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Differences in virulence of *Phytophthora capsici* isolates from a worldwide collection on host fruits

Leah L. Granke • Lina M. Quesada-Ocampo • Mary K. Hausbeck

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Abstract *Phytophthora capsici* causes root, crown, and fruit rot of vegetable and tropical hosts. Cucumber, zucchini, tomato, and pepper fruits were inoculated using 6-mm-diameter agar plugs of *P. capsici*, incubated in clear plastic boxes at room temperature (25 ± 2ºC and 100% relative humidity), and virulence was estimated by measuring the lesion diameter, pathogen growth diameter, and pathogen sporulation density three (cucumber, zucchini) or four (tomato, pepper) days later. When isolates were grouped by genetic cluster, significant differences in virulence were observed on cucumber and zucchini, with isolates belonging to genetic cluster five causing larger lesions than isolates from genetic cluster six. On tomato, no significant differences were observed for isolates grouped by genetic cluster, but isolates from vegetable crops were generally more virulent than isolates from tropical hosts. Isolates from fabaceous hosts sporulated better on cucumber fruits than isolates from Solanaceous hosts. Isolates from vegetable hosts sporulated better on zucchini than isolates from tropical hosts. No significant differences in lesion diameter were noted on pepper when isolates were grouped by host family of origin or genetic cluster, but differences in pathogen sporulation were apparent by host family. Our findings suggest that isolate characteristics such as host family of origin and genetic cluster membership may be used to guide initial isolate selection for cucurbit fruit resistance screening. Final isolate selection should incorporate the phenotypic and genetic diversity of *P. capsici,* including isolates with differing virulence to the host organ of interest.

Keywords: Genetic structure, Pathogenicity, *Phytophthora tropicalis,* Population genetics, Population structure, Virulence structure

Introduction

The oomycete plant pathogen *Phytophthora capsici* Leonian is distributed worldwide (Erwin and Ribeiro 1996) and can be a significant limiting factor to vegetable production (Hausbeck and Lamour 2004). Foliar blighting and root, crown, and fruit rot of a variety of cucurbitaceous and solanaceous hosts are caused by *P. capsici* (Erwin and Ribeiro 1996). Recently, snap bean (*Phaseolus vulgaris* L.) (Gevens et al. 2008), lima bean (*Phaseolus lunatus* L.) (Davidson et al. 2002), and Fraser fir (*Abies fraseri* (Pursh) Poir.) (Quesada-Ocampo et al. 2009) have been added as new hosts, and weeds may be susceptible (French-Monar et al. 2006). In addition, *P.*

Leah L. Granke and Lina M. Quesada-Ocampo made equal contributions to this work

L. L. Granke • L. M. Quesada-Ocampo • M. K. Hausbeck
Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA
email: hausbec1@msu.edu

capsici causes disease on tropical hosts such as cacao (*Theobroma cacao L.)*, rubber (*Hevea brasiliensis* Mull. Arg.), macadamia (*Macadamia integrifolia* Maiden and Betche), papaya (*Carica papaya* L.), and black pepper (*Piper nigrum* L.) (Erwin and Ribeiro 1996). Management of *P. capsici* is challenging and is limited by the long-term survival of oospores in the soil (Lamour and Hausbeck 2003), an increasing list of susceptible hosts (Quesada-Ocampo et al. 2009), fungicide-resistant pathogen populations (Lamour and Hausbeck 2000), and a lack of commercially acceptable resistant cultivars (Lee et al. 2001, Gevens et al. 2006, Foster and Hausbeck 2010).

The existence of diverse pathogen populations and physiological races within *P. capsici* has limited development of resistant cultivars of economically important hosts (Walker and Bosland 1999, Oelke and Bosland 2003). Since resistant host varieties may perform well in the presence of isolates from a certain region, but not with isolates from another region, breeders need to use a wide variety of *P. capsici* isolates to develop resistant varieties (Foster and Hausbeck 2010). The spatial distribution of *P. capsici* isolates differing in virulence will also dictate how resistant varieties should be deployed (Foster and Hausbeck 2010). Previous studies have reported differences in virulence and pathogenicity among *P. capsici* isolates on vegetable hosts (Kim and Hwang 1992, Oelke and Bosland 2003, Palloix et al. 1988, Reifschneider et al. 1986, Ristaino 1990). Isolate virulence responses vary by host. For example, no significant difference in virulence was noted for *P. capsici* isolates 12889, OP97, SP98, and SFF3 from Michigan when they were used to inoculate cucumber (*Cucumis sativus* L.) fruits (Gevens et al. 2006) and Fraser fir roots and stems (Quesada-Ocampo et al. 2009). However, when these same isolates were inoculated onto pepper (*Capsicum annuum* L.) roots and fruits (Foster and Hausbeck 2010) and tomato (*Solanum lycopersicum* L.) roots (Quesada-Ocampo and Hausbeck 2010), variations in virulence were noted.

