameter of oospores for the 36 isolates ranged from 31.1-48.8 µm, averaging 37.0 µm. Individual oospores ranged from 18-55 µm (Table 8). Diameter of oospores were highly correlated with oogonia diameter (r^2 = .97). Oospore size by host followed a similar pattern to that reported for oogonia. Average diameters were generally the same for alfalfa, almond, clover, apple, soybean, and rose (35-37 µm), slightly higher for grape soil and Douglas-fir (40-41 µm).

Sporangia Morphology

Length and width measurements of sporangia are listed in Table 9. Mean lengths and widths of the 36 isolates ranged from 42 to 73 µm,

Table 8. Diameter of oospores by host of origin for 36 isolates of *Phytophthora megasperma*.[1]

Host and Isolate	Diameter Range	Mean
Alfalfa		
W1	30.3-41.8	36.0
S1	22.2-39.2	32.0
P1	35.6-47.2	41.0
M1	27.6-32.9	31.4
PC3	18.0-39.6	31.1
PC5	18.0-39.2	31.1
S2	23.1-38.3	33.3
P3	36.5-49.8	42.3
5b	24.0-46.3	33.2
DA	27.6-41.0	33.9
Mean		34.5
Almond		
K2	32.0-49.8	41.0
K3	30.3-39.3	33.5
Mean		37.3
Grape Soil		
K10	26.7-43.6	45.5
K11	36.5-55.2	34.6
Mean		40.1
Douglas-fir		
B3A	18.2-45.4	35.3
B217	28.5-44.5	36.3
345	26.2-49.0	37.7
336	32.9-49.8	41.0
C-17-2D2	42.1-55.2	48.8
520	30.3-49.0	44.1
Mean		40.5
Soybean		
908	27.6-42.7	35.8
909	28.5-47.0	35.5
Mean		35.7
Clover		
105	21.0-45.4	34.8
117	27.6-47.2	36.8
102	27.6-49.0	38.8
Mean		36.8

Table 8 continued.

Host and Isolate	Diameter Range	Mean
Apple		
T14	28.5-41.8	35.5
T47	30.3-45.4	37.5
Mean		36.5
Rose		
PA	32.0-44.5	34.2
PB	36.5-42.7	39.5
Mean		36.8
Mixture		
K1	29.6-46.3	41.3
K8	39.2-54.3	45.0
K9	26.7-43.6	36.4
T32[2]	36.0-38.0	35.9
T56	27.6-45.4	37.2
T28	33.8-50.7	42.0
NF1	35.6-51.6	43.6
Grand Mean		37.0
Overall Range	18.0-55.2	

[1]Values based on 90 observations/isolate from clarified V-8 agar after 1 mo. at 20°C.

[2]SSI of this isolate could not be obtained so values are means of ten observations of parent cultures.

Table 9. Sporangial characteristics by host of origin for 36 isolates of Phytophthora megasperma.[1]

Host and Isolate	Range Length		Range Width	Mean	Ratio Length/Width (Ave.)	Shape[2]
Alfalfa						
W1	35.6-62.3	X	28.8-46.3	48.6 X 34.1	1.4:1	2,3,4
S1	41.8-73.9	X	26.7-37.4	53.9 X 38.5	1.4:1	2,3,4
P1	49.8-74.8	X	35.6-55.2	63.1 X 44.1	1.4:1	2,3,4,7
M1	24.9-73.0	X	24.9-49.5	49.5 X 35.1	1.4:1	2,4
PC3	46.3-66.8	X	32.9-49.8	55.7 X 41.1	1.6:1	2,4
PC5	42.7-62.3	X	32.9-49.8	53.5 X 40.2	1.4:1	2,3,4,5
S2	44.5-71.2	X	31.2-49.8	54.9 X 39.0	1.4:1	2,3,4
P3	56.1-80.1	X	37.4-57.0	66.5 X 45.1	1.4:1	4,2,3
5b	45.4-74.8	X	22.9-49.0	57.7 X 40.0	1.7:1	2,6,4
DA	28.5-71.2	X	21.6-47.2	50.4 X 36.1	1.4:1	2,4,3
Mean				55.4 X 39.4	1.4:1	
Almond						
K2	46.3-80.1	X	31.2-57.9	65.1 X 43.9	1.5:1	2,4,5
K3	55.2-87.2	X	35.6-58.7	66.9 X 47.0	1.5:1	4,2,6
Mean				66.0 X 45.5	1.5:1	
Grape Soil						
K10	48.6-91.8	X	31.2-57.9	66.1 X 44.9	1.5:1	2,4,3,6
K11	48.1-83.7	X	32.9-60.5	61.6 X 44.6	1.4:1	2,4,3
Mean				63.9 X 44.8	1.45:1	

Table 9 continued.

Host and Isolate	Range Length		Range Width	Mean	Ratio Length/Width (Ave.)	Shape[2]
Douglas-fir						
B3A	40.1-66.8	X	28.5-42.7	52.3 X 34.6	1.4:1	2,4,3
B217	41.8-75.7	X	24.9-46.3	56.5 X 35.6	1.5:1	2,3,4,5
345	41.8-69.4	X	24.5-45.4	55.1 X 36.2	1.6:1	2,3,5,4
336	43.2-85.4	X	32.9-50.7	59.8 X 41.0	1.5:1	2,4,3,7
C-17-2D2	49.0-81.0	X	35.6-51.6	64.2 X 42.9	1.5:1	2,4,3
520	34.2-85.4	X	27.0-53.4	59.1 X 38.6	1.5:1	2,4,3,5,6
Mean				57.8 X 38.2	1.5:1	
Soybean						
908	46.3-91.7	X	35.6-55.2	72.7 X 44.5	1.5:1	2,3,4
909	44.5-102.4	X	29.4-49.8	67.9 X 39.6	1.6:1	2,3,7
Mean				70.3 X 42.1	1.55:1	
Clover						
105	32.4-56.1	X	22.3-36.0	42.4 X 29.9	1.4:1	2,3,4,5
117	30.6-57.9	X	21.6-44.5	44.6 X 32.3	1.4:1	2,4
102	32.4-62.3	X	21.6-36.0	44.6 X 30.6	1.5:1	3,2,4
Mean				42.1 X 30.9	1.43:1	
Apple						
T14	43.6-92.6	X	24.0-57.9	64.2 X 41.4	1.6:1	2,3,4
T47	32.4-77.4	X	23.4-49.0	57.1 X 38.2	1.5:1	2,4,3
Mean				60.7 X 39.8		

Table 9 continued.

Host and Isolate	Range Length		Range Width	Mean	Ratio Length/Width (Ave.)	Shape[2]
Rose						
PA	35.6-96.1	X	26.7-60.5	68.2 X 41.7	1.6:1	2,3,7
PB	41.8-81.0	X	28.5-60.5	62.0 X 49.4	1.3:1	2,1
Mean				65.1 X 45.6	1.45:1	
Mixture						
K1	41.8-78.3	X	32.0-54.3	61.7 X 42.0	1.5:1	4,2,5
K8	40.1-87.2	X	31.2-58.3	60.4 X 40.6	1.5:1	2,4,3
K9	48.6-91.8	X	31.2-57.9	62.0 X 44.3	1.5:1	2,4,3,6
T32[3]	52.9-71.2	X	40.1-44.5	66.8 X 42.2	1.4:1	4,2
T56	54.3-108.6	X	33.8-52.5	65.5 X 40.4	1.6:1	2,4,3,5
T28	39.6-99.6	X	28.8-46.3	62.7 X 38.7	1.6:1	2,3,4,5
NF1	49.0-89.0	X	31.2-55.2	62.0 X 43.1	1.4:1	4,2,3,5
Grand Mean				58.7 X 39.8	1.48:1	
Overall Range	24.9-108.6	X	21.6-60.5		1.3-1.7:1	

[1]Values represent 90 observations. Sporangia produced in 7 day old pea broth colonies washed with distilled water and incubated overnight at 20°C.

[2]Sporangia shapes 1,2,3,4,5,6,7 = spherical, ovoid, ellipsoid, ovoid-obpyriform, obturbinate, obovoid and irregular, respectively. Shapes listed in decreasing order of number observed. Shapes after Newhook et al. (1979) plate 1.

[3]SSI of this isolate could not be obtained so values are based on ten observations of parent isolate.

and 30 to 49 µm respectively, averaging 58.7 X 39.8 µm. Length/width ratios were 1.3:1 to 1.7:1 (average 1.5:1). Sporangia shapes were predominately ovoid, with fewer ellipsoid or ovoid-obpyriform.

Average width and length values were nearly equal for apple (61 X 40 µm), alfalfa (55 X 39 µm), and Douglas-fir (58 X 38 µm) isolates. Somewhat larger sporangia were produced by isolates from almond (66 X 46 µm), grape soil (64 X 45 µm) and rose (65 X 46 µm). Clover isolates produced the smallest sporangia (42 X 31 µm), soybean isolates, the largest (70 X 42 µm). Correlation between average length and width of sporangia was highly significant (r^2 = .85). Length to width ratios were nearly equal in all host groups (1.4-1.5).

Colony Morphology

Isolate colonies grown on clarified V-8 agar varied from appressed to fluffy aerial (range 1-3), homogeneous or slightly patterned (range 1-1.8), and colony margins ranged from even or slightly undulating to rough or irregular (range 1-2) (Table 10).

Alfalfa, apple, clover and rose isolates were generally more aerial on clarified V-8 agar. Colony margins of soybean and grape soil isolates were rough or irregular, others were generally even or slightly undulating.

Growth Characteristics

Maximum, minimum, and optimum temperatures for growth (mm/day) of the isolates at seven temperatures are listed in Table 11. Most isolates grew between 5 and 30°C; only Douglas-fir (B3A, 345, B217) and soybean isolates (908, 909) grew more than 1.0 mm/day at 35°C. Optimum growth for all isolates occurred between 20 and 30°C.

Table 10. Colony morphology of isolates of *Phytophthora megasperma*.[1]

Host and Isolate	Aerial[2]	Pattern[3]	Margin[4]
Alfalfa			
W1	1.2	1.0	1.8
S1	3.0	1.0	1.0
P1	1.1	1.0	1.9
M1	1.0	1.0	2.0
PC3	2.1	1.0	1.1
PC5	3.0	1.0	1.0
S2	2.4	1.0	1.7
P3	1.0	1.4	2.0
5b	2.3	1.0	1.3
DA	2.7	1.0	1.3
Mean	2.0	1.0	1.5
Almond			
K2	1.3	1.0	1.9
K3	1.1	1.0	1.8
Mean	1.2	1.0	1.9
Grape Soil			
K10	1.1	1.0	1.8
K11	1.0	1.4	2.0
Mean	1.2	1.2	1.9
Douglas-fir			
B3A	1.3	1.2	1.6
B217	2.1	1.7	1.2
345	1.8	1.8	1.0
336	1.0	1.4	1.3
C-17-2D2	1.0	1.0	1.9
520	1.6	1.0	1.5
Mean	1.5	1.4	1.4
Soybean			
908	1.0	1.6	1.9
909	1.1	1.1	1.8
Mean	1.2	1.4	1.9
Clover			
105	2.3	1.0	1.2
117	2.4	1.0	1.2
102	3.0	1.0	1.1
Mean	2.6	1.0	1.2

Table 10 continued.

