Variation in Phenotypic Characteristics of *Phytophthora capsici* Isolates from a Worldwide Collection

L. L. Granke, Visiting Research Associate, L. M. Quesada-Ocampo, Visiting Research Associate, and M. K. Hausbeck, Professor, Department of Plant Pathology, Michigan State University, East Lansing 48824

Abstract

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To determine variation within *Phytophthora capsici*, 124 *P. capsici* isolates from 12 countries were characterized for sporangial length and width, pedicle length, oospore diameter, sporangia and chlamydospore production, and growth at 32, 35, and 38°C. Sporangia were 23 to 35 µm wide and 38 to 60 µm long; differences in width and length were noted when isolates were grouped by genetic cluster and continent of origin. Length:breadth ratio (1.34 to 2.07) and pedicle length (20 to 260 µm long) varied widely among isolates; differences were apparent by continent and host family of origin. Oospore diameters varied among isolates (22 to 37 µm), but no differences were noted by isolate

genetic cluster, host family of origin, continent of origin, mating type, or sensitivity to mefenoxam. Differences in sporangia production were observed among isolates grouped by continent, and isolates from non-vegetable hosts produced fewer sporangia than isolates from vegetable hosts. When cultures were incubated in a liquid medium, 35 *P. capsici* isolates formed chlamydospores. Most (122 of 124) of the isolates were able to grow at 35°C, but all of the isolates grew poorly at 38°C. The results of this study indicate substantial variation in morphological and physiological characteristics among *P. capsici* isolates.

The oomycete plant pathogen *Phytophthora capsici* Leonian is a serious pathogen that limits production of vegetable crops in the Cucurbitaceae, Solanaceae, and Fabaceae (16). The pathogen is distributed worldwide (9) and may also cause disease on nonvegetable hosts such as cacao (*Theobroma cacao* L.), rubber (*Hevea brasiliensis* Mull. Arg.), macadamia (*Macadamia integrifolia* Maiden & Betche), papaya (*Carica papaya* L.), and black pepper (*Piper nigrum* L.) (9). *P. capsici* is heterothallic and requires both the A1 and A2 mating types (MTs) to be present for oospore production (19). Challenges to managing disease caused by *P. capsici* include long-term survival of oospores in the soil (20), an increasing list of susceptible hosts (25), fungicide-resistant pathogen populations (18), spread of the pathogen via irrigation water (13), and a lack of commercially acceptable resistant cultivars (11,12,21).

Identifying the causal agent of *Phytophthora* root, stem, and fruit rot may be challenging, as many of the hosts susceptible to *P. capsici* are susceptible to other *Phytophthora* species and *P. capsici* displays considerable phenotypic variation (9). This is especially true for nonvegetable hosts such as cacao, which may be infected with *P. capsici*, *P. palmivora*, *P. megakarya*, *P. citrophthora*, *P. nicotianae* (2), and *P. tropicalis* (8). However, diseases on vegetable crops may also be caused by species other than *P. capsici*; root rot of tomato is caused by *P. capsici* and *P. nicotianae* (15,27). Nonetheless, correct identification of the causal agent is important for disease management and also for the correct use of pathogen isolates in resistance screening programs. Previous studies have used morphology (3), species-specific polymerase chain reaction (PCR) (30), PCR and sequencing (4,26), and enzyme digestion (4,22) to separate *P. capsici* from closely related species.

Differences in morphological and physiological characters of *P. capsici* isolates have been previously reported (3,4,17,22,28,31). Sporangial lengths have been previously reported to range from 33

to 66 µm and widths from 17 to 43 µm (3,17,22,28). Overall, the length:breadth (LB) ratio of sporangia ranged from <1.3 to 2.2 in prior work (3,9,22,28). Pedicle lengths for *P. capsici* isolates are known to vary widely; pedicles for isolates from a variety of hosts ranged from 32 to 138 µm long (17,22,28). Chlamydospores formed in liquid cultures of some isolates from nonvegetable hosts (4,22) and pumpkin (*Cucurbita moschata* Duchesne ex Poir.) (17), but were not observed for isolates from pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), lima bean (*Phaseolus lunatus* L.), cucumber (*Cucumis sativus* L.), or squash (*Cucurbita pepo* L.) (4,28). Because of the variability in morphological characteristics among isolates, identifying *Phytophthora* species based on morphology alone may be challenging.

Much genetic variability exists within *P. capsici* (10), and collections of isolates have been separated into subpopulations using isozymes (22,23), traditional phylogenetic methods (4,8), and Bayesian clustering (26). When a group of 114 isolates from vegetable and nonvegetable hosts were separated into two subgroups (CAPA and CAPB) using isozymes, similarities in sporangial morphology were noted within each group (22). However, a few isolates from each subgroup showed sporangial morphology characteristic of the opposite group. Only isolates in CAPB (*P. tropicalis*) produced chlamydospores (22); whereas CAPA (*P. capsici* sensu stricto) isolates did not produce chlamydospores in this study (22). Other studies have correlated genetic subgroups with host of origin and geography (4,8,23), but did not investigate correspondence with morphological characteristics. When Bayesian clustering was used to subdivide a worldwide collection of *P. capsici* isolates (26), no direct correlation was observed between genetic clusters and host of origin, geography, or mefenoxam sensitivity, but population structure was apparent when isolates were grouped by these categories. Previous studies have not investigated if morphological and physiological differences correspond with genetic clusters or subpopulations. Since characterizing population subdivision with molecular tools can be a time-consuming and costly procedure, it would be helpful if phenotypic characteristics could be used to guide the sampling process.

There are conflicting opinions as to whether or not *P. tropicalis* should be considered a separate species from *P. capsici* (3,4). While morphological (3) and genetic (8,35) differences may be

Corresponding author: M. K. Hausbeck, E-mail: hausbeck1@msu.edu

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observed among *P. capsici* and *P. tropicalis*, overlap may also be observed between some isolates of these species. Previous studies (3,4,22) have suggested that growth at 35°C may be used to separate *P. capsici* from *P. tropicalis*. Identification of *Phytophthora* species using morphological characteristics is challenging in general (5), and species like *P. capsici* may have much intra-specific variation in morphological characteristics (9,28). Even when molecular methods are used, isolates may be misidentified unless information from multiple genes is used in the analysis. Quesada-Ocampo et al. (26) found isolates belonging to *P. capsici*, *P. tropicalis*, and others with an intermediate genotype within a set of 255 *P. capsici* sensu lato isolates. While *P. capsici* and *P. tropicalis* isolates were assigned to their respective species for every gene, the intermediate isolates were assigned to *P. capsici* for some genes and to *P. tropicalis* for others. These intermediate isolates would have been incorrectly identified as *P. capsici* or *P. tropicalis* if only a single gene was used. Hence, investigating the intraspecific diversity of phenotypic characteristics of a large set of *P. capsici* isolates from diverse hosts and geographic locations may aid understanding the natural phenotypic variation of *P. capsici* and the overlap between *P. capsici* and *P. tropicalis*, which could help guide separation of these closely related species.