It has been understood since the 1970s (Polach and Webster 1972) that *P. capsici* populations may differ in pathogenicity, but whether or not virulence may be associated with other isolate characteristics is unknown. Interestingly, when 24 *P. capsici* isolates from processing pumpkin were used to inoculate pumpkin seedlings, isolates grouped into six virulence groups, which corresponded directly with six random amplified polymorphic DNA (RAPD) groups (Islam et al. 2004). Robust host screenings should include isolates that represent the phenotypic and genotypic diversity of *P. capsici,* and isolates with varying levels of virulence should be included. Toward this end, it would be useful to understand if isolate virulence is associated with other isolate characteristics, especially host family of origin, geographic origin, and isolate membership in a genetic cluster. The objective of this study was to determine whether differences in virulence on cucumber, zucchini (*Cucuribita pepo* L.), tomato, and pepper fruits were apparent when *P. capsici* isolates were grouped by host family of origin, geography, and genetic cluster membership. In addition, we examined differences in pathogenicity between *P. capsici* and *Phytophthora tropicalis* isolates and isolates with an intermediate genotype between *P. capsici* and *P. tropicalis*. The species identity, mating type, mefenoxam sensitivity, and genetic cluster membership of isolates included in this study have been previously reported (Quesada-Ocampo et al. 2011), and a preliminary report of isolate virulence to zucchini has been published (Quesada-Ocampo et al. 2010).

Materials and methods

Isolate selection and maintenance. *Phytophthora capsici* isolates were obtained from colleagues or selected from the *Phytophthora* culture collection maintained in the laboratory of Dr. Hausbeck at Michigan State University. *P. capsici* isolates originated from 12 countries in five continents and were obtained from 17 vegetable and tropical host species representing six host families (Table 1). In addition, nine *P. tropicalis* and five isolates with an intermediate genotype between *P. capsici* and *P. tropicalis* were also used (Table 1). Type cultures for *P. capsici* 13692 (ATCC.15399, New Mexico) and *P. tropicalis* 13602 (ATCC.76651, Hawaii) were included.

Actively growing, single-spore cultures on unclarified V8 agar (UCV8A, 3 g $CaCO_3$, 15 g agar, 160 ml unfiltered V8 juice and 840 ml distilled water) were obtained from long-term stock cultures.

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

Isolate	Origin[a]	Host Family	MT[b]	MS[c]	GC[d]	Source[e]
13371	Africa	Sterculiaceae	A1	S	1	K.H. Lamour
13222	N. America	Solanaceae	A1	S	2	A. P. Keinath
13712	S. America	Solanaceae	A2	S	2	K.H. Lamour
13713	S. America	Solanaceae	A2	S	2	K.H. Lamour
13714	S. America	Solanaceae	A2	S	2	K.H. Lamour
13672	Asia	Cucurbitaceae	A1	S	3	P. J. Ann
13673	Asia	Cucurbitaceae	A2	S	3	P. J. Ann
455	N. America	Cucurbitaceae	A1	S	3	M. K. Hausbeck
9964	N. America	Cucurbitaceae	A1	S	3	M. K. Hausbeck
13339	N. America	Cucurbitaceae	A1	S	3	R. L. Wick
13423	N. America	Cucurbitaceae	A1	S	3	M. K. Hausbeck
13456	N. America	Cucurbitaceae	A1	S	3	M. K. Hausbeck
1177	N. America	Cucurbitaceae	A2	S	3	M. K. Hausbeck
11571	N. America	Cucurbitaceae	A2	S	3	M. K. Hausbeck
12017	N. America	Cucurbitaceae	A2	S	3	M. K. Hausbeck
12162	N. America	Cucurbitaceae	A2	S	3	M. K. Hausbeck
13338	N. America	Cucurbitaceae	A2	S	3	R. L. Wick
13677	N. America	Cucurbitaceae	A2	S	3	S. Miller
102	N. America	Cucurbitaceae	A2	I	3	M. K. Hausbeck
10412	N. America	Cucurbitaceae	A2	I	3	M. K. Hausbeck
11783	N. America	Cucurbitaceae	A2	I	3	M. K. Hausbeck
13671	Asia	Piperaceae	A2	IS	3	P. J. Ann
13666	Asia	Solanaceae	A1	S	3	P. J. Ann
13668	Asia	Solanaceae	A1	S	3	P. J. Ann
13662	Asia	Solanaceae	A1	S	3	N. Kabir
13639	Asia	Solanaceae	A1	S	3	T. C. Wang
13641	Asia	Solanaceae	A1	S	3	T. C. Wang
13638	Asia	Solanaceae	A2	S	3	T. C. Wang
13642	Asia	Solanaceae	A2	S	3	T. C. Wang
13665	Asia	Solanaceae	A2	S	3	P. J. Ann
13667	Asia	Solanaceae	A2	S	3	P. J. Ann
13669	Asia	Solanaceae	A2	S	3	P. J. Ann
13692	N. America	Solanaceae	A1	S	3	ATCC
11457	N. America	Solanaceae	A1	IS	3	M. K. Hausbeck
12328	N. America	Solanaceae	A1	IS	3	M. K. Hausbeck
10044	N. America	Solanaceae	A2	S	3	M. K. Hausbeck
12842	N. America	Solanaceae	A2	S	3	M. K. Hausbeck
13471	N. America	Solanaceae	A2	I	3	M. K. Hausbeck
13199	N. America	Cucurbitaceae	A1	S	4	K. W. Seebold
13219	N. America	Cucurbitaceae	A1	S	4	A. P. Keinath
13220	N. America	Cucurbitaceae	A1	S	4	A. P. Keinath
13247	N. America	Cucurbitaceae	A1	S	4	K. L. Ivors
13205	N. America	Cucurbitaceae	A2	S	4	C. D. Smart
13705	N. America	Cucurbitaceae	A2	S	4	N. Gregory
13704	N. America	Fabaceae	A2	S	4	N. Gregory