Host and Isolate	Aerial[2]	Pattern[3]	Margin[4]
Apple			
T14	1.8	1.0	1.6
T47	2.0	1.2	1.6
Mean	1.9	1.1	1.6
Rose			
PA	2.3	1.0	1.0
PB	1.6	1.8	1.1
Mean	2.0	1.4	1.1
Mixture			
K1	1.8	1.1	1.2
K8	2.2	1.1	1.3
K9	1.1	1.0	1.8
T32[5]	1.0	1.0	1.0
T56	1.0	1.0	1.8
T28	1.2	1.0	1.6
NF1	1.0	1.0	1.9
Grand Mean	2.6	1.1	1.5
Overall Range	1.0-3.0	1.0-1.8	1.0-2.0

[1]Observations on clarified V-8 agar after 1 mo. at 20°C.

[2]Aerialness based on ratings 1 = appressed; 2 = slight; 3 = fluffy.

[3]Colony pattern based on ratings 1, 2, 3 = none, faint or definite petal or star pattern, respectively.

[4]Colony margin based on 1 = even or slightly undulating and 2 = rough or irregular.

[5]SSI of this isolate could not be obtained so values are based only on parent isolate.

Table 11. Rate of growth (mm/day) of 36 isolates of P. megasperma at 7 temperatures.[1]

Host and Isolate	Temperature (°C)						
	5	10	15	20	25	30	35
Alfalfa							
W1	0.1	0.7	2.6	3.2	4.4	4.7	0.5
S1	0.3	0.8	3.2	4.6	5.2	4.0	0.5
P1	1.3	2.0	3.4	4.1	4.0	3.0	0.0
M1	0.5	0.9	1.5	2.6	4.2	4.0	0.2
PC3	0.2	0.6	2.2	3.5	4.5	4.1	0.5
PC5	0.3	0.6	2.5	3.8	4.7	4.0	0.5
S2	0.2	0.6	2.3	3.5	3.4	3.7	0.6
P3	1.2	1.9	3.3	4.2	4.1	3.0	0.0
5b	0.2	0.7	2.3	3.1	3.9	3.2	0.6
DA	0.3	0.9	3.1	4.5	5.0	4.4	0.3
Mean	0.5	1.0	2.6	3.7	4.3	3.8	0.4
Almond							
K2	1.4	2.0	3.4	3.9	3.1	0.7	0.0
K3	1.4	2.4	3.8	4.5	4.3	2.9	0.0
Mean	1.4	2.2	3.6	4.2	3.7	1.8	0.0
Grape Soil							
K10	1.8	2.2	3.2	4.0	4.1	3.0	0.0
K11	1.6	2.1	3.9	4.7	4.5	1.6	0.0
Mean	1.7	2.1	3.6	4.4	4.3	2.3	0.0
Douglas-fir							
B3A	0.3	1.6	4.2	6.6	6.9	7.4	1.5
B217	0.7	1.5	5.3	6.8	7.2	7.1	1.5
345	0.5	1.7	4.2	6.6	6.7	7.0	1.6

Table 11 continued.

Host and Isolate	Temperature (°C)						
	5	10	15	20	25	30	35
Douglas-fir continued							
336	1.4	2.1	3.0	3.9	3.9	1.9	0.0
C-17-2D2	1.4	2.1	3.9	5.1	5.2	2.1	0.0
520	1.2	2.0	3.7	4.7	4.7	1.3	0.0
Mean	0.9	1.9	3.9	5.6	5.8	4.5	0.8
Soybean							
908	0.0	0.3	2.8	3.1	2.1	1.6	1.0
909	0.5	0.4	2.9	3.1	2.1	1.8	1.3
Mean	0.2	0.4	2.9	3.1	2.1	1.7	1.2
Clover							
105	0.0	0.4	2.6	3.4	3.8	1.4	0.0
117	0.3	1.1	2.7	3.2	2.8	0.5	0.0
102	0.2	0.7	2.4	3.2	3.2	1.8	0.0
Mean	0.2	0.7	2.6	3.3	3.3	1.2	0.0
Apple							
T14	1.0	2.0	3.0	3.8	3.9	1.5	0.0
T47	0.7	1.2	3.2	3.8	3.8	1.3	0.0
Mean	0.9	1.6	3.1	3.8	3.9	1.4	0.0
Rose							
PA	0.0	0.5	1.3	3.0	4.1	1.7	0.0
PB	0.0	2.4	4.5	4.5	6.2	3.7	0.0
Mean		1.2	3.9	3.8	5.2	2.7	0.0

Table 11 continued.

Host and Isolate	Temperature (°C) 5	10	15	20	25	30	35
Mixture							
K1	1.3	1.9	3.4	3.8	4.1	3.0	0.1
K8	1.5	2.3	3.5	4.3	4.6	2.1	0.0
K9	1.8	2.2	3.2	4.0	4.1	3.0	0.0
T32[2]	0.0	0.2	1.9	2.0	0.2	0.0	0.0
T56	1.0	1.3	3.6	4.5	4.0	1.0	0.0
T28	1.0	1.6	3.0	4.2	3.3	1.1	0.0
NF1	1.3	2.0	3.3	4.3	3.9	2.9	0.0
Grand Mean	0.7	1.4	3.1	4.1	4.3	2.8	0.3
Overall Range	0.0-1.8	1.3-2.3	1.9-3.6	2.0-4.5	3.3-4.6	0.0-3.0	0.0-0.1

[1]Isolates grown on corn meal agar with 20 ppm pimaricin for 7 days (11 days at 5°C). Values represent means of 18 replications (2 each from parent culture and 8 single spore isolates).

[2]SSI of this isolate could not be obtained so means based on 2 replications of parent isolate.

Optimum temperature for growth when separated by host was 20°C for almond, grape soil, and soybean; 25°C for alfalfa, rose and Douglas-fir. Clover isolates grew equally well at 20 and 25°C. At 5°C, rose isolates did not grow but grape soil isolates grew well (1.7 mm/day). Soybean isolates grew little (.4 mm/day) at 10°C compared to almond (2.0 mm/day), grape soil (2.0 mm/day) and Douglas-fir (2.0 mm/day). Average growth rates at 30°C were higher for alfalfa (4.0 mm/day), Douglas-fir (5.0) and rose (3.0 mm/day) than almond (2.0 mm/day), apple (1.0 mm/day), grape soil (2.0 mm/day), soybean (2.0 mm/day), and clover (1.0 mm/day). Little or no growth occurred at 35°C by isolates from almond, apple, grape soil, clover, and rose.

Cluster Analysis

A cluster analysis identifying 4 groups was subjected to stepwise discriminate analysis (Table 12). The resulting groups could not be separated by size of oogonia or any other single characteristic. Instead, isolates were grouped consistently with other isolates from the same host of origin, except the isolates from almond and grape soil. Some isolates from both Douglas-fir and alfalfa were placed into separate clusters. Alfalfa isolates from near Corvallis were grouped with other large-spored isolates from several hosts. The remaining alfalfa isolates were grouped with clover isolates. Large-spored Douglas-fir isolates clustered with the local alfalfa isolates and others with large spores. Small-spored Douglas-fir isolates formed a group of their own. Table 13 briefly summarizes the differences between DF Group 1 and DF Group 2 and between alfalfa 1 and 2. The four groups were made up of the following isolates: Cluster A consisted of alfalfa (W1, S1, M1, PC3,

Table 12. Cluster analysis of 36 isolates of Phytophthora megasperma from 4 continents and 14 hosts.[1]

	maximum oogonia[2]	minimum oogonia	mean oogonia	oogonia wall thickness	maximum oospore	minimum oospore	mean oospore	maximum sporangia length[3]	minimum sporangia length	maximum sporangia width	minimum sporangia width	mean sporangia length	mean sporangia width	oogonia production[4]	sporangia ratio length/width	growth at 5°C (mm/day)[5]	optimum temperature	growth at optimum temperature (mm/day)	growth at 35°C (mm/day)
	µm																		
Cluster A[6]	51.7	31.1	40.9	1.3	41.8	24.3	33.9	66.5	37.9	44.7	29.9	50.5	36.1	1.8	1.4	0.2	25.2	4.2	0.3
Cluster B[7]	58.3	41.3	49.8	1.3	51.3	35.5	43.4	84.6	50.4	54.6	32.4	62.9	42.4	2.8	1.5	1.4	21.8	4.4	0.0
Cluster C[8]	50.5	33.9	42.4	1.2	43.0	29.9	36.4	87.9	45.3	55.1	31.5	64.7	42.9	2.6	1.5	0.8	21.9	4.0	0.2
Cluster D[9]	55.2	27.9	43.0	1.3	46.3	24.5	36.4	70.6	41.2	44,8	26.4	54.6	35.5	3.7	1.5	0.5	29.2	7.2	1.5
Grand Mean	53.4	34.6	44.0	1.3	45.2	29.3	37.6	79.0	44.1	50.9	29.6	59.0	40.1	2.5	1.5	0.8	23.5		0.3

[1]Groups were significantly different (p = 0.05) from each other using a stepwise discriminate multivariate analysis with all variables. Values based on 90 observations/isolate.

[2]Oogonia observed after 1 mo. on clarified V-8 agar at 20°C.

[3]Sporangia developed on 7 day old pea broth colonies grown at 20°C, wash with distilled water and when incubated overnight in soil extract water. Values based on 90 observations/isolate.

[4]Oogonia production ratings 1,2,3,4,5, = 0, 5, 5-20, 20-50, >50 respectively, in field of view at 125X.

[5]Growth rate values based on 18 replications/isolate on corn meal agar.

[6]Group A includes alfalfa (W1, S1, M1, PC3, PC5, S2, 5b, DA) and clover (105, 117, 102) isolates: 11 total.

[7]Group B includes Douglas-fir (336, C-17-2D2, 520); Noble fir (NF1); alfalfa (P1, P3); pear (K8); poplar (T28); grape soil (K10) and almond (K2) isolates; 10 total.