The objective of this study was to determine variations in sporangial morphology, sporangial and chlamydospore production, and growth at 32, 35, and 38°C for *P. capsici* populations from diverse hosts and geographic locations. Isolates were grouped within arbitrarily defined geographic, host, mefenoxam resistance, and mating type categories or by predominant genetic cluster membership, and differences between subgroups in each category were investigated. A small set of *P. tropicalis* isolates and isolates with an intermediate genotype between *P. capsici* and *P. tropicalis* were included for comparison. A preliminary report of morphological characters for some of the isolates included in this study has been published (14), and the species identity, mating type, mefenoxam sensitivity, and genetic cluster membership of isolates included in this study have been previously reported (26).

Materials and Methods

Isolate selection, maintenance, and genetic cluster assignment. A total of 124 *P. capsici* isolates were chosen from the culture collection maintained in the laboratory of Dr. Hausbeck at Michigan State University (MSU). Isolates from 12 countries and 17 host species were collected or were donated by colleagues in various countries (Table 1). Single-spore cultures were retrieved from long-term storage and maintained on unclarified V8 agar (UCV8, 3 g CaCO₃, 15 g agar, 160 ml unfiltered V8 juice, and 840 ml distilled water) as previously described (25). *P. capsici* was confirmed using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (33) and molecular techniques (26). We included the type cultures for *P. capsici* 13692 (ATCC.15399, New Mexico) and *P. tropicalis* 13602 (ATCC.76651, Hawaii) and additional *P. tropicalis* and intermediate isolates for a total of 138 isolates (124 *P. capsici*, 9 *P. tropicalis*, and 5 isolates with an intermediate genotype).

The model-based Bayesian clustering algorithm implemented in Structure 2.3X (24) was used in a previous study to assess population subdivision for a group of 236 *P. capsici* sensu stricto isolates, 124 of which are included in this study (26). Each isolate was assigned proportionate membership (Q) in a genetic cluster by Structure 2.3X. For statistical analysis, each isolate was assigned to the cluster in which it had predominant membership. If an isolate's membership was not >0.1 proportion membership higher in one genetic cluster than another, that isolate was designated as highly admixed and excluded from statistical analysis ($n = 9$), but not from population structure figures. The graphic editing program Population Sorting Tool (PST) (J. J. Morrice, unpublished data) was used to create population structure figures for *P. capsici* with phenotypic information and isolates sorted by Q.

Phenotypic characterization. The length, breadth, and pedicel length of 10 arbitrarily chosen sporangia were measured for each

isolate. To dislodge sporangia, a droplet of water was added to each single culture, agitated, and poured onto a glass slide. An optical micrometer and compound microscope were used to measure sporangial length and breadth at $\times 200$ magnification and pedicel length at $\times 100$ magnification. The length:breadth (LB) ratio was calculated for each isolate. The experiment was conducted twice.

Sporangial production on culture medium was estimated for each isolate. To estimate sporangial production, a 6-mm-diameter mycelial disk was transferred from the growing edge of a 7-day-old culture onto three replicate 60-mm-diameter plastic petri dishes containing UCV8. Plates were incubated in a single layer under constant fluorescent lighting at room temperature ($25 \pm 2^\circ\text{C}$) for 7 days. For each plate, 1 ml of sterile distilled water was added to the culture, sporangia were dislodged using a sterilized bacterial loop, and the resulting suspension was pipetted into a 1.7-ml microcentrifuge tube. Water was added to the tube as needed to ensure that 1 ml of suspension was present in each tube. For each replicate of each isolate, sporangia were enumerated using a hemacytometer, and the number of sporangia per square centimeter of medium was calculated. The experiment was conducted twice.

Since the mating type of each isolate had been previously determined (26), isolates were paired with a known standard of the opposite mating type (either isolate 101 or 13349) on UCV8 to form oospores. The diameters of 10 oospores per isolate were measured using an optical micrometer at $\times 200$ magnification by taking a measurement of the length and width at the widest point (excluding the antheridium).

To determine if isolates would produce chlamydospores in liquid medium, the methods of Ristaino (28) were followed with modifications. Briefly, a single 8-mm-diameter plug from the growing edge of each isolate was transferred to 25 ml of clarified V8 broth (C-V8, 163 ml clarified V8 juice, 3 g CaCO₃, 1 liter distilled water, 86 mg ampicillin, 26 mg rifampicin) in a 50-ml Corning tube and incubated vertically in the dark at room temperature ($\sim 25 \pm 2^\circ\text{C}$) for 24 h. The tubes were shaken vigorously for 5 min using a shaker and then incubated vertically for 5 days in the dark at room temperature. The C-V8 broth was removed from the tube and was replaced with ~ 45 ml of sterile distilled water. Cultures were incubated vertically in an incubator at $18 \pm 2^\circ\text{C}$ for 3 weeks with no light and checked for the presence of chlamydospores. Each culture was individually removed from the tube in which it was incubated and placed into a plastic 80-mm-diameter petri dish. The mycelia were teased apart using forceps and a dissecting needle to enable visualization, and the culture was scanned under $\times 100$ magnification for chlamydospores.

To examine isolate growth at temperatures near the maximum growth temperature (9), a 6-mm-diameter agar plug from the growing edge of a 5-day-old culture of each isolate was inoculated to the center of six 60-mm-diameter UCV8 plates. Two replicate plates per isolate were Parafilm and incubated in the dark in growth chambers set at 32, 35, and 38°C, respectively. The temperature in each growth chamber was monitored for the duration of each experiment using a WatchDog model 450 data logger (Spectrum Technologies, Plainfield, IL). Data loggers were set to record temperature ($^\circ\text{C}$) at 10-min intervals. The accuracy ratings of the data loggers were $\pm 0.6^\circ\text{C}$. Data were downloaded into a computer at the end of each experiment. The diameter of pathogen growth was measured after 5 days incubation and recorded. The experiment was conducted twice. The recorded temperature in each growth chamber was 32.2 ± 0.2 , 35.3 ± 0.0 , and $38.3 \pm 0.1^\circ\text{C}$ for the first experiment and 32.8 ± 0.1 , 35.7 ± 0.2 , and $38.7 \pm 0.2^\circ\text{C}$ for the second experiment, respectively.

Data analysis. All statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Cary, NC). Isolates were grouped by species and subjected to analysis of variance. Tukey's HSD was used for separation of means to account for unequal sample sizes between subgroups in a category ($P = 0.05$). Isolate was considered a random factor in the ANOVA analyses. The response variables were sporangial width, sporangial