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Isolate	Origin[a]	Host Family	MT[b]	MS[c]	GC[d]	Source[e]
13709	N. America	Fabaceae	A2	S	4	N. Gregory
13699	Asia	Solanaceae	A2	S	4	S. Uemtsu
13700	Asia	Solanaceae	A2	S	4	S. Uemtsu
13230	N. America	Solanaceae	A1	S	4	A. P. Keinath
13228	N. America	Solanaceae	A1	I	4	A. P. Keinath
13390	N. America	Solanaceae	A2	S	4	K.H. Lamour
13236	N. America	Solanaceae	A2	S	4	M. E. Matheron
13654	N. America	Solanaceae	A2	S	4	N. Kabir
13656	N. America	Solanaceae	A2	S	4	N. Kabir
13226	N. America	Solanaceae	A2	I	4	A. P. Keinath
13693	S. America	Solanaceae	A2	S	4	R. Bernal
13694	S. America	Solanaceae	A2	S	4	R. Bernal
13695	S. America	Solanaceae	A2	S	4	R. Bernal
13206	N. America	Cucurbitaceae	A1	S	5	C. D. Smart
13243	N. America	Cucurbitaceae	A1	S	5	K. L. Ivors
13245	N. America	Cucurbitaceae	A1	S	5	K. L. Ivors
13248	N. America	Cucurbitaceae	A1	S	5	K. L. Ivors
13249	N. America	Cucurbitaceae	A1	S	5	K. L. Ivors
11885	N. America	Cucurbitaceae	A1	I	5	M. K. Hausbeck
11923	N. America	Cucurbitaceae	A1	I	5	M. K. Hausbeck
13706	N. America	Cucurbitaceae	A2	IS	5	N. Gregory
13202	N. America	Cucurbitaceae	A2	S	5	C. D. Smart
13678	N. America	Cucurbitaceae	A2	S	5	S. Miller
13708	N. America	Cucurbitaceae	A2	IS	5	N. Gregory
11861	N. America	Cucurbitaceae	A2	IS	5	M. K. Hausbeck
10251	N. America	Fabaceae	A1	S	5	M. K. Hausbeck
10193	N. America	Fabaceae	A1	S	5	M. K. Hausbeck
10858	N. America	Fabaceae	A1	S	5	M. K. Hausbeck
11478	N. America	Fabaceae	A1	I	5	M. K. Hausbeck
10213	N. America	Fabaceae	A1	I	5	M. K. Hausbeck
13707	N. America	Fabaceae	A2	IS	5	N. Gregory
13616	N. America	Solanaceae	A1	S	5	A. J. Gevens
13617	N. America	Solanaceae	A1	S	5	A. J. Gevens
13229	N. America	Solanaceae	A1	IS	5	A. P. Keinath
12889	N. America	Solanaceae	A1	I	5	M. K. Hausbeck
13647	N. America	Solanaceae	A2	S	5	J. P. Prince
13618	N. America	Solanaceae	A2	S	5	A. J. Gevens
13675	N. America	Solanaceae	A2	S	5	S. Miller
11469	N. America	Solanaceae	A2	IS	5	M. K. Hausbeck
13397	N. America	Proteaceae	A2	S	6	K.H. Lamour
13624	Europe	Solanaceae	A1	S	6	G. Tamietti
13632	Europe	Solanaceae	A1	S	6	G. Tamietti
13620	Europe	Solanaceae	A1	S	6	R. B. Kung
13658	Europe	Solanaceae	A1	S	6	N. Kabir
13621	Europe	Solanaceae	A2	S	6	R. B. Kung