[8]Group c includes soybean (908, 909); apple (T14, T47); rose (PB, PA); cherry (K1), almond (K5); juniper (K9); grape soil (K11); hollyhock (T32); Brassica (T56) isolates; 12 total.

[9]Group D includes Douglas-fir (B3A, B217, 345) isolates; 3 total.

Table 13. Morphological and growth characteristics of Phytophthora megasperma from two hosts.[1]

Structure	Douglas-fir		Alfalfa	
	DF Group 1[2]	DF Group 2[2]	Alfalfa 1[3]	Alfalfa 2[3]
Mean oogonia diameter[4]	43.0	51.0	39.4	47.7
Mean oospore diameter[4]	36.3	44.6	32.7	41.7
Mean sporangia size[4]	55.6 X 35.4	61.0 X 40.8	53.0 X 38.0	64.8 X 45.0
Mean sporangia ratio[4] (length/width)	1.6:1	1.5:1	1.5:1	1.4:1
Growth (mm/day)[5]				
5°C	0.5	1.3	1.3	0.3
20°C	6.7	4.6	4.2	3.6
35°C	1.3	0.0	0.0	0.5
Host(s)[6]	Douglas-fir and soybean	Douglas-fir	Alfalfa	Alfalfa and Douglas-fir

[1]Results based on cluster analysis where isolates were joined by similar characteristics based on smallest dissimilarity values. Grouping based on information on Table 13.

[2]DF Group 1 consists of isolates B3A, B217 and 345; DF Group 2 consists of isolates 336, C-17-2D2 and 520.

[3]Alfalfa 1 contains isolates W1, S1, M1, PC3, PC5, S2, 5b and DA; Alfalfa 2 contains isolates P1 and P3.

[4]Based on 90 observations.

[5]Based on 18 plates.

[6]Information from Chapter III.

PC5, S2, 5b, DA) and clover (102, 105, 117) isolates. Cluster B included isolates from Douglas-fir (336, C-17-2D2, 520), alfalfa (P1, P3), Nobel fir (NF1), almond (K2), poplar (T28), grape soil (K10) and pear (K8). Cluster C consisted of soybean (908, 909), apple (T14, T47), rose (PA, PB), cherry (K1), almond (K3), juniper (K9), grape soil (K11), hollyhock (T32), and Brassica (T56). Cluster D was comprised solely of isolates from Douglas-fir (B3A, B217, 345). The four groups were significantly different ($p = 0.05$) from each other when variables were compared simultaneously. Largest separation ($F > 10$) occurred between clusters A and B, A and C, B and D, and C and D. Clusters A and D, and B and C were not as distinguishable ($F = 5.6$ and 2.7, respectively).

DISCUSSION

The recent report (Kuan and Erwin 1979) questioning the lack of evidence for varietal subgrouping of P. megasperma by diameter of oogonia (<45 µm and >45 µm) is confirmed by our results. When we compared diameter of oogonia, no distinct break occurred between small and large-spored isolates (Figure 2). The addition of information reported by Kuan and Erwin (1980) and that obtained through a comprehensive literature search for P. megasperma characteristics (Table 14) did not support a subdivision based on this character.

A number of isolates have been described with oogonia near the 45 µm diameter established for varietal separation (Kuan and Erwin 1980; Drechsler 1931; Robertson and Dance 1971). In addition, the literature contained references to surprisingly few isolates with oogonia above 45 µm (3 of 21, Table 14) and none larger than 48 µm until recently

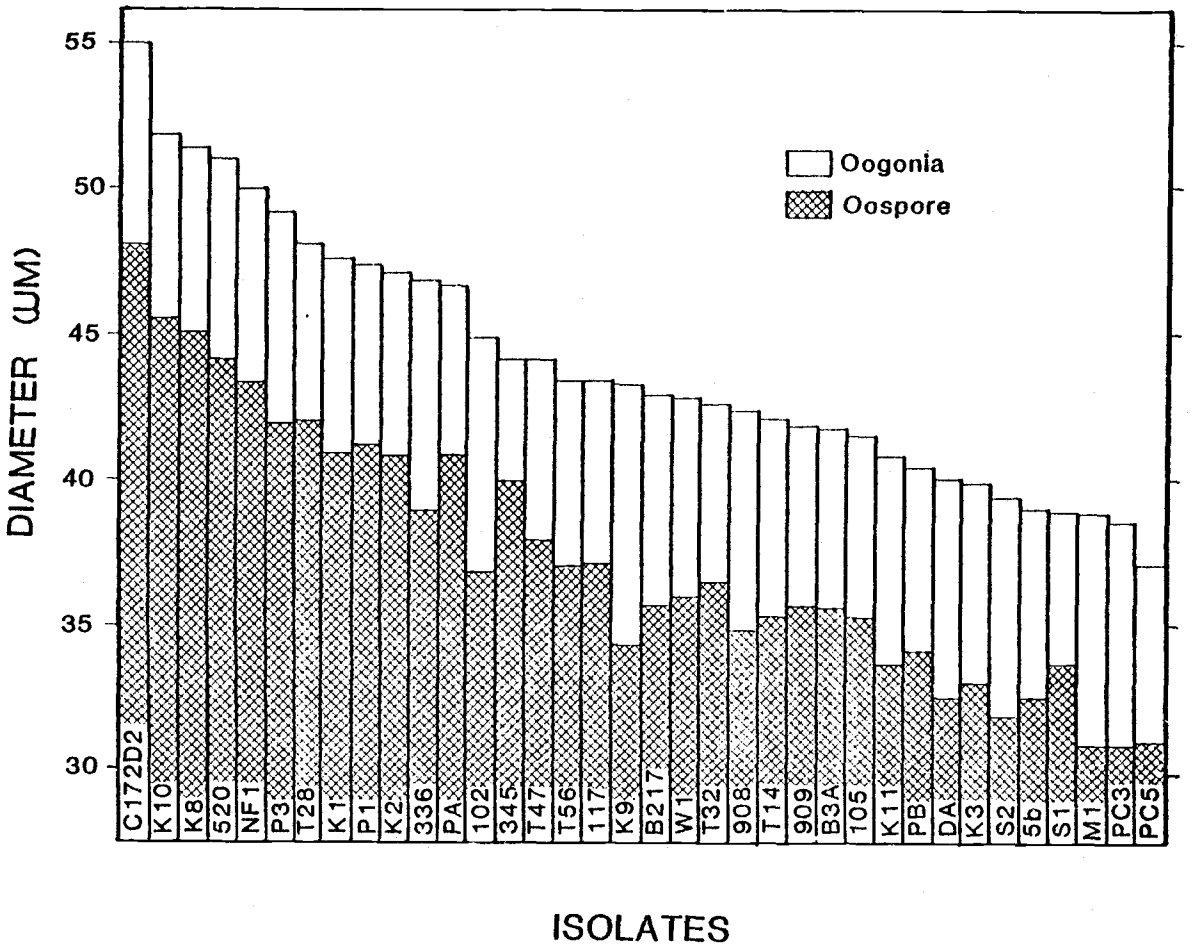

Figure 2. Average oogonia and oospore diameters of 36 isolates of Phytophthora megasperma. Isolates grown on clarified V-8 agar at 20°C for 1 mo. Values represent means of 90 observations.

Table 14. Literature Descriptions of Phytophthora megasperma.

Citation	Host	Oogonia[1]			Oospore[1]		Sporangia[1]					Growth (temp.)		
		Range	Ave.	Antheridia[2]	Range	Ave.	Range length/width	Ave.	Ratio[3]	Shape[4]	Prolif.[5]	Min.	Opt.	Max.
Skotland, 1955	Soybean	29-42	[6]	P	23-32		35-48X16-32			2	Yes		24	
Kaufmann and Gerdemann, 1958	Soybean	28-45	37	P	19-38	31	23-88X17-52	58X38	1.5:1	2,3		>5	20	<35
Hildebrand, 1959	Soybean	29-46	36	mostly P	23-35	31	43-65X32-53	53X35	1.5:1	5	Yes	>5	25	32.5
Morgan and Hartwig, 1965	Soybean	26-37	37	mostly P	26-33	29	35-65X16-28	42X26	1.6:1	5	Yes	5	28	35
Morgan and Hartwig, 1965	Soybean	26-37	37	mostly P	26-36	29	36-66X17-25	39X25	1.6:1	5	Yes	>5	28	<35
Erwin, 1954	Alfalfa	23-39	31	A	19-37	25	27-63X23-38	39X31	1.3:1	4	Yes	8	25	30-33
Bushong and Gerdemann, 1959	Alfalfa			mostly A						4,5	Yes	5	25	35
Erwin, 1965	Alfalfa			% P 7-33			40-60X24-30			3,2				
Irwin, 1974	Alfalfa	27-54	35	both	24-46	31	32-51X23-35	41X30	1.4:1	4	Yes			
Leath and Baylor, 1975	Alfalfa	26-39	32	both	17-30	24	25-58X17-37	37X30	1.2:1		Yes			
Welty and Busbice, 1976	Alfalfa					29		198X118[7]	1.7:1	1				
Ribeiro et al. 1978	Alfalfa	----------None Produced----------					37-54X29-42	47X33	1.4:1	3	Yes	<9	39	<42[7]
Matsumoto and Araki, 1978	Alfalfa		34	mostly P		29		42X31	1.4:1	4		5-7	22-28	32-35
Johnson and Keeling, 1969	Suberranean Clover		31	mostly A		27		54X32	1.7:1	2	Yes		24	
Drechsler, 1931	Hollyhock	16-61	47	mostly P	11-54	41	15-60X16-45				Yes			

Table 14 continued.

Citation	Host	Oogonia[1] Range	Oogonia[1] Ave.	Antheridia	Oospore Range	Oospore Ave.	Sporangia[1] Range length/width	Sporangia[1] Ave.	Ratio[3]	Shape[4]	Prolif.[5]	Growth (temp.) Min.	Growth (temp.) Opt.	Growth (temp.) Max.
Tompkins, et al. 1936	Cauliflower	32-45	36	mostly P	28-38	33	42-56X28-40	49X34	1.4:1	5			25	<35
vanderZwett and Forbes, 1961	Sugarcane	26-42	35	mostly P	19-38		27-56X20-39	42X27	1.6:1	2,3	Yes	5	20-22	35
Boesewinkel, 1974	Asparagus	19-46	28	mostly P		25	22-65X14-32	41X19	2.2:1	5	Yes	5	22	<30
Mircetich and Matheron, 1976	Cherry	29-45	38	P	28-42	35	39-67X23-35	51X30	1.7:1	5,2	Yes	5	24-30	<33
Nagai, et al. 1978	Rose	35-55	43	88% P	28-49	39	23-70X18-42	47X30	1.6:1	2,3	Yes	>5	25	<35
Robertson and Dance, 1971	Apple	40-50	45	both	36-42	30	37-54X30-37	49X27	1.8:1	3	Yes		22	
Brasier and Strouts, 1976	Horse Chestnut	37-54	48	mostly P				61X36	1.7:1		Yes			

[1] Oogonia, oospore and sporangia values in microns.