Table 1. Isolates of *Phytophthora* species used in this study

Isolate	Origin ^v		Host family	MT ^w	MS ^x	GC ^y	Source ^z
101	N. America	US, MI	Cucurbitaceae	A1	S	7	M. K. Hausbeck
102	N. America	US, MI	Cucurbitaceae	A2	I	3	M. K. Hausbeck
455	N. America	US, MI	Cucurbitaceae	A1	S	3	M. K. Hausbeck
1177	N. America	US, MI	Cucurbitaceae	A2	S	3	M. K. Hausbeck
9790	N. America	US, MI	Solanaceae	A1	IS	AD	M. K. Hausbeck
9964	N. America	US, MI	Cucurbitaceae	A1	S	3	M. K. Hausbeck
10044	N. America	US, MI	Solanaceae	A2	S	3	M. K. Hausbeck
10193	N. America	US, MI	Fabaceae	A1	S	5	M. K. Hausbeck
10213	N. America	US, MI	Fabaceae	A1	I	5	M. K. Hausbeck
10251	N. America	US, MI	Fabaceae	A1	S	5	M. K. Hausbeck
10412	N. America	US, MI	Cucurbitaceae	A2	I	3	M. K. Hausbeck
10858	N. America	US, MI	Fabaceae	A1	S	5	M. K. Hausbeck
11457	N. America	US, MI	Solanaceae	A1	IS	3	M. K. Hausbeck
11469	N. America	US, MI	Solanaceae	A2	IS	5	M. K. Hausbeck
11478	N. America	US, MI	Fabaceae	A1	I	5	M. K. Hausbeck
11571	N. America	US, MI	Cucurbitaceae	A2	S	3	M. K. Hausbeck
11783	N. America	US, MI	Cucurbitaceae	A2	I	3	M. K. Hausbeck
11861	N. America	US, MI	Cucurbitaceae	A2	IS	5	M. K. Hausbeck
11885	N. America	US, MI	Cucurbitaceae	A1	I	5	M. K. Hausbeck
11923	N. America	US, MI	Cucurbitaceae	A1	I	5	M. K. Hausbeck
12017	N. America	US, MI	Cucurbitaceae	A2	S	3	M. K. Hausbeck
12162	N. America	US, MI	Cucurbitaceae	A2	S	3	M. K. Hausbeck
12328	N. America	US, MI	Solanaceae	A1	IS	3	M. K. Hausbeck
12842	N. America	US, MI	Solanaceae	A2	S	3	M. K. Hausbeck
12889	N. America	US, MI	Solanaceae	A1	I	5	M. K. Hausbeck
13199	N. America	US, KY	Cucurbitaceae	A1	S	4	K. W. Seebold
13200	N. America	US, KY	Cucurbitaceae	A2	S	8	K. W. Seebold
13201	N. America	US, KY	Cucurbitaceae	A2	S	8	K. W. Seebold
13202	N. America	US, NY	Cucurbitaceae	A2	S	5	C. D. Smart
13204	N. America	US, NY	Solanaceae	A1	S	AD	C. D. Smart
13205	N. America	US, NY	Cucurbitaceae	A2	S	4	C. D. Smart
13206	N. America	US, NY	Cucurbitaceae	A1	S	5	C. D. Smart
13207	N. America	US, CA	Solanaceae	A2	S	9	C. L. Blomquist
13208	N. America	US, CA	Solanaceae	A2	S	9	C. L. Blomquist
13212	N. America	US, SC	Cucurbitaceae	A2	S	8	A. P. Keinath
13213	N. America	US, SC	Cucurbitaceae	A2	S	8	A. P. Keinath
13214	N. America	US, SC	Cucurbitaceae	A2	S	8	A. P. Keinath
13219	N. America	US, SC	Cucurbitaceae	A1	S	4	A. P. Keinath
13220	N. America	US, SC	Cucurbitaceae	A1	S	4	A. P. Keinath
13222	N. America	US, SC	Solanaceae	A1	S	2	A. P. Keinath
13224	N. America	US, SC	Solanaceae	A2	S	AD	A. P. Keinath
13226	N. America	US, SC	Solanaceae	A2	I	4	A. P. Keinath
13228	N. America	US, SC	Solanaceae	A1	I	4	A. P. Keinath
13229	N. America	US, SC	Solanaceae	A1	IS	5	A. P. Keinath
13230	N. America	US, SC	Solanaceae	A1	S	4	A. P. Keinath
13233	N. America	US, LA	Solanaceae	A1	S	AD	D. M. Ferrin
13236	N. America	US, AR	Solanaceae	A2	S	4	M. E. Matheron
13239	N. America	US, TX	Cucurbitaceae	A2	S	9	T. Isakeit
13243	N. America	US, NC	Cucurbitaceae	A1	S	5	K. L. Ivors
13245	N. America	US, NC	Cucurbitaceae	A1	S	5	K. L. Ivors
13247	N. America	US, NC	Cucurbitaceae	A1	S	4	K. L. Ivors
13248	N. America	US, NC	Cucurbitaceae	A1	S	5	K. L. Ivors
13249	N. America	US, NC	Cucurbitaceae	A1	S	5	K. L. Ivors
13338	N. America	US, MA	Cucurbitaceae	A2	S	3	R. L. Wick
13339	N. America	US, MA	Cucurbitaceae	A1	S	3	R. L. Wick
13347	N. America	US, GA	Cucurbitaceae	A1	S	8	P. Ji
13349	N. America	US, MI	Cucurbitaceae	A2	S	7	M. K. Hausbeck
13366	S. America	Brazil	Sterculiaceae	A1	S	IN	K. H. Lamour
13367	S. America	Brazil	Sterculiaceae	A2	S	IN	K. H. Lamour
13368	S. America	Brazil	Sterculiaceae	A2	S	IN	K. H. Lamour
13371	Africa	Cameroon	Sterculiaceae	A1	S	1	K. H. Lamour
13377	N. America	Mexico	Sterculiaceae	A1	S	TR	K. H. Lamour
13384	Asia	India	Piperaceae	A2	S	TR	K. H. Lamour
13385	N. America	US, HI	Proteaceae	A2	S	TR	K. H. Lamour
13390	N. America	Mexico	Solanaceae	A2	S	4	K. H. Lamour
13397	N. America	US, HI	Proteaceae	A2	S	6	K. H. Lamour

(continued on next page)

^v Geographical origin (continent, country, state). Standard U.S. state abbreviations are used.^w Mating type of an isolate: A1 or A2.^x Sensitivity to mefenoxam: I = insensitive, IS = intermediately insensitive, S = sensitive.^y Predominant genetic cluster (GC) membership. AD indicates a highly admixed *P. capsici* isolate. TR indicates *P. tropicalis*. IN indicates isolates with an intermediate genotype.^z ATCC = American Type Culture Collection.

Table 1. (continued from previous page)