[2] Antheridial type P = paragynous, A = amphigynous.

[3] Ratio determined by dividing length by width.

[4] After Newhook (1979), sporangia shapes 1,2,3,4,5,6,7 = spherical, ovoid, ellipsoid, ovoid-obpyriform, obturbinate, obovoid, and irregular respectively.

[5] Proliferation either internal or external.

[6] Blanks are missing values.

[7] These values were *not* considered for the proposed taxonomic change for P. megasperma.

(Kuan and Erwin 1979; Hamm and Hansen 1980). Apparently few strains with large oogonia (>45 µm) exist and these belong to a continuum of sizes encompassing both described varieties (Figure 2; Kuan and Erwin 1979, Figure 3). Similarly, no other single morphological or growth characteristic examined here (Figure 3) or by Kuan and Erwin (1979) was identified that justified varietal subgrouping when used singly. In fact, this pooled information (Table 14) shows that wider limits are necessary for these morphological and growth characteristics than previously used to describe the species (Drechsler 1931).

When morphological and growth characteristics were compared simultaneously, multivariate analysis separated these isolates by host of origin (Table 12). Soybean, clover, and rose isolates were constantly grouped with other isolates from the same host. Isolates from these hosts have been reported to be host specific (Nagai, et al. 1978; Kuan and Erwin 1980; Pratt 1981). In addition, though isolates from Douglas-fir and alfalfa were each separated into two groups (DF Group 1 and 2, alfalfa 1 and 2), distinctive pathogenic reactions were found between these groups of isolates (Chapter III).

Apparently a relationship exists between distinct morphological and growth characteristics and distinct host specificity. A correlation of this kind may have important pathological implications but should not be used as a basis for taxonomic subgrouping of this species. This relationship implies that probable pathogenicity of an isolate of P. megasperma could be determined from its morphological characteristics. No pathogenicity test would be required.

Taxonomic subgrouping based on this relationship, however, has no practical value because the groups cannot be simply defined. When

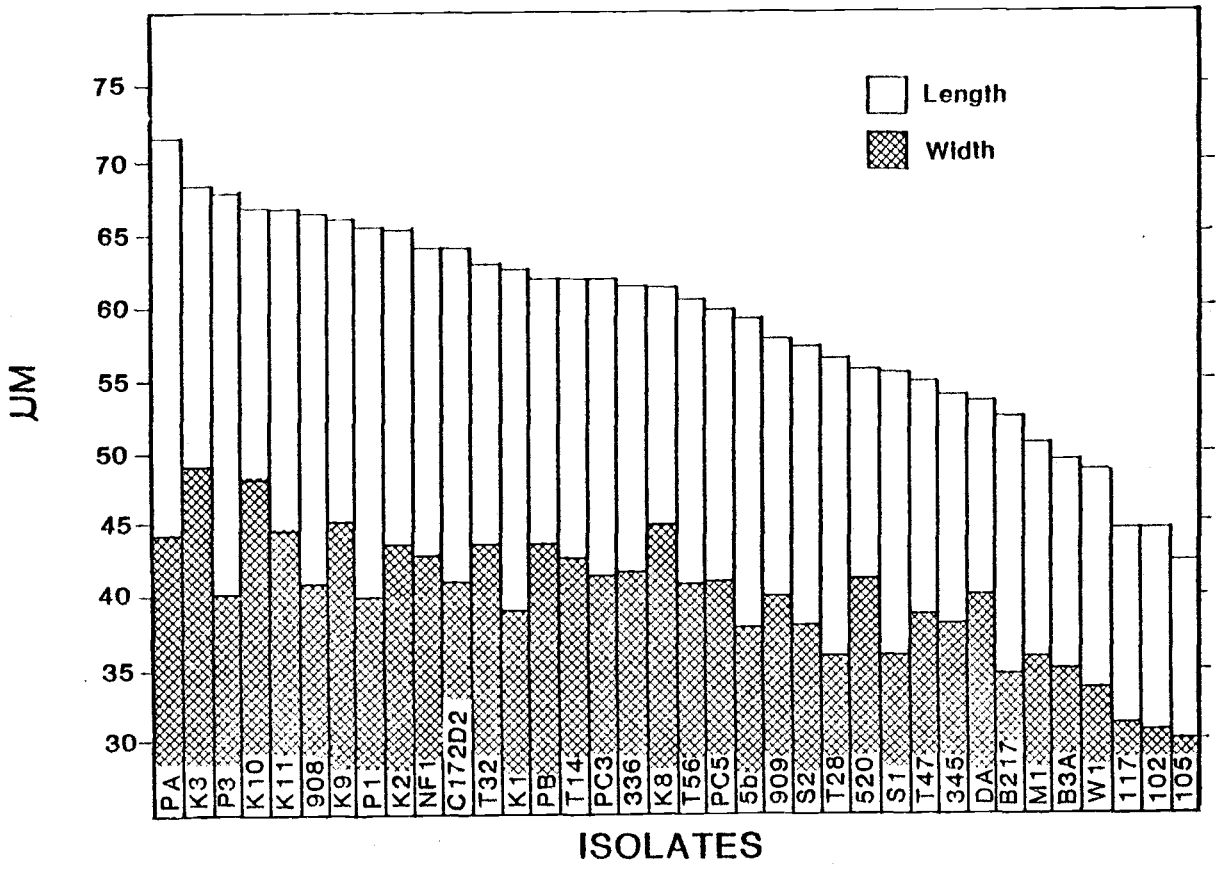

Figure 3. Average sporangia length and width of 36 isolates of Phytophthora megasperma. Sporangia produced on 7 day old pea broth colonies grown at 20°C after overnight washing with soil extract water. Values represent means of 90 observations.

characteristis are compared singly or collectively between clusters A (alfalfa and clover) and D (DF Group 1) or clusters B (DF Group 2) and C (soybean and rose) of Table 12, few differences are evident even though these clusters were significantly different.

Lack of a morphological break to justify varietal separation is not surprising. P. megasperma was initially described in the Eastern United States (Drechsler 1931) years before reports of similar isolates (Tompkins et al. 1936; Kaufmann and Gerdemann 1958; Hildebrand 1959). Drechsler described the species based on isolates from one geographical area and therefore could not have identified the range of variation present in the population. Tompkins et al. (1936) were first to recognize variation from the original description in isolates from cauliflower but their revision was either overlooked or neglected by Kaufmann and Gerdemann (1958) who chose to name similar isolates P. sojae. When Hildebrand (1959) identified P. megasperma var. sojae he was unaware of the range of oogonia diameters possible in the species and undoubtedly put too much emphasis on host specificity as a character for varietal separation. Finally, when Waterhouse (1963; 1970) purposed varietal distinction based on size of oogonia (<45 µm and >45 µm) she apparently had no new information and maintained Hildebrand's suggested subgrouping (var. sojae), but removed host specificity as a characteristic for division. The division of 45 µm seems arbituary since 10 isolates whose oogonia average below 45 µm and therefore would be P. megasperma var. sojae produced SSI whose average oogonia were above 45 µm and therefore would be identified as P. megasperma var. megasperma. Obviously, separation of P. megasperma isolates by oogonia diameter alone is no longer justified (Kuan and Erwin 1979; Figure 2).

Since specific host pathogenicity is not yet accepted as a taxonomic characteristic varietal designation should be abandoned. The following descriptive revision of P. megasperma Drechsler is suggested based on the ranges of characteristics reported here and elsewhere. Sporangia mostly ovoid, fewer ellipsoid or ovoid-obpyriform; measuring 15-109 X 6-76 µm, mostly 37-68 X 24-45 µm (ave. 53 X 35 µm); ratio 1.2-1.8:1, averaging 1.55:1; proliferation internal or external; and rarely formed on solid substrate. Oogonia large, ranging 16-64 µm, mostly 28-55 µm (mean 40.5 µm), and usually produced readily in single strain culture on V-8 agar; oogonia smooth walled, walls 1.0-1.7 µm (mean 1.3 µm) thick. Oospores smooth, measuring 11-55 µm, mostly 25-59 µm (mean 34 µm). Antheridia mostly paragynous, occasionally amphigynous in some isolates. Colony on V-8 agar appressed to slightly aerial, with no floral pattern and rough or undulating margin. Growth normally occurs from <5 to >35°C, optimum 15-30°C (mostly 20-25°C). Growth rate can vary greatly. Table 15 summarizes Drechslers' and Waterhouses' descriptions and contrasts these proposed changes. No alteration of Waterhouses' key (1963) is necessary to incorporate these changes except to remove the varietal distinctions.

Cultural or other conditions may cause morphological characteristics to vary beyond the limits described. Ten isolates used here were also used by Kuan and Erwin (1979) for their morphological comparisons, with somewhat different results. Oogonia and oospores averaged 7 and 10% smaller, respectively, in these tests though the same culture medium (clarified V-8 agar) was used. For that reason, the forementioned description is not an absolute quantification of variation of P. megasperma characteristics and should be used flexibly.

Table 15. Comparisons between previous descriptions and the proposed taxonomic change for Phytophthora megasperma.

P. megasperma var. megasperma (Drechsler 1931 & Waterhouse 1963)	P. megasperma var. sojae (Hildebrand 1959)	Proposed Change[1]
oogonia	oogonia	oogonia
Range 16-61 µm Most 42-52 µm Average 47.4 µm	Range 39.4-45.7 µm Average 36.3 µm	Range 16-64 µm Most 28-55 µm Average 40.5 µm
Wall Thickness Average 1.2 µm		Wall Thickness Average 1.3 µm Mostly paragynous antheridia
oospores	oospores	oospores
Range 11-54 µm Most 37-47 µm Average 41.4 µm	Range 22.8-35.1 µm Average 30.7 µm	Range 11-55 µm Most 24-49 µm Average 34 µm
sporangia	sporangia	sporangia
Range 15-60 X 6-45 µm	Range 42.5-65.4 X 31.8-52.5 µm Average 52.5 X 35.1 µm	Range 15-109 X 6-67 µm Most 37-68 X 24-45 µm Average 53 X 35 µm
Shape ovoid-obpyriform	Shape ovoid-obpyriform	Shape ovoid, fewer ellipsoid or ovoid-obpyriform Ratio Range 1.2-1.8:1 Mean 1.55:1 Formed rarely on solid medium
Growth Range 2-30 C Optimum 15?-27.5 C	Growth Range 5-35 C Optimum 25 C	Growth Range <5-35 C Optimum 20-30 C

[1]Values incorporate data from Tables 8-10 and 12 reported here and those obtained through a comprehensive literature search (Table 14).