Isolate	Origin ^y		Host family	MT ^w	MS ^x	GC ^y	Source ^z
13398	N. America	Mexico	Sterculiaceae	A2	S	TR	K. H. Lamour
13401	N. America	US, CA	Cucurbitaceae	A2	S	8	K. H. Lamour
13407	Europe	Norway	Cucurbitaceae	A2	S	8	M. L. Herrero
13423	N. America	US, MI	Cucurbitaceae	A1	S	3	M. K. Hausbeck
13456	N. America	US, MI	Cucurbitaceae	A1	S	3	M. K. Hausbeck
13471	N. America	US, MI	Solanaceae	A2	I	3	M. K. Hausbeck
13479	Europe	Spain	Solanaceae	A1	S	8	A. Lacasa
13602	N. America	US, HI	Proteaceae	A1	S	TR	ATCC
13603	N. America	US, HI	Caricaceae	A2	S	TR	J. Y. Uchida
13606	Australia	Australia	Annonaceae	A1	S	IN	B. McNeil
13607	Australia	Australia	Apocynaceae	A1	S	TR	B. McNeil
13608	Australia	Australia	Annonaceae	A2	S	TR	B. McNeil
13609	Australia	Australia	Annonaceae	A2	S	TR	B. McNeil
13613	N. America	US, DE	Fabaceae	A1	I	7	G. Majeau
13614	N. America	US, DE	Fabaceae	A1	S	IN	G. Majeau
13616	N. America	US, FL	Solanaceae	A1	S	5	A. J. Gevens
13617	N. America	US, FL	Solanaceae	A1	S	5	A. J. Gevens
13620	Europe	Italy	Solanaceae	A1	S	6	R. B. Kung
13621	Europe	Italy	Solanaceae	A2	S	6	R. B. Kung
13624	Europe	Italy	Solanaceae	A1	S	6	G. Tamietti
13632	Europe	Italy	Solanaceae	A1	S	6	G. Tamietti
13634	Europe	Italy	Solanaceae	A1	S	9	G. Tamietti
13638	Asia	Taiwan	Solanaceae	A2	S	3	T. C. Wang
13639	Asia	Taiwan	Solanaceae	A1	S	3	T. C. Wang
13640	Asia	Taiwan	Solanaceae	A1	S	AD	T. C. Wang
13641	Asia	Taiwan	Solanaceae	A1	S	3	T. C. Wang
13642	Asia	Taiwan	Solanaceae	A2	S	3	T. C. Wang
13644	N. America	US, CA	Solanaceae	A2	S	9	J. P. Prince
13645	N. America	US, CA	Solanaceae	A2	S	9	J. P. Prince
13647	N. America	US, CA	Solanaceae	A2	S	5	J. P. Prince
13649	N. America	US, CA	Solanaceae	A1	S	6	J. P. Prince
13652	N. America	US, CA	Solanaceae	A2	S	9	N. Kabir
13653	N. America	US, NM	Solanaceae	A1	S	AD	N. Kabir
13654	N. America	Mexico	Solanaceae	A2	S	4	N. Kabir
13655	N. America	US, FL	Solanaceae	A2	IS	6	N. Kabir
13656	N. America	US, NM	Solanaceae	A2	S	4	N. Kabir
13657	N. America	Mexico	Solanaceae	A2	S	8	N. Kabir
13658	Europe	Italy	Solanaceae	A1	S	6	N. Kabir
13659	N. America	Mexico	Solanaceae	A2	S	AD	N. Kabir
13660	Asia	Korea	Solanaceae	A1	S	8	N. Kabir
13662	Asia	Thailand	Solanaceae	A1	S	3	N. Kabir
13663	N. America	US, FL	Solanaceae	A1	S	6	N. Kabir
13665	Asia	Taiwan	Solanaceae	A2	S	3	P. J. Ann
13666	Asia	Taiwan	Solanaceae	A1	S	3	P. J. Ann
13667	Asia	Taiwan	Solanaceae	A2	S	3	P. J. Ann
13668	Asia	Taiwan	Solanaceae	A1	S	3	P. J. Ann
13669	Asia	Taiwan	Solanaceae	A2	S	3	P. J. Ann
13670	Asia	Taiwan	Piperaceae	A1	I	AD	P. J. Ann
13671	Asia	Taiwan	Piperaceae	A2	IS	3	P. J. Ann
13672	Asia	Taiwan	Cucurbitaceae	A1	S	3	P. J. Ann
13673	Asia	Taiwan	Cucurbitaceae	A2	S	3	P. J. Ann
13674	N. America	US, OH	Cucurbitaceae	A1	A	7	S. Miller
13675	N. America	US, OH	Solanaceae	A2	S	5	S. Miller
13677	N. America	US, OH	Cucurbitaceae	A2	S	3	S. Miller
13678	N. America	US, OH	Cucurbitaceae	A2	S	5	S. Miller
13692	N. America	US, NM	Solanaceae	A1	S	3	ATCC
13693	S. America	Uruguay	Solanaceae	A2	S	4	R. Bernal
13694	S. America	Uruguay	Solanaceae	A2	S	4	R. Bernal
13695	S. America	Uruguay	Solanaceae	A2	S	4	R. Bernal
13699	Asia	Japan	Solanaceae	A2	S	4	S. Uemtsu
13700	Asia	Japan	Solanaceae	A2	S	4	S. Uemtsu
13704	N. America	US, NJ	Fabaceae	A2	S	4	N. Gregory
13705	N. America	US, NJ	Cucurbitaceae	A2	S	4	N. Gregory
13706	N. America	US, NJ	Cucurbitaceae	A2	IS	5	N. Gregory
13707	N. America	US, NJ	Fabaceae	A2	IS	5	N. Gregory
13708	N. America	US, NJ	Cucurbitaceae	A2	IS	5	N. Gregory
13709	N. America	US, NJ	Fabaceae	A2	S	4	N. Gregory
13710	N. America	US, NJ	Fabaceae	A2	S	8	N. Gregory
13711	N. America	US, NJ	Fabaceae	A1	S	8	N. Gregory
13712	S. America	Peru	Solanaceae	A2	S	2	K. H. Lamour
13713	S. America	Peru	Solanaceae	A2	S	2	K. H. Lamour
13714	S. America	Peru	Solanaceae	A2	S	2	K. H. Lamour

length, LB ratio, pedicle length, sporangial density, and growth diameter at 32, 35, and 38°C. A Box-Cox transformation (PROC TRANSREG) was used for sporangial width. The sporangial length, pedicle length, LB ratio, and growth diameter were log transformed and the sporangial density was square root transformed prior to analyses to satisfy the assumption of normality.

P. tropicalis and isolates with an intermediate genotype were removed from the dataset prior to further statistical analysis, leaving only *P. capsici* sensu stricto isolates. Data were subjected to analysis of variance, and Tukey's HSD was used for separation of means to account for unequal sample sizes between subgroups in a category ($P = 0.05$). The contrast option in PROC MIXED was used to compare isolates from vegetable crops and nonvegetable hosts. Isolates were arbitrarily grouped by predominant genetic cluster, continent of origin, mefenoxam sensitivity, mating type, and host family of origin; these were the main factors in ANOVA analyses. Isolate was considered a random factor. The response variables were sporangial width, sporangial length, LB ratio, pedicle length, oospore diameter, and sporangial density. A Box-Cox transformation (PROC TRANSREG) was used for sporangial width. The sporangial length, pedicle length, and LB ratio were log transformed, and the sporangial density was square root transformed prior to analyses to satisfy the assumption of normality.

Results

P. capsici sporangia were caducous and papillate with long pedicles ranging in length from 24 to 177 μm (mean $68 \pm 41 \mu\text{m}$). Sporangia for isolates determined to be *P. tropicalis* or intermediate isolates were also caducous and papillate with long pedicles ranging from 20 to 260 μm (mean $92 \pm 44 \mu\text{m}$) and 20 to 152 μm (mean $67 \pm 33 \mu\text{m}$), respectively. Overall, the mean pedicle length was significantly larger for *P. tropicalis* than for *P. capsici*, with intermediate isolates showing an intermediate mean pedicle length (Table 2). The mean sporangial length and width for each *P. capsici* isolate ranged from 38.0 to 59.5 μm long and 22.5 to 34.5 μm wide. The overall mean length for all *P. capsici* isolates was $47.1 \pm 8.4 \mu\text{m}$ long, and the overall mean width was $28.1 \pm 4.4 \mu\text{m}$. The LB ratio of all of the *P. capsici* isolates was between 1.3 and 2.1, with a mean ratio of 1.7 ± 0.3 (Table 2). Twenty-six *P. capsici* isolates had LB ratios >1.8 , and these isolates belonged to all nine genetic clusters (Fig. 1A).

P. tropicalis isolates presented a mean length and width of 39.6 to 49.0 μm long and 21.7 to 26.3 μm wide, and intermediate isolates ranged from 32.8 to 50.1 μm long and 18.2 to 24.6 μm wide. The overall mean length and width for all *P. tropicalis* isolates was $44.0 \pm 7.3 \mu\text{m}$ long and $23.8 \pm 3.4 \mu\text{m}$ wide. Intermediate isolates presented an overall mean length and width of $41.4 \pm 9.8 \mu\text{m}$ long and $20.7 \pm 3.5 \mu\text{m}$ wide. The LB ratio of all of the *P. tropicalis* isolates was 1.7 to 2.2, with a mean of 1.9 ± 0.3 . The LB ratio of the intermediate isolates was 1.7 to 2.1, with an overall mean of 2.0 ± 0.3 (Table 2). Overall, *P. capsici* had a larger mean spo-

rangial width and smaller LB ratio than *P. tropicalis* or intermediate isolates. Intermediate isolates had a smaller mean sporangial width than *P. capsici* or *P. tropicalis*. *P. tropicalis* and intermediate isolates had a statistically similar LB ratio. *P. capsici* had the largest mean sporangial length, which was similar to the mean length for *P. tropicalis* isolates. Intermediate isolates had shorter sporangia than *P. capsici*, but these were similar to *P. tropicalis* (Table 2).

When *P. capsici* isolates were grouped by predominant genetic cluster, no differences were observed in LB ratio ($P = 0.1965$) or pedicle length ($P = 0.6148$), but differences were observed in the mean sporangial width ($P = 0.0001$) and length ($P = 0.0387$) of isolates (Table 3). Isolates belonging predominantly to clusters four and six presented the smallest and largest sporangia, respectively. The mean sporangial length of isolates in the other genetic clusters was not significantly different than the mean sporangial length for clusters four and six. Clusters three, six, seven, and eight had the greatest mean sporangial width. Isolates belonging to clusters one, two, five, and nine had sporangia that were not significantly different in width from isolates belonging to other clusters (Table 3).

When isolates were grouped by host family of origin, differences were observed in LB ratio ($P = 0.0031$) and pedicle length ($P = 0.0017$), but not in sporangial width ($P = 0.6580$) and length ($P = 0.1552$) (Table 4). Isolates from solanaceous and cucurbitaceous hosts had the largest and smallest mean LB ratio, respectively. Isolates from hosts belonging to the Piperaceae, Proteaceae, Sterculiaceae, and Fabaceae did not significantly differ in LB ratio from isolates from other host families. Isolates from vegetable hosts belonging to the Solanaceae, Fabaceae, and Cucurbitaceae had shorter pedicles than isolates from piperaceous hosts. Isolates from hosts belonging to the Proteaceae and Sterculiaceae had mean pedicle lengths that were not significantly different than isolates from other host families.

Differences in LB ratio ($P = 0.0002$), pedicle length ($P = 0.0146$), and sporangial length ($P < 0.0001$) were apparent when *P. capsici* isolates were grouped by continent. Isolates from Asia had longer sporangia, longer pedicles, and larger LB ratios than isolates from North America. In addition, North American isolates had shorter sporangia than isolates from Europe and a smaller LB ratio than isolates from South America. Isolates from Asia and Europe had longer sporangia than isolates from North America. Isolates from Asia and South America had larger LB ratios than isolates from North America. Isolates from Asia also had longer pedicles than isolates from North America. Differences in sporangial width were less clear ($P = 0.0341$), with statistically similar means for each continent using the more conservative Tukey's HSD method (Table 5).

When isolates were grouped by mating type and sensitivity to mefenoxam, no differences were observed for sporangial width ($P > 0.0664$) and length ($P > 0.0993$), or for pedicle length ($P > 0.6126$). LB ratios were not different for isolates grouped by sensitivity to mefenoxam ($P = 0.4140$), but the LB ratio was statistically

Table 2. Characteristics of *Phytophthora* isolates included in this study

<i>Phytophthora</i> spp.	<i>P. capsici</i>	Intermediate ^x	<i>P. tropicalis</i>	<i>P</i> value
n ^y	124	5	9	
Pedicle length (μm) ^z	67.9 ± 41.5 b	67.2 ± 32.9 ab	92.2 ± 44.3 a	0.0066
Sporangial length (μm) ^z	47.1 ± 8.4 a	41.4 ± 9.8 b	44.0 ± 7.3 ab	0.0027
Sporangial width (μm) ^z	28.1 ± 4.4 a	20.7 ± 3.5 c	23.8 ± 3.4 b	<0.0001
Length:width ratio ^z	1.7 ± 0.3 b	2.0 ± 0.3 a	1.9 ± 0.3 a	<0.0001
Chlamydospores (n)	35	1	2	
Growth at 32°C (n)	124	5	9	
Growth at 32°C (cm) ^z	4.2 ± 0.6 a	2.1 ± 1.7 c	3.1 ± 1.6 b	<0.0001
Growth at 35°C (n)	122	2	8	
Growth at 35°C (cm) ^z	1.7 ± 2.5 a	0.2 ± 0.4 b	0.3 ± 0.2 b	<0.0001
Growth at 38°C (n)	0	0	0	
Growth at 38°C (cm) ^z	0.0 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.1493
Sporangial density (sporangia/cm ²) ^z	$515,151 \pm 10,717$ ab	$980,720 \pm 88,599$ a	$417,638 \pm 48,832$ b	0.0200

^x Isolates that were identified as *P. capsici* in some genes and *P. tropicalis* in others.

^y n = number of isolates.

^z Mean $\pm$ standard deviation. Means that share letters within a row are not significantly different (Tukey's HSD, $P < 0.05$).

greater ($P = 0.0269$) for A2 isolates (1.72 ± 0.01) than for A1 isolates (1.66 ± 0.01).

While no significant differences were observed for sporangial density on culture medium when *P. capsici* isolates were grouped by genetic cluster ($P = 0.3204$), mating type ($P = 0.0599$), sensitivity to mefenoxam ($P = 0.4862$), or host family ($P = 0.3577$), differences were observed for isolates when grouped by continent ($P = 0.0033$, Table 5). Isolates collected in North America produced more sporangia in culture than isolates from South America. Isolates from Asia, Europe, and Africa produced a statistically similar number of sporangia as isolates from other continents (Table 5). While only four *P. capsici* isolates from nonvegetable hosts were included in this study, these isolates generally produced fewer sporangia than isolates from vegetable crops ($P = 0.0253$, data not shown). The five intermediate isolates included in this study produced more sporangia on culture media than *P. tropicalis* isolates. Mean sporangial production by *P. capsici* on culture media was not significantly different than production by *P. tropicalis* or isolates with an intermediate genotype (Table 2).

All of the *P. capsici* isolates produced oospores when paired with known A1 or A2 mating types on UCV8. The average *P. capsici* oospore diameter was $28.5 \pm 3.4 \mu\text{m}$, and oospores ranged in diameter from 21.7 to 36.9 μm . No differences were noted in *P. capsici* oospore diameter when the isolates were grouped by genetic cluster ($P = 0.5650$), host family of origin ($P = 0.3324$), mating type ($P = 0.4655$), mefenoxam sensitivity ($P = 0.0655$), or by continent ($P = 0.0686$) (data not shown).