This work, the examination of single spore isolates in Chapter I, and the descriptions by others listed in Table 14 identifies both inadequate and useful characters for identifying P. megasperma. Inconsistent characters are optimum growth temperature, growth rates (mm/day), hyphal swellings, colony morphology, and pathogenicity. Optimum temperature ranged from 20-30°C; one isolate ranged as high as 42°C (Ribeiro 1978). Growth rate was variable between isolates at all temperatures; growth rate at optimum temperatures varied from 1.2 to 7.4 mm/day among isolates. Hyphal swellings in these isolates were only occasionally observed. General colony appearance varied as shown by Kuan and Erwin (1979). Additional evidence showing unreliability of growth rate and colony morphology has been found in examining single spore isolates (Chapter I). Pathogenicity can be important but not diagnostic since isolates of P. megasperma from the same location have been found to differ in host ranges (Hamm and Hansen 1981; Chapter III). Though not consistent throughout the population, these characteristics are generally useful for comparisons between isolates from the same geographical location and host, or as additional characters. This is not unreasonable since host of origin is related to morphological and growth characteristics (Table 12).

In contrast, sporangia, though quite variable in width and length, had consistent length/width ratios and were uniformly nonpapillate with proliferation either internally or externally. Sporangia were consistently shaped ovoid, ellipsoid, or ovoid-obpyriform. Numerous large oogonia are produced in most colonies; antheridia were mostly paragynous. The use of length/width ratio of sporangia, diameter of oogonia, production of oogonia (homothallism) and production of mostly

paragynous antheridia as important taxonomic characteristics is also supported by relative lack of variation in these characters among single spore isolates (Chapter I). Oogonia were formed by 94% of the 315 colonies observed and of those 96% formed >51% paragynous antheridia. The use of length/width ratio of sporangia is further supported by the similarities of this characteristic between DF Group 1 and 2, and between alfalfa 1 and 2 (Table 13; Hamm and Hansen 1981) where other characteristics were consistantly different.

Pratt (1981) recently questioned the importance of oogonia formation (homothallism or heterothallism) and antheridia attachment in his discussion of the taxonomy of P. megasperma and P. erythroseptica. He reported that isolates of P. erythroseptica did not produce oogonia while other colonies isolated in close proximity formed oogonia readily. In addition, he found that isolates produced larger numbers of paragynous antheridia on solid media than in liquid culture. Our observations were limited to clarified V-8 agar where nearly all isolates formed oogonia and produced predominately paragynous antheridia. The discrepancy between his report and that reported here may be due to the isolates Pratt used for comparisons. His observations were from arrowleaf clover and alfalfa isolates which we found to have a greater tendency to form amphigynous antheridia. In addition we also found alfalfa isolates to have a greater tendency not to form oogonia (Chapter I). These conditions could give an undue weight to these characteristics as a diagnostic tool in P. megasperma. Some variation in these characteristics should be expected since variation in antheridial type due to media has been previously reported (Erwin 1965) and because single spore isolates have been found to develop oogonia when parent

isolates did not (Chapter I). Variation can be expected in all characteristics (Chapter I) and should be considered when identifying unknown isolates. Ribeiro (1978) came to a similar conclusion when he identified isolates that failed to produce oogonia as P. megasperma recognizing that other distinctive characteristics could be used for comparisons.

The wide variation of morphological and growth characteristics within the population of P. megasperma demonstrates how unwise differentiation of isolates into distinct groups can occur when variation in the population is inadequately ascertained. For that reason we have proposed new limits for the inclusion of isolates in P. megasperma and suggested the removal of varietal designations. Future identification of any new taxon within this group should be done only after: (1) careful comparisons of previously described taxa; (2) an attempt to acquire similar isolates from throughout the known range; and (3) morphological and growth comparisons have been made between parent and several single zoospore isolates. Unless clearly justified the identification of a new taxon only hinders classification when (if) intermediate forms appear.

CHAPTER III

HOST SPECIFICITY OF *PHYTOPHTHORA MEGASPERMA* FROM DOUGLAS-FIR, SOYBEAN, ALFALFA, AND ARROWLEAF CLOVER

INTRODUCTION

Phytophthora megasperma Drechs. has been isolated from many hosts since its original description on hollyhock (Drechsler 1931). However, recent investigations have involved primarily alfalfa and soybean, and host specificity of isolates from those crops has been demonstrated. Hildebrand (1959) recognized that isolates from soybean, which he designated *P. megasperma* var. *sojae*, differed from *P. megasperma* in having smaller oogonia (36 µm versus 47 µm, respectively). Hildebrand's isolates were pathogenic to soybean but not to alfalfa or 47 other crop varieties representing 28 plant species. Subsequently, host specificity was demonstrated for isolates from alfalfa, soybean and arrowleaf clover (Kuan and Erwin 1980; Pratt 1981). Kuan and Erwin (1980) erected *formae speciales* to subdivide *P. megasperma* based on host specificity. Isolates from soybean 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 (Hansen et al. 1979; 1980). The present work had a two-fold purpose: First, to compare the pathogenicity and virulence of *P. megasperma* isolates from Douglas-fir with isolates from alfalfa, soybean and clover reported to be host specific, and secondly, to determine if *P. megasperma* isolates from other hosts exhibited host specificity.

MATERIALS AND METHODS

Two separate series of pathogenicity tests (1 and 2) were used to evaluate pathogenicity and host specificity. The isolates of Test 1 included four from alfalfa, five from soybean (two race 1, three race 3), three from clover, and 19 from Douglas-fir (Table 17). Test 2 included 36 isolates originating from 4 continents and 14 hosts (Table 1). Isolates from test 2 were identical to those used for determining single spore isolate variability (Chapter I) and taxonomic comparisons (Chapter II). Potential hosts evaluated were Douglas-fir, alfalfa, and soybean during Test 1; and Douglas-fir, alfalfa, soybean, Noble fir, clover, broccoli and cauliflower during Test 2. Isolates from each host from Test 1 were among those used in Test 2. Oregon isolates from alfalfa, Douglas-fir, and Noble fir had previously been identified by their nonpapillate, ovoid to obpyriform sporangia with internal proliferation in liquid culture, smooth-walled oogonia (30-60 µm diameter) and mostly paragynous antheridia (Waterhouse 1963, 1970; Newhook et al. 1979). Procedures used to test pathogenicity during Tests 1 and 2 are described separately.

Test 1

Pathogenicity to Douglas-fir. 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 1.4 cm longitudinal slit to the cambium and placing a small quantity of mycelium from colonies grown in pea broth (Trione 1959) 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 lengths of the resulting lesions were measured after 42 days. Average minimum and maximum greenhouse 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 distilled water autoclaved in 500 ml flasks) incubated 3 wk. CMS inoculum was mixed 1:32 with a steamed, clayloam soil for each isolate. 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 to Soybean. Pathogenicity of P. megasperma isolates to soybean (Glycine max (L.)) was compared by stem inoculations (Laviolette and Athow 1977) of cultivar Harosoy. The Harosoy seeds were planted in 2.5 liter pots, thinned to five plants per pot after 6 days and inoculated at 9 days. Small portions of oatmeal agar inoculum (Laviolette and Athow 1977) 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 (1977) and cultivar Tracy were inoculated in an additional soybean test 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 seed 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.

Pathogenicity to Alfalfa and Arrowleaf Clover. Seeds of alfalfa (Medicago sativa L. 'Vernal') and arrowleaf clover (Trifolium vesiculosum Savi 'Meechee') were germinated 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 were transplanted to each of three 540-ml cups of infested soil for each isolate. Controls were transplanted similarly into sterile CMS soil mix. Pots were arranged randomly in the greenhouse and watered daily to field capacity. Average minimum and maximum daily temperatures were 20 and 28°C, respectively. Root symptoms were rated and top symptoms and fresh weights were recorded for plants after 4 wk. 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.

Test 2

Pathogenicity to Douglas-fir. Materials and methods were identical

to those of Test 1 except for the following. Stem lesion lengths were measured after 52 days using 14 mo old seedlings. Root inoculation used CMS inoculum mixed 1:16 with soil. Average minimum and maximum greenhouse temperatures were 19 and 24°C, respectively.

Pathogenicity to Soybean. Nine day old Harosoy plants were grown and inoculated as for Test 1. Symptom development was recorded after 5 days.

Pathogenicity to Alfalfa and Arrowleaf Clover. For root inoculation of alfalfa and arrowleaf clover, a CMS to soil ratio of 1:16 was used. Symptoms were rated after 8 wk. Average minimum and maximum daily temperatures were 18 and 25°C, respectively.

Pathogenicity to Broccoli and Cauliflower. Single 4 mo old broccoli (Brassica oleracea var. italica) and cauliflower (Brassica oleracea var. botrytis) plants were transplnated to 150-ml plastic tubes (3 plants per isolate). CMS inoculum was prepared as described previously and mixed 1:16 with a pasteurized clay-loam soil. Controls were transplanted similarly using sterile CMS. Pots were arranged randomly in the greenhouse and watered daily. Root symptoms were rated after 4 wk for broccoli and after 8 wk for cauliflower. Root disease scores were 0, 1, 2, 3 and 4 for <1%, 1-25%, 26-50%, 51-75%, 76-100% root disease respectively. Average minimum and maximum greenhouse temperature were 20 and 25°C for both cauliflower and broccoli tests.

Pathogenicity to Noble fir. Pathogenicity to Noble fir (Abies procera Rehd.) was determined by stem inoculation of 7 mo old seedlings. Trees were inoculated (3 per isolate) using the procedure described for the Douglas-fir stem test. Pots were randomly placed in the greenhouse and watered daily. Average minimum and maximum greenhouse temperature

was 20 and 25°C, respectively.

Significance of differences between disease scores was determined by use of Duncan's new multiple range test (Steel and Torrie 1960).

RESULTS

Pathogenicity to Douglas-fir. Isolates from Douglas-fir and alfalfa formed two distinct groups based on their pathogenicity. During Test 1 six Douglas-fir isolates (DF Group 1) were pathogenic to both Douglas-fir and soybean. Thirteen isolates (DF Group 2) caused damage only on Douglas-fir (Tables 16 and 17). These results were repeated in Test 2 with representative isolates from each host used in Test 1. Likewise, two alfalfa isolates during Test 2 (alfalfa 2) caused damage only on Douglas-fir, while eight other alfalfa isolates (alfalfa 1) caused disease only on alfalfa (Tables 19 and 20). All alfalfa isolates in Test 1 were included in alfalfa 1.