Chlamydospores were produced by 35 isolates from hosts in the Cucurbitaceae (14 of 46), Fabaceae (2 of 12), and Solanaceae (19 of 63). No chlamydospores were produced by the four isolates of *P. capsici* retrieved from nonvegetable hosts (Piperaceae, Proteaceae, and Sterculiaceae) (data not shown). Isolates that produced chlamydospores belonged to all of the clusters except one and two (Fig. 1B). Isolates belonging to cluster three seemed to have a greater probability of producing chlamydospores than isolates belonging to other clusters (Fig. 1B); 14 of 35 isolates that produced chlamydospores belonged predominantly to cluster three. Chlamydospores were produced

Fig. 1. Genetic structure of *Phytophthora capsici* isolates grouped by **A**, length:breath ratio, and **B**, chlamydospore production. Each bar represents a single *P. capsici* isolate, which may be partitioned into colored segments representing an individual's proportionate genetic membership in a given Kth genetic cluster. Colors correspond to clusters as follows: dark red, one; purple, two; yellow, three; light green, four; dark blue, five; aqua, six; dark green, seven; light blue, eight; and pink, nine.

Table 3. Differences in morphological characteristics of *Phytophthora capsici* isolates grouped by predominant genetic cluster membership

GC ^w	n ^x	Sporangial length (μm) ^y	Sporangial width (μm) ^y	LB ratio ^y	Pedicle length (μm) ^y	Sporangial density ^{y,z}
1	1	45.4 $\pm$ 9.8 ab	25.0 $\pm$ 3.1 ab	1.8 $\pm$ 0.3	76.0 $\pm$ 39.9	144,792 $\pm$ 21,161
2	4	48.9 $\pm$ 7.6 ab	27.9 $\pm$ 3.7 ab	1.8 $\pm$ 0.3	56.4 $\pm$ 24.3	393,490 $\pm$ 74,862
3	33	47.4 $\pm$ 8.0 ab	28.8 $\pm$ 4.4 a	1.7 $\pm$ 0.3	70.5 $\pm$ 39.3	477,424 $\pm$ 17,332
4	20	44.5 $\pm$ 8.5 b	25.9 $\pm$ 4.4 b	1.7 $\pm$ 0.3	63.1 $\pm$ 35.8	600,844 $\pm$ 28,329
5	25	46.4 $\pm$ 8.4 ab	27.5 $\pm$ 4.0 ab	1.7 $\pm$ 0.2	64.7 $\pm$ 37.9	542,742 $\pm$ 22,217
6	9	51.6 $\pm$ 10.0 a	29.3 $\pm$ 3.1 a	1.8 $\pm$ 0.3	75.4 $\pm$ 38.3	387,060 $\pm$ 36,153
7	4	46.5 $\pm$ 7.7 ab	30.0 $\pm$ 5.0 a	1.6 $\pm$ 0.2	64.4 $\pm$ 44.1	467,031 $\pm$ 53,939
8	13	48.1 $\pm$ 7.2 ab	29.3 $\pm$ 3.9 a	1.7 $\pm$ 0.2	66.5 $\pm$ 35.8	553,157 $\pm$ 34,796
9	5	47.7 $\pm$ 7.4 ab	27.6 $\pm$ 4.0 ab	1.7 $\pm$ 0.2	82.7 $\pm$ 49.9	544,500 $\pm$ 50,666

^w GC = genetic cluster. Isolates (n = 10) that were highly admixed were excluded from these analyses.

^x n = number of isolates.

^y Mean $\pm$ standard deviation. Means with no letter or that share letters within a column are not significantly different (Tukey's HSD, $P < 0.05$).

^z Sporangia/cm².

by one of five isolates with an intermediate genotype and two of nine *P. tropicalis* isolates (Table 2).

All of the *P. capsici*, *P. tropicalis*, and intermediate isolates grew well at 32°C. At 35°C, two of 124 *P. capsici* (13634, 13693), one of nine *P. tropicalis* (13607), and three of five intermediate (13366, 13367, 13368) isolates were unable to grow (Table 2). When cultures were incubated at 32 or 35°C, the mean growth diameter of *P. capsici* was greater than the mean growth diameter of *P. tropicalis* and isolates with an intermediate genotype. When isolates were incubated at 38°C for 5 days, all of the isolates grew poorly (<0.5 cm), and there were no significant differences in mean growth diameter among species (Table 2).

Discussion

The *P. capsici* isolates in this study showed significant variation in morphological and physiological characteristics. The mean sporangial length and width for each *P. capsici* isolate ranged from 38 to 60 µm long and 23 to 35 µm wide, in agreement with other studies that reported sporangia to range from 33 to 66 µm long and 17 to 43 µm wide (3,17,22,28,36). Ristaino (28) noted that *P. capsici* isolates from cucurbit hosts (56 to 63 µm long) generally produced longer sporangia than isolates collected from pepper (39 to 60 µm long). In our study, differences in sporangial length and width were not apparent when isolates were grouped by host family of origin, in contrast to what was previously observed by Ristaino. The range of sporangial lengths in our study were 39 to 53 µm for isolates from cucurbit hosts and 38 to 59 µm for isolates from solanaceous hosts (38 to 59 µm for isolates from pepper). Islam et al (17) found lengths of 42 to 55 µm for isolates from processing pumpkin, which is more similar to lengths observed in our study than to those reported by Ristaino for cucurbit isolates (28). Significant differences in the mean sporangial width and length of *P. capsici* isolates were noted when isolates were grouped by genetic cluster or geography, but not when isolates were grouped by mating type and sensitivity to mefenoxam. *P. tropicalis* isolates presented a mean sporangial length and width of 39.6 to 49.0 µm long and 21.7 to 26.3 µm wide, and intermediate isolates had sporangia that ranged from 32.8 to 50.1 µm long and 18.2 to 24.6 µm wide in our study. Mchau and Coffey (22) reported sporangial lengths and widths of 33 to 66 and 19 to 32 µm, respectively, for *P. capsici* CAPB isolates, which likely correspond to *P. tropicalis*.

The LB ratio of all of the *P. capsici* isolates in this study ranged from 1.3 to 2.1. Mchau and Coffey (22) found a similar range of LB ratios, with isolates belonging to CAPA (likely *P. capsici* sensu

stricto) having ratios of 1.3 to 2.0 and isolates belonging to CAPB, which likely represents *P. tropicalis* isolates, having ratios of 1.4 to 2.1. Other previous studies reported LB ratios of 1.4 to 1.8 for isolates from pepper (28,36) and 1.3 to 1.8 for isolates from cucurbits (17,28). We found LB ratios ranging from 1.4 to 2.1 for isolates from solanaceous hosts and 1.3 to 1.8 for isolates from cucurbitaceous hosts, which generally agrees with previous findings. Differences in mean LB ratios were apparent when *P. capsici* isolates were grouped by host family of origin, mating type, and continent of origin, but not when isolates were grouped by predominant genetic cluster or mefenoxam sensitivity. Aragaki and Uchida (3) suggest that generally isolates belonging to *P. capsici* should have an LB ratio <1.8, and isolates belonging to *P. tropicalis* should have an LB ratio >1.8. In our study, 21% of *P. capsici* isolates (26 of 124), 67% of *P. tropicalis* (6 of 9), and 80% of intermediate isolates (4 of 5) had LB ratios >1.8, and would be identified as *P. tropicalis* if this one morphological feature was used exclusively. Most of the *P. capsici* isolates with an LB ratio >1.8 were originally isolated from solanaceous hosts (23 of 26). Clearly, LB ratio is an important morphological trait for separation of *P. capsici* from *P. tropicalis*; however, since some *P. capsici* and most intermediate isolates possessed LB ratios >1.8, separation of *P. capsici* and intermediate isolates from *P. tropicalis* requires a more thorough examination of isolates, and molecular methods using multiple genes are likely necessary.