In both tests, DF Group 1 isolates caused extensive root rot on Douglas-fir seedlings. Reddish brown subcortical lesions on the tap root extended above the soil line on most trees. During Test 1 ten of 13 DF Group 2 isolates caused little or no discoloration of tap roots. In Test 2, significantly more root rot occurred on trees inoculated with DF Group 2, Noble fir, and alfalfa 2 isolates than on control trees but root rot was less than caused by DF Group 1. Lateral roots only were discolored near apical areas during Test 1 when inoculated with alfalfa 1, soybean, clover or DF Group 2 isolates. During Test 2 only isolates from DF Groups 1 and 2, alfalfa 2, and Noble fir caused lateral root damage.

Stem lesions produced by DF Groups 1 and 2 were distinctive in both

Table 16. Reciprocal pathogenicity of Phytophthora megasperma isolates during Test 1 from Douglas-fir, Vernal alfalfa, Harosoy soybean, and clover.

Host of origin and isolate no.	Source	Douglas-fir		Alfalfa	Soybean[1]			Clover
		Lesion growth (mm/day)[1]	Root rating[2]	Root rating[2]	% wilted	% mortality		Root rating[2]
					1 day	2 days	9 days	
Douglas-fir--Group 1[3]								
B3A	Oregon	1.7	4.0	1.7	60	0	47	1.0
304	Oregon	2.0	0.3	1.4	73	0	73	0.3
B217	Oregon	0.7	3.7	1.1	67	0	73	1.2
306	Oregon	1.3	3.7	1.8	73	0	53	1.4
284	Oregon	2.7	3.7	1.3	40	0	67	0.7
345	Oregon	1.9	3.0	2.0	93	0	73	0.4
Douglas-fir--Group 2[3]								
C-17-2N-5	Oregon	0.4	0.0	1.8	0	0	0	1.3
341	Oregon	0.3	1.7	1.6	0	0	0	1.5
C-17-2D2	Oregon	0.3	2.0	1.4	0	0	0	3.3
BIC	Oregon	0.3	0.0	0.9	0	0	0	0.7
C-17-2N-4	Oregon	0.4	0.0	1.8	0	0	0	1.3
307	Oregon	0.3	0.0	2.3	0	0	0	0.4
336	Washington	0.4	2.7	1.8	0	0	0	1.9
260	Oregon	0.3	0.0	1.3	0	0	0	1.1
B+2	Oregon	0.3	0.7	1.3	0	0	0	0.8
316	Oregon	0.4	0.0	1.7	0	0	0	0.6
520	Oregon	0.3	0.3	1.1	0	0	0	1.3
337	Oregon	0.3	0.3	1.5	0	0	0	0.3
C-17-2N-6	Oregon	0.3	0.3	1.3	0	0	0	0.9

Table 16 continued.

Host of origin and isolate no.	Source	Douglas-fir		Alfalfa	Soybean[1]			Clover
		Lesion growth (mm/day)[1]	Root rating[2]	Root rating[2]	% wilted	% mortality		Root rating[2]
					1 day	2 days	9 days	
Soybean								
36 Race 1	C. Grau--WI	0.2	0.1	1.4	<1	0	67	0.6
908 Race 1	C. Grau--WI	0.2	1.3	1.6	<1	33	87	0.8
16 Race 3	C. Grau--WI	0.3	0.0	2.3	<0	13	73	1.2
411 Race 3	C. Grau--WI	0.1	0.0	1.3	20	20	67	1.0
909 Race 3	C. Grau--WI	0.2	0.7	1.4	<1	27	100	0.6
Alfalfa								
5b	D. Maxwell--WI	0.2	0.0	2.5	0	0	0	2.6
DA	D. Maxwell--WI	0.2	1.0	3.0	0	0	0	0.3
Jefferson 1A	R. Pratt-MS	0.3	0.3	3.4	0	0	0	1.0
Ozaukee-2A	R. Pratt-MS	0.3	0.7	2.2	0	0	0	1.8
Clover								
105	R. Pratt--MS	0.2	0.7	2.5	0	0	0	0.5
117	R. Pratt--MS	0.3	0.7	2.0	0	0	0	0.5
102	R. Pratt--MS	0.4	0.5	1.3	0	0	0	1.0
Control		0.0	0.3	0.3	0	0	0	0.8

[1] Stem inoculation.

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

[3] 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.

Table 17. Differences in pathogenicity of Phytophthora megasperma isolates during Test 1 to Douglas-fir, Vernal alfalfa, and Harosoy soybean.

Host of origin	Douglas-fir		Alfalfa	Soybean[1]			Clover
	Lesion growth (mm/day)[1]	Mean root rating[2]	Mean root rating[2]	% wilted	% mortality		Mean root rating[2]
				1 day	2 days	9 days	
Douglas-fir--Group 1[3]	1.70d[4]	3.10c	1.6b	68c	0a	64b	0.7a
Douglas-fir--Group 2[3]	0.34c	0.61ab	1.5b	0a	0a	0a	1.2a
Soybean	0.21b	0.55a	1.6b	4b	19b	79b	0.8a
Alfalfa	0.25b	0.50a	2.8c	0a	0a	0a	1.4a
Clover	0.28b	0.61ab	1.9b	0a	0a	0a	1.0a
Control	0.00a	0.30a	0.3a	0a	0a	0a	0.8a

[1]Stem inoculation.

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

[3]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.

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

Table 18. Reciprocal pathogenicity of *Phytophthora megasperma* isolates from Douglas-fir, Vernal alfalfa, Harosoy soybean, and Noble fir during Test 2.

Host of origin and isolate no.	Douglas-fir		Alfalfa	Soybean[1]	Noble fir
	Lesion growth (mm/day)[1]	Mean root rating[2]	Mean root rating[2]	% Mortality	Lesion growth (mm/day)[1]
Douglas-fir--Group 1					
B3A	1.00	3.7	1.0	93	0.64
B217	1.20	3.7	2.1	67	1.40
345	1.20	4.0	1.1	93	1.70
Douglas-fir--Group 2					
336	0.15	0.7	1.7	0	0.13
C-17-2D2	0.06	1.7	1.1	0	0.17
520	0.05	2.7	1.3	0	0.10
Soybean					
908 Race 1	0.13	0.0	0.5	93	0.22
909 Race 3	0.03	0.0	0.9	93	0.09
Alfalfa--Group 1[4]					
5b	0.06	0.3	1.8	0	0.06
DA	0.04	0.0	2.2	0	0.13
W1	0.03	1.3	1.3	0	0.12
S1	0.01	0.0	3.8	0	0.26
M1[3]	-	0.0	2.9	0	-
PC[5]	0.08	0.0	3.2	0	0.16
PC	0.06	0.7	2.5	0	0.08
S2	0.19	0.0	2.4	0	0.06
Alfalfa--Group 2[4]					
P1	0.00	2.0	0.7	0	0.16
P3	0.01	1.3	0.9	0	0.08

Table 18 continued.

Host of origin and isolate no.	Douglas-fir Lesion growth (mm/day)[1]	Douglas-fir Mean root rating[2]	Alfalfa Mean root rating[2]	Soybean[1] % Mortality	Noble fir Lesion growth (mm/day)[1]
Clover					
105	0.00	0.0	0.8	0	0.00
117	0.01	0.0	1.2	0	0.12
102	0.00	0.0	1.5	0	0.02
Apple					
T14	0.00	0.0	1.5	0	0.06
T47	0.00	0.0	0.8	0	0.23
K4	0.00	0.0	1.3	0	0.00
Almond					
K2	0.00	0.0	1.9	0	0.40
K3	0.00	0.0	1.4	0	0.09
Pear					
K8	0.36	0.0	0.9	0	0.36
Juniper					
K9	0.00	2.7	1.0	0	0.08
Grape Soil					
K10	0.13	0.7	1.0	0	0.16
K11	0.01	0.0	0.9	0	0.34
Type (Hollyhock)					
T32	0.00	0.0	1.4	0	0.0
Noble fir					
NF1	0.00	1.0	1.7	0	0.16
Poplar					
T28	0.06	0.0	0.7	0	0.06

Table 18 continued.

Host of origin and isolate no.	Douglas-fir Lesion growth (mm/day)[1]	Douglas-fir Mean root rating[2]	Alfalfa Mean root rating[2]	Soybean[1] % Mortality	Noble fir Lesion growth (mm/day)[1]
Rose					
PB	-	-	-	0	-
PA	-	-	-	0	-
Cherry					
K1	0.17	0.0	1.4	0	0.17
Brassica					
T56	0.00	0.0	1.2	0	0.0
Control	0.00	0.0	1.2	0	0.0

[1]Stem inoculation.

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

[3]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.

[4]One of two groups of P. megasperma isolated from alfalfa. Alfalfa 1 pathogenic only to alfalfa. Alfalfa 2 pathogenic to alfalfa and Douglas-fir.

[5]IMI = Imperial Mycological Institute, Kew, Surrey, England.

[6]ATCC = American Type Cultural Collection, Rockville, Maryland.

Table 19. Differences in pathogenicity of Phytophthora megasperma isolates during Test 2 to Douglas-fir, Vernal alfalfa, Harosoy soybean, and Noble fir.

Host of origin	Douglas-fir		Alfalfa	Soybean[1]	Noble fir
	Lesion growth (mm/day)[1]	Mean root rating[2]	Mean root rating[2]	% Mortality	Lesion growth (mm/day)[1]
Douglas-fir--Group 1[3]	1.3b[5]	3.8f	1.4a[6]	76b	1.20e
Douglas-fir--Group 2[3]	0.09a	1.7d	1.4a	0a	0.13ab
Alfalfa 1[4]	0.08a	0.2a	2.5c	0a	0.13ab
Alfalfa 2[4]	0.01a	1.7d	0.8	0a	0.10ab
Soybean	0.08a	0.0a	0.7	93c	0.16ab
Grape Soil	0.07a	0.3ab	0.9	0a	
Poplar	0.03a	0.0a	0.7	0a	0.06a
Cherry	0.01a	0.0a	1.4a	0a	0.17ab
Clover	0.0a	0.0a	1.2	0a	0.07a
Hollyhock	0.0a	0.0a	0.9	0a	0.00a
Brassica	0.0a	0.0a	1.2	0a	0.00a
Noble fir	0.0a	0.7c	1.7ab	0a	0.16ab
Apple	0.0a	0.0a	1.4a	0a	0.02a
Juniper	0.0a	2.7e	1.0	0a	0.08a
Almond	0.0a	0.0a	1.7ab	0a	0.24c
Rose	-	-	-	0a	-
Pear	0.1a	0.0a	0.9	0a	0.36d
Control	0.0a	0.0a	1.2a	0a	0.00a

Table 19 continued.

[1]Stem inoculation.

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

[3]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.

[4]One of two groups of P. megasperma isolated from alfalfa. Alfalfa 1 pathogenic only to alfalfa. Alfalfa 2 pathogenic to alfalfa and Douglas-fir.

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

[6]Duncan's new multiple range test compares those means with root rot values greater than controls.

[7]Isolated from soil adjacent to grape.

Tests 1 and 2. Lesion growth (mm/day) averaged 1.7 and 1.3 for DF Group 1 and .34 and 1.0 for DF Group 2 during Tests 1 and 2, respectively.

Two of 8 alfalfa isolates added to Test 1 and the Noble fir isolate were pathogenic to Douglas-fir during soil tests. These two alfalfa isolates, P1 and P3 (alfalfa 2), caused root damage equal to DF Group 2. The Noble fir isolate caused slightly less root damage than DF Group 2, but was significantly greater than controls. Neither alfalfa 2 nor the Noble fir isolate caused stem lesions significantly larger than controls.

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 17-20). Other isolates caused few symptoms during this period. Mortality during Test 1 was first observed at 48 hr on plants inoculated with soybean isolates and 96 hr on plants inoculated with DF Group 1 isolates (Table 17). Final mortality caused by soybean and DF Group 1 isolates was 79 and 64%, and 93 and 76% for Test 1 and Test 2, respectively.

Pathogenicity to Soybean Race Differentials. As predicted by Laviolette and Athow (1977) 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 21). Group 1 DF isolates 304 and 306 were pathogenic to all soybean cultivars tested, including Tracy. The single isolate of DF Group 2 (341) was avirulent to all soybean

Table 20. Mortality of soybean diffential cultivars when inoculated in the hypocotyl with isolates of Phytophthora megasperma from Douglas-fir and soybean.[1]

Cultivar	Soybean 908 Race 1	Soybean 909 Race 3	DF Group 1 304	DF Group 1 306	DF Group 2 341	Control
	% Mortality					
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
P.I. 103.091	0	0	33	75	0	0
P.I. 869.972-1[2]	0	0	100	75	0	0
Tracy	0	0	25	67	0	0

[1]Six plants inoculated per isolate for Altona, 10 for Harosoy 63, and 13 for all other cultivars.

[2]Used in place of P.I. 171.442.

differentials and Tracy. No mortality occurred in the controls.

Pathogenicity to Alfalfa. Alfalfa isolates (alfalfa 1) caused significantly more stunting, wilting and tap root necrosis on alfalfa than other isolates during both series of tests (Table 17-20). No appreciable root rot symptoms were caused by alfalfa 2 isolates. 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 during Test 1 were greater than the average fresh weight from plants inoculated with alfalfa isolates. Fresh weights of control plants were greater than each of the other treatments.

Pathogenicity to Noble fir. Douglas-fir Group 1 isolates caused significantly larger lesions on Noble fir than isolates from other hosts (Tables 19-20). Isolates from pea, grape soil, and almond caused smaller lesions but these were significantly greater than controls. Lesions caused by Noble fir, DF Group 2, soybean, alfalfa 1, and alfalfa 2 isolates and control inoculations were not significantly different.

Pathogenicity to Clover, Cauliflower, and Broccoli. Root disease ratings from cauliflower, broccoli, and clover (Tests 1 and 2) tests are listed (Tables 17, 18 and 22). No isolate or group of isolates caused significantly greater root rot than occurred on control plants in any of the tests.

DISCUSSION

Results of this study confirm the occurrence of host specificity among some isolates of P. megasperma and support the grouping of these

Table 21. Differences in pathogenicity of *Phytophthora megasperma* isolates during Test 2 to clover, cauliflower and broccoli.[1]

Host of origin	Clover Mean disease rating[2]	Cauliflower Mean disease rating[2]	Broccoli Mean disease rating[2]
Douglas-fir--Group 1	0.8	0.1	2.5
Douglas-fir--Group 2	0.9	0.8	4.3
Alfalfa 1	0.5	0.9	1.4
Alfalfa 2	0.8	0.4	1.7
Soybean	0.0	1.0	3.3
Grape Soil[3]	0.5	0.7	1.8
Poplar	0.0	0.0	1.0
Cherry	0.9	0.3	2.3
Clover	0.1	1.4	4.1
Hollyhock	0.0	2.3	5.0
Brassica sp.	1.2	0.0	3.5
Noble fir	0.7	0.0	1.0
Apple	0.8	1.4	2.8
Juniper	0.0	0.0	2.0
Almond	0.3	0.2	2.5
Rose	-	-	-
Pear	0.9	0.7	1.7
Control	0.4	0.3	4.5

[1]No significant differences in root rot occurred between inoculated treatments and controls.

[2]Seedlings transplanted to infested soil. Root discolorations ratings 0,1,2,3,4 = <1%, 1-25%, 26-50%, 51-75%, 76-100%, respectively.

[3]Isolate from soil adjacent to grape.

isolates into formae speciales (Kuan and Erwin 1980; Pratt 1981). Seemingly, this contrasts with earlier reports where P. megasperma isolates caused damage on more than one plant species (Hildebrand 1959; Bhelwa 1962; Morgan 1964; Jones and Johnson 1969; Nagai et al. 1978). 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, 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 (Synder and Hansen 1980). Results of Nagai et al. (1978), 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 soybean and alfalfa (group 1) were pathogenic only to their original hosts while DF Group 1 isolates were strongly pathogenic to Douglas-fir, soybean, and Noble fir, but not to alfalfa or the other hosts tested. Alfalfa 2 isolates, originally isolated from diseased alfalfa caused root rot on Douglas-fir but not on any other host tested. 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 17-21). However, none of the soybean

isolates exhibited similar pathogenicity either to Douglas-fir or Noble 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 (Laviolette and Athow 1977). 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 sizes produced by DF Group 2 isolates were significantly larger during Test 1 on Douglas-fir than lesions produced by isolates from the leguminous hosts. DF Group 2 isolates were avirulent to both soybean and alfalfa. Interestingly, however, during Test 1 DF Group 2 isolates did not cause significantly greater root disease of Douglas-fir than isolates from soybean, clover or alfalfa. Low root disease ratings of DF Group 2 isolates on Douglas-fir during Test 1 probably resulted from the short test period. This is apparent by the increased root disease scores obtained during Test 2 using a higher inoculum concentration. DF Group 2 isolates are prevalent in Northwest tree nurseries (Hansen et al. 1979, 1980) where conditions are favorable for disease development over long periods.

The apparent lack of pathogenicity of arrowleaf clover isolates on arrowleaf clover is perplexing. Host specificity of these same isolates to arrowleaf clover has been determined (Pratt 1981). During Test 1, maximum greenhouse temperatures (37°C) were above the maximum in vitro growth temperature of these isolates (30°C). However, during lower greenhouse temperatures during Test 2, clover isolates again caused

no significant disease. No reason is known why these isolates failed to cause root rot on arrowleaf clover. Inoculations of alfalfa, Douglas-fir, soybean and Noble fir with arrowleaf clover isolates during milder conditions indicates that they are not pathogenic to those hosts.

The isolate from Brassica also failed to cause significant root rot on cauliflower, or broccoli, contrary to what was expected. The original host variety of this isolate, however, could not be identified, making the test for host specificity impractical. This isolate still exhibited evidence of host specificity by failing to cause disease on Douglas-fir, alfalfa, soybean or Noble fir.

Lack of pathogenicity to Noble fir by the Noble fir isolate and to alfalfa by alfalfa 2 isolates is interesting. The Noble fir isolate originated from a severely root rotted seedling and was expected to cause large stem lesions on Noble fir. But average lesion length by this isolate was no greater than isolates from DF Group 2 and several other hosts. Host specific reactions different than expected also occurred from alfalfa 2 isolates. The two isolates of alfalfa 2, isolated from diseased alfalfa plants caused no damage to alfalfa during root tests (Tables 19 and 20). Both Noble fir and alfalfa 2 isolates, however, did cause disease on root inoculated Douglas-fir seedlings comparable to DF Group 2 (Tables 19 and 20). These isolates (alfalfa 2, DF Group 2 and Noble fir) were among those intensively compared for morphological and growth similarities and found to be identical (Chapter II). That work identified a general correlation between host of origin and similar morphological and growth characteristics.

Stem inoculations of the soybean race differential cultivar produced an unexpected range of symptoms. Previous workers (Laviolette and Athow 1977) 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 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.

The identification of isolates virulent to alfalfa (alfalfa 1) highly virulent to Douglas-fir (DF Group 1), slightly virulent to Douglas-fir (DF Group 2), and those intermediately virulent to Douglas-fir and alfalfa (alfalfa 1), confirms differing host ranges of P. megasperma isolates. In addition these isolates show that host ranges are not related to original geographical region of isolation. Alfalfa 1 isolates from Oregon, Washington and Wisconsin caused similar reactions during these tests. The reciprocal condition occurred when isolates belonging to DF Group 1 and 2 were isolated from the same location but caused differential reactions.

The use of host specificity designators for 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

(Van der Zwet and Forbes 1961; Erwin 1965; Boesewinkel 1974; Kuan and Erwin 1980) 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 and alfalfa 2 isolates to Douglas-fir and alfalfa was unexpected. These hosts, though unrelated phylogenetically and biogeographically, shared 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, alfalfa 1, and alfalfa 2 isolates also exhibited differential host specificity. Designation of host specificity for alfalfa 1 has already been done (Kuan and Erwin 1979), but designation of Douglas-fir Group 2 and alfalfa 2 is delayed pending soil inoculations of Noble fir using DF Group 2 isolates and more comprehensive tests on Douglas-fir and alfalfa with alfalfa 2 isolates. These results emphasize the broadest possible range of isolates and hosts should be used to establish taxonomic groupings based on morphology or host specificity.

GENERAL CONCLUSIONS

The investigations described in this thesis have provided significant new information on the variation, morphology and pathogenicity of Phytophthora megasperma.

Variation occurred in all morphological and growth characteristics, both within (Chapter I) and between (Chapter II) isolates. The amount of variation was especially high between SSI and their parents for length and width measurements of sporangia, less for diameters of oogonia and length/width ratio of sporangia. Growth rate and colony and hyphal morphology produced little variation between SSI and parents (Chapter I) but these characteristics varied extensively between isolates (Chapter II).

The amount of variation was used to determine the reliability of these characteristics for isolate identification. Diameter of oogonia and oospores, type of antheridia, production of oogonia (homothallism) and shape and length/width ratio of sporangia were considered useful. In contrast, length and width of sporangia values varied greatly. Colony and hyphal morphology, growth rate, hyphal swellings or host specificity were of little value except for some comparisons between isolates from the same geographical region or host of origin.