Pedicle length has been noted to be a highly variable feature in previous studies (17,22,28), and pedicle lengths also varied widely in our study. Pedicle lengths ranged from 24 to 177 µm (mean 68 ± 41 µm) for *P. capsici*, from 20 to 260 µm (mean 92 ± 44 µm) for *P. tropicalis*, and from 24 to 177 µm (mean 67 ± 33 µm) for intermediate isolates. Mean pedicle lengths were 65 and 87 µm for *P. capsici* and *P. tropicalis*, respectively, in a previous study by Aragaki and Uchida (3). Pedicle lengths for *P. capsici* isolates ranged from 32 to 123 µm in previous studies (17,22,28), which is a slightly more limited range than was observed in our study. When isolates were grouped by host family or continent of origin, significant differences were observed in pedicle lengths, but differences were not apparent when isolates were grouped by other categories.

When cultures were incubated in liquid media, 35 of 124 (28%) *P. capsici* isolates and two of nine (22%) *P. tropicalis* isolates produced chlamydospores. In Aragaki and Uchida's study (3), no *P. capsici* isolates and most (46 of 53, 87%) *P. tropicalis* isolates produced chlamydospores. Similarly, no isolates from temperate hosts, but the majority (8 of 10) of isolates from nonvegetable

Table 4. Differences in morphological characteristics of *Phytophthora capsici* isolates grouped by host family of origin

Host ^w	n ^x	Sporangial length (µm) ^y	Sporangial width (µm) ^y	LB ratio ^y	Pedicle length (µm) ^y	Sporangial density ^{y,z}
Pi	3	48.6 ± 5.5	27.4 ± 3.2	1.8 ± 0.2 ab	144.6 ± 110.6 a	320,577 ± 41,642
Pr	1	48.9 ± 8.5	30.9 ± 3.1	1.6 ± 0.2 ab	99.9 ± 38.3 ab	93,750 ± 27,594
St	1	45.4 ± 9.8	25.0 ± 3.1	1.8 ± 0.3 ab	76.0 ± 39.9 ab	144,792 ± 21,161
So	63	48.5 ± 8.8	28.1 ± 4.4	1.7 ± 0.3 a	69.9 ± 38.3 b	533,760 ± 15,072
Fa	12	46.7 ± 7.3	28.4 ± 5.1	1.7 ± 0.3 ab	59.7 ± 30.0 b	529,236 ± 27,694
Cu	44	45.6 ± 7.8	28.3 ± 4.2	1.6 ± 0.2 b	63.4 ± 38.6 b	512,296 ± 18,820

^w Host family of origin. Pi = Piperaceae, Pr = Proteaceae, St = Sterculiaceae, So = Solanaceae, Fa = Fabaceae, Cu = Cucurbitaceae.

^x n = number of isolates.

^y Mean ± standard deviation. Means with no letter or that share letters within a column are not significantly different (Tukey's HSD, *P* < 0.05).

^z Sporangia/cm².

Table 5. Differences in morphological characteristics of *Phytophthora capsici* isolates grouped by geography

Continent	n ^x	Sporangial length (µm) ^y	Sporangial width (µm) ^y	LB ratio ^y	Pedicle length (µm) ^y	Sporangial density ^{y,z}
N. America	91	46.0 ± 8.2 b	28.0 ± 4.3	1.7 ± 0.3 b	64.6 ± 37.1 b	555,371 ± 12,837 a
Asia	18	50.5 ± 7.6 a	28.8 ± 4.6	1.8 ± 0.3 a	85.8 ± 58.7 a	518,067 ± 26,753 ab
Europe	8	51.9 ± 9.4 a	29.9 ± 4.4	1.7 ± 0.2 ab	72.7 ± 42.1 ab	318,646 ± 29,040 ab
S. America	6	48.4 ± 7.1 ab	26.1 ± 3.7	1.9 ± 0.3 a	56.9 ± 22.7 ab	220,139 ± 25,856 b
Africa	1	45.4 ± 9.8 ab	25.0 ± 3.1	1.8 ± 0.3 ab	76.0 ± 39.9 ab	144,792 ± 21,161 ab

^x n = number of isolates.

^y Mean ± standard deviation. Means with no letter or that share letters within a column are not significantly different (Tukey's HSD, *P* < 0.05).

^z Sporangia/cm².

hosts, some of which may have been *P. tropicalis*, formed chlamydospores in another study (4). Chlamydospores were not produced by isolates from solanaceous or cucurbitaceous hosts in the study by Ristaino (28), but were produced by 38% (21 of 57) of the isolates from pumpkin in the study by Islam et al. (17). Mchau and Coffey (22) found that 16 of 29 (55%) CAP2 isolates produced chlamydospores, but none of the CAP1 isolates that likely represent *P. capsici* sensu stricto isolates produced chlamydospores. Hence, chlamydospore production also does not appear to be reliable for separation of *P. tropicalis* from *P. capsici*. The studies by Ristaino (28) and Islam et al. (17) incubated the cultures under similar conditions to the current study to check for chlamydospore production; in the study by Aragaki and Uchida (3), the isolates were incubated under warmer conditions, and this may account for some of the variation among studies. Curiously, previous studies have reported chlamydospore production by isolates from nonvegetable hosts (1,32) and from pumpkin (17); in our study, chlamydospores were produced by isolates from the Cucurbitaceae, Fabaceae, and Solanaceae, and no chlamydospores were produced by the four isolates of *P. capsici* retrieved from nonvegetable hosts. However, *P. tropicalis* and intermediate isolates may have been included in some earlier studies (1,32), which challenges comparisons between studies. In our study, cluster three contained a greater proportion of isolates that produced chlamydospores than other clusters. Isolates belonging predominantly to cluster three were collected from North America and Asia. Cluster three contains the type culture for *P. capsici* from New Mexico, which did not produce chlamydospores in our study.

Oospore diameters for *P. capsici* isolates ranged from 22 to 37 μm in our study, in agreement with previous studies (17,22), and is a slightly larger range than was reported by Ristaino (28). Oospore diameters did not differ significantly when isolates were grouped by genetic cluster, host family of origin, mating type, mefenoxam sensitivity, or geography. Previous studies (3,4,22) have suggested that growth at 35°C is a good character to separate *P. capsici* from *P. tropicalis*. A few isolates in these previous studies did not conform to this standard, but most did. In our study, most of the *P. capsici* isolates were able to grow at 35°C as in previous studies. Eight of the nine *P. tropicalis* isolates were also able to grow at 35°C in our study, which was unexpected. Overall, the growth diameter of *P. capsici* isolates at 35°C was significantly greater than the growth diameter of *P. tropicalis* isolates.