Large continuous ranges of these morphological characteristics did not support the varietal subgroups reported for P. megasperma but did support the following descriptive revision for P. megasperma: Sporangia mostly ovoid, fewer ellipsoid or ovoid-obpyriform; measuring 15-109 X 6-67 µm, mostly 37-68 X 25-45 µm (ave. 53 X 35 µm); proliferation internal or external; and rarely formed on solid substrate. Oogonia large, ranging 16-64 µm, mostly 28-55 µm (mean 40.5 µm), and usually

produced readily in single strain culture in V-8 agar; oogonial walls 1.0-1.7 µm (mean 1.3 µm) thick. Oospores smooth, measuring 11-55 µm, mostly 25-59 µm (mean 34 µm). Antheridia mostly paragynous, occasionally amphigynous in some cultures. Colony on V-8 agar non- to slightly aerial, little or no floral pattern, with a rough or undulating margin. Growth usually occurs from 5 to 35°C, optimum 15 to 30°C (most 20-25). Growth rate can vary greatly. No alteration of Waterhouses' key was necessary to incorporate these changes except to remove the varietal distinctions based on oogonium diameter.

A relationship between isolate host of origin and distinct morphological and growth characteristics was found (Chapter II). This relationship proved to be of little value for taxonomic subgrouping because differences between these subdivisions were small.

The occurrence of host specificity within P. megasperma was confirmed by this work; however, no longer can this be implied to mean pathogenicity to a single host. Isolates from both Douglas-fir and alfalfa infected two hosts (Chapter III). In addition, two of the host groups studied here may well represent the formation of host specific subgroups from a common parent. SSI from alfalfa and clover produced an unusually high proportion of amphigynous antheridia. In addition, SSI from these hosts were nearly identical by the other morphological and growth characteristics measured (Chapter II) and differed only in host specificity (Chapter III). These crops (alfalfa and clover) are often grown in close proximity, which could allow for cross exposure to occur. Under these conditions the fungus could then become pathogenic by the activation of genes not previously used or by another mechanism. If host specific pathogenicity can

change as theorized, then the usefulness of host specific designations (Chapter III) would be limited.

BIBLIOGRAPHY

Apple, J. L. 1957. Pathogenic, cultural, and physiological variation within Phytophthora parasitica var. nicotanae. Phytopathology 47:733-740.

Bhelwa, B. H. 1962. Seed decay, seedling blight, and root rot of Cicer arietinum caused by Phytophthora cryptogea. M.S. Dissertation, Ohio State University. 94 p.

Boesewinkel, H. J. 1974. Phytophthora on asparagus in New Zealand. Plant Dis. Rep. 58:525-529.

Brasier, C. M. and R. G. Strouts. 1976. New records of Phytophthora on trees in Britain. Eur. J. For. Path. 6:129-136.

Buddenhaggen, I. W. 1958. Induced mutations and variability in Phytophthora cactorum. Amer. Jour. Bot. 45:355-365.

Bushong, J. W. and J. W. Gerdemann. 1959. Root rot of alfalfa caused by Phytophthora cryptogea in Illinois. Plant Dis. Rep. 43:1178-1183.

Caten, C. E. and J. L. Jinks. 1968. Spontaneous variability of single isolates of Phytophthora infestans. I. Cultural variation. Can. J. Bot. 46:329-341.

Caten, C. E. 1970. Spontaneous variability of single isolates of Phytophthora infestans. II. Pathogenic variation. Can. J. Bot. 48:897-905.

Caten, E. C. 1971. Single zoospore variation in Phytophthora infestans and attenuation of strains in culture. Trans. Br. Mycol. Soc. 56:1-7.

Cooley, W. W. and P. R. Lohnes. 1971. Multivariate data analysis. Wiley, New York. 364 p.

Drechsler, C. 1931. A crown rot of hollyhocks caused by Phytophthora megasperma n. sp. J. Wash. Acad. Sci. 21:513-526.

Erwin, D. C. 1954. Root rot of alfalfa caused by Phytophthora crytogea. Phytopathology 44:700-704.

Erwin, D. C. 1965. Reclassification of the causal agent of root rot of alfalfa from Phytophthora cryptogea to P. megasperma. Phytopathology 55:1139-1143.

Galindo, J. A. 1965. Sexuality and genetics of Phytophthora infestans. (abstract) Am. Potato J. 40:304.

Gallegly, M. E. 1970. Genetics of Phytophthora. In Tucker memorial symposium on Phytophthora. Phytopathology 60:1135-1141.

Hamm, P. B. and E. M. Hansen. 1980. Morphological groups of Phytophthora megasperma from Douglas-fir and their pathogenicity to alfalfa and soybean. Phytopathology (abstract) (In Press).

Hamm, P. B. and E. M. Hansen. 1981. Host specificity of Phytophthora megasperma from Douglas-fir, soybean and alfalfa. Phytopathology 71:65-68.

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.

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.

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.

Hilty, J. W. and A. F. Schmitthenner. 1962. Pathogenic and cultural variability of single zoospore isolates of Phytophthora megasperma var. sojae. Phytopathology 52:859-862.

Irwin, J. A. G. 1974. Reaction of lucerne cultivars to Phytophthora megasperma, the cause of a root rot in Queensland. Australian Journal of Exp. Agriculture and Animal Husbandry 14:561-

Johnson, H. W. and B. L. Keeling. 1969. Pathogenicity of Phytophthora megasperma isolated from subterranean clover roots. Phytopathology 59:1279-1283.

Jones, J. P. and H. W. Johnson. 1969. Lupine, a new host for Phytophthora megasperma var. sojae. Phytopathology 59:504-507.

Kaufmann, J. J. and J. W. Gerdemann. 1958. Root rot of soybeans caused by Phytophthora sojae. n. sp. Phytopathology 48:201-208.

Kuan, Ta-Li and D. C. Erwin. 1979. Differentiation of soybean and alfalfa isolates of Phytophthora megasperma by use of formae speciales. Phytopathology 69:333-338.

Lance, G. N. and W. T. Williams. 1967. A general theory of classificatory sorting strategies. I. Hierarchical systems. Computer J. 9:373-380.

Laviolette, F. A. and K. L. Athow. 1977. Three new physiologic races of Phytophthora megasperma var. sojae. Phytopathology 67:367-368.

Leach, S. S. and A. E. Rich. 1969. The possible role of parasexuality and cytoplasmic variation in race differentiation in Phytophthora infestans. Phytopathology 59:1360-1365.

Leath, K. T. and J. E. Baylor. 1975. Phytophthora root rot of alfalfa in Pennsylvania. Plant Dis. Reptr. 59:972-974.

MacIntyre, D. and C. G. Elliott. 1974. Selection for growth-rate during asexual and sexual propagation in Phytophthora cactorum. Genet. Res. 24:295-309.

Matsumoto, N. and T. Araki. 1978. Alfalfa root rot caused by Phytophthora megasperma Drechsler in Japan. Ann. Phytopathology Soc. Jpn. 44:214-217.

Mircetich, S. M. and M. E. Matheron. 1976. Phytophthora root rot and crown rot of cherry trees. Phytopathology 66:549-558.

Morgan, F. L. 1964. Infection of cranesbill by the soybean Phytophthora. Plant Dis. Rep. 48:140-141.

Morgan, F. L. and E. E. Hartwig. 1965. Physiologic specialization in Phytophthora megasperma var. sojae. Phytopathology 55: 1277-1279.

Nagai, Y., T. Takeuck and T. Watanabe. 1978. A stem blight of rose caused by Phytophthora megasperma. Phytopathology 68:684-688.

Newhook, F. J., G. M. Waterhouse, and D. J. Stamps. 1979. Tabular key to the species of Phytophthora DeBary. Commonwealth Mycol. Inst. Mycol. Paper No. 143. 20 p.

Pratt, R. G. 1981. Morphology, pathogenicity and host range of Phytophthora megasperma, P. erythroseptica, and P. parasiticia from Arrowleaf clover. Phytopathology (In Press).

Ribeiro, O. K., D. C. Erwin, and R. A. Khan. 1978. A new high-temperature Phytophthora pathogenic to roots of alfalfa. Phytopathology 68:155-161.

Robertson, G. I. and H. M. Dance. 1971. The association of Phytophthora megasperma with crown rot of apple trees. N. Z. J. of Ag. Res. 14:509-614.

Romero, S. and D. C. Erwin. 1969. Variation in pathogenicity among single zoospore cultures of Phytophthora infestans. Phytopathology 59:1310-1317.

Shaw, D. S. and C. G. Elliott. 1961. Streptomycin resistance and morphological variation in Phytophthora cactorum. J. Gen. Microbiology 51:75-84.

Shepherd, C. J. and B. H. Pratt. 1974. Temperature-growth relations and genetic diversity of A2 mating-type isolates of Phytophthora cinnamomi in Australia. Aust. J. Bot. 22:231-249.

Skotland, C. B. 1955. A Phytophthora damping-off disease of soybean. Plant Dis. Reptr. 39:682-683.

Snyder, W. C. and H. N. Hansen. 1940. The species concept in Fusarium. Amer. J. Bot. 27:64-67.

Staflue, F. A., C. E. B. Bonner, R. McVaugh, R. D. Meikle, R. C. Rollins, R. Ross, J. M. Schopf, G. M. Schulze, and R. Devilmorin. 1972. International code of botanical nomenclature. Utrecht, The Netherlands. 426 p.

Stamps, D. J. 1953. Variation in a strain of Phytophthora cactorum. Trans. Br. Mycol. Soc. 36:248-254.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc. New York. 481 p.

Trione, E. J. 1959. The pathology of Phytophthora lateralis on native Chamaecyparis lawsoniana. Phytopathology 49:306-310.

Tompkins, C. M., C. M. Tucker, and M. W. Gardner. 1936. Phytophthora root rot of cauliflower. J. Agric. Res. 53:685-592.

Van der Zwet, T. and I. L. Forbes. 1961. Phytophthora megasperma, the principal cause of seed-piece rot of sugarcane in Louisiana. Phytopathology 51:634-640.

Wallin, J. R. 1957. Response of potatoes to monozoospore lines of Phytophthora infestans. Plant Dis. Reptr. 41:612-615.

Waterhouse, G. M. 1963. Key to the species of Phytophthora DeBary. Commonwealth Mycol. Inst. Mycol. Paper No. 92. 22 p.

Waterhouse, G. M. 1970. The genus Phytophthora DeBary. Commonwealth Mycol. Inst. Mycol. Paper No. 122. 59 p.

Welty, R. E. and T. H. Busbice. 1976. Phytophthora root rot of alfalfa in North Carolina. Plant Dis. Reptr. 60:14-18.