As sporangia and zoospores are responsible for polycyclic disease development (16), knowing the sporangial production potential of isolates may be useful to predict an isolate's ability to spread. Differences in sporangial production on culture medium were observed when isolates were grouped by geography (continent). Differences in sporangial production were not observed when isolates were grouped by genetic cluster, mating type, sensitivity to mefenoxam, or host family. A previous study by Café-Filho and Ristaino (6) found no significant differences in sporangial production on culture medium when a limited number of isolates were grouped by sensitivity to mefenoxam, in agreement with our results. The few isolates from nonvegetable hosts included in our study generally produced fewer sporangia than isolates from vegetable hosts; in a previous study looking at isolate virulence, a similar trend was observed, where isolates from nonvegetable hosts were generally less virulent on vegetable fruits than isolates from vegetable hosts (L. L. Granke, L. M. Quesada-Ocampo, and M. K. Hausbeck, unpublished). Sporangial density in culture medium was not correlated with isolate virulence (lesion diameter on cucumber, zucchini, tomato, and pepper fruits), but it was correlated with sporangial density (rated visually on a 0 to 2 scale, 0 = no sporangia, 1 = light sporulation, and 2 = heavy sporulation) on infected fruits (data not shown, Pearson's correlation, $P = 0.05$).

A wide range of variability was observed in morphological traits among *P. capsici* isolates in this study. When *P. capsici* isolates were compared to a limited number of *P. tropicalis* and intermediate isolates, clear phenotypic differences were not apparent for all isolates of each species. Hence, reliance on morphology alone

could lead to misidentification of some isolates. In addition, previous studies have found differences in sporangial morphology of an oomycete pathogen between host species (29,34) and by plant part on a single host (7), which further supports using molecular markers for identification.

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Literature Cited

- Alizadeh, A., and Tsao, P. H. 1985. Chlamydospore formation in *Phytophthora palmivora* MF4. Trans. Br. Mycol. Soc. 85:71-79.
- Appiah, A. A., Flood, J., Archer, S. A., and Bridge, P. D. 2004. Molecular analysis of the major *Phytophthora* species on cacao. Plant Pathol. 53:209-219.
- Aragaki, M., and Uchida, J. Y. 2001. Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. Mycologia 93:137-145.
- Bowers, J. H., Martin, F. N., Tooley, P. W., and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology 97:492-503.
- Brasier, C. M. 1983. Problems and prospects in *Phytophthora* research. Pages 351-364 in: *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
- Café-Filho, A. C., and Ristaino, J. B. 2008. Fitness of isolates of *Phytophthora capsici* resistant to mefenoxam from squash and pepper fields in North Carolina. Plant Dis. 92:1439-1443.
- Delanoe, D. 1972. Biologie et Epidemiologie du mildiou du tournesol (in French). CETIOM Inform. Tech. 26:1-61.
- Donahoo, R. S., and Lamour, K. H. 2008. Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. Mycologia 100:911-920.
- Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.
- Forster, H., Oudemans, P., and Coffey, M. D. 1990. Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. Exp. Mycol. 14:18-31.
- Foster, J. M., and Hausbeck, M. K. 2010. Resistance of pepper to *Phytophthora* crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24-30.
- Gevens, A. J., Ando, K., Lamour, K. H., Grumet, R., and Hausbeck, M. K. 2006. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. Plant Dis. 90:1276-1282.
- Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology 97:421-428.
- Granke, L. L., Quesada-Ocampo, L. M., and Hausbeck, M. K. 2010. Phenotypic diversity of *Phytophthora capsici* isolates from a worldwide collection. Pages 233-236 in: Cucurbitaceae 2010 Proceedings, American Society for Horticultural Science, Charleston SC.
- Grote, D., and Claussen, W. 2001. Severity of root rot on tomato plants caused by *Phytophthora nicotianae* under nutrient- and light-stress conditions. Plant Pathol. 50:702-707.
- Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Dis. 88:1292-1303.
- Islam, S. Z., Babadoost, M., Lambert, K. N., Ndeme, A., and Fouly, H. M. 2004. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. Plant Dis. 89:191-197.
- Lamour, K. H., and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90:396-400.
- Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.
- Lamour, K. H., and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Dis. 87:841-845.
- Lee, B. K., Kim, B. S., Chang, S. W., and Hwang, B. K. 2001. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. Plant Dis. 85:497-500.

22. Mchau, G. R. A., and Coffey, M. D. 1995. Evidence for the existence of two distinct subpopulations in *Phytophthora capsici* and a redescription of the species. *Mycol. Res.* 99:89-102.
23. Oudemans, P., and Coffey, M. D. 1991. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. *Mycol. Res.* 95:1025-1046.
24. Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotypic data. *Genetics* 155:945-959.
25. Quesada-Ocampo, L. M., Fulbright, D. W., and Hausbeck, M. K. 2009. Susceptibility of Fraser fir to *Phytophthora capsici*. *Plant Dis.* 93:135-141.
26. Quesada-Ocampo, L. M., Granke, L. L., Mercier, M. R., Olsen, J., and Hausbeck, M. K. 2011. Investigating the genetic structure of *Phytophthora capsici* populations. *Phytopathology* 101:1061-1073.
27. Quesada-Ocampo, L. M., and Hausbeck, M. K. 2010. Resistance in tomato and wild relatives to crown and root rot caused by *Phytophthora capsici*. *Phytopathology* 100:619-627.
28. Ristaino, J. B. 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* 80:1253-1259.
29. Runge, F., and Thines, M. 2011. Host matrix has major impact on the morphology of *Pseudoperonospora cubensis*. *Eur. J. Plant Pathol.* 129:147-156.
30. Silvar, C., Duncan, J. M., Cooke, D. E. L., Williams, N. A., Diaz, J., and Merino, F. 2005. Development of specific PCR primers for identification and detection of *Phytophthora capsici* Leon. *Eur. J. Plant Pathol.* 112:43-52.
31. Tamietti, G., and Valentino, D. 2001. Physiological characterization of a population of *Phytophthora capsici* Leon. from northern Italy. *J. Plant Pathol.* 83:199-205.
32. Uchida, J. Y., and Aragaki, M. 1985. Occurrence of chlamydospores in *Phytophthora capsici*. *Mycologia* 77:832-835.
33. Waterhouse, G. M. 1963. Key to the Species of *Phytophthora* de Bary. Commonwealth Mycological Society, Kew, Surrey, UK.
34. Waterhouse, G. M., and Brothers, M. P. 1981. The taxonomy of *Pseudoperonospora*. *Mycol. Pap.* 148:1-28.
35. Zhang, Z. G., Zhang, J. Y., Zheng, X. B., Yang, Y. W., and Ko, W. H. 2004. Molecular distinctions between *Phytophthora capsici* and *Ph. tropicalis* based on ITS sequences of ribosomal DNA. *J. Phytopathol.* 152:358-364.
36. Zhijun, L. I., Weiping, L., Jinrong, Z., and Jianjun, L. 2007. Isolation and identification of *Phytophthora capsici* in Guangdong province and measurement of their pathogenicity and physiological race differentiation. *Front. Agric. China* 1:377-381.