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

Isolate	Origin[a]	Host Family	MT[b]	MS[c]	GC[d]	Source[e]
13663	N. America	Solanaceae	A1	S	6	N. Kabir
13649	N. America	Solanaceae	A1	S	6	J. P. Prince
13655	N. America	Solanaceae	A2	IS	6	N. Kabir
13674	N. America	Cucurbitaceae	A1	A	7	S. Miller
101	N. America	Cucurbitaceae	A1	S	7	M. K. Hausbeck
13349	N. America	Cucurbitaceae	A2	S	7	M. K. Hausbeck
13613	N. America	Fabaceae	A1	I	7	G. Majeau
13407	Europe	Cucurbitaceae	A2	S	8	M.L. Herrero
13347	N. America	Cucurbitaceae	A1	S	8	P. Ji
13212	N. America	Cucurbitaceae	A2	S	8	A. P. Keinath
13213	N. America	Cucurbitaceae	A2	S	8	A. P. Keinath
13214	N. America	Cucurbitaceae	A2	S	8	A. P. Keinath
13200	N. America	Cucurbitaceae	A2	S	8	K. W. Seebold
13201	N. America	Cucurbitaceae	A2	S	8	K. W. Seebold
13401	N. America	Cucurbitaceae	A2	S	8	K.H. Lamour
13711	N. America	Fabaceae	A1	S	8	N. Gregory
13710	N. America	Fabaceae	A2	S	8	N. Gregory
13660	Asia	Solanaceae	A1	S	8	N. Kabir
13479	Europe	Solanaceae	A1	S	8	A. Lacasa
13657	N. America	Solanaceae	A2	S	8	N. Kabir
13239	N. America	Cucurbitaceae	A2	S	9	T. Isakeit
13240	N. America	Cucurbitaceae	A2	S	9	T. Isakeit
13634	Europe	Solanaceae	A1	S	9	G. Tamietti
13644	N. America	Solanaceae	A2	S	9	J. P. Prince
13645	N. America	Solanaceae	A2	S	9	J. P. Prince
13652	N. America	Solanaceae	A2	S	9	N. Kabir
13207	N. America	Solanaceae	A2	S	9	C. L. Blomquist
13208	N. America	Solanaceae	A2	S	9	C. L. Blomquist
13670	Asia	Piperaceae	A1	I	AD	P. J. Ann
13640	Asia	Solanaceae	A1	S	AD	T. C. Wang
13233	N. America	Solanaceae	A1	S	AD	D. M. Ferrin
13653	N. America	Solanaceae	A1	S	AD	N. Kabir
9790	N. America	Solanaceae	A1	IS	AD	M. K. Hausbeck
13204	N. America	Solanaceae	A1	S	AD	C. D. Smart
13659	N. America	Solanaceae	A2	S	AD	N. Kabir
13224	N. America	Solanaceae	A2	S	AD	A. P. Keinath
13606	Australia	Annonaceae	A1	S	IN	B. McNeil
13614	N. America	Fabaceae	A1	S	IN	G. Majeau
13366	S. America	Sterculiaceae	A1	S	IN	K.H. Lamour
13367	S. America	Sterculiaceae	A2	S	IN	K.H. Lamour
13368	S. America	Sterculiaceae	A2	S	IN	K.H. Lamour
13609	Australia	Annonaceae	A2	S	TR	B. McNeil
13603	N. America	Caricaceae	A2	S	TR	J. Y. Uchida
13384	Asia	Piperaceae	A2	S	TR	K.H. Lamour
13602	N. America	Proteaceae	A1	S	TR	ATCC

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

Isolate	Origin[a]	Host Family	MT[b]	MS[c]	GC[d]	Source[e]
13385	N. America	Proteaceae	A2	S	TR	K.H. Lamour
13377	N. America	Sterculiaceae	A1	S	TR	K.H. Lamour
13398	N. America	Sterculiaceae	A2	S	TR	K.H. Lamour

[a] Geographic origin (continent)
[b] Mating type of an isolate: A1 or A2
[c] Sensitivity to mefenoxam: I: insensitive, IS: intermediately insensitive, S: sensitive
[d] Predominant genetic cluster (GC) membership. AD indicates a highly admixed *P. capsici sensu stricto* isolate. TR indicates *P. tropicalis*. IN indicates an isolate with an intermediate genotype
[e] ATCC: American type culture collection

Each isolate was inoculated onto and isolated from wounded pepper fruits according to the methods of Quesada-Ocampo et al. (2009) before use, except for two *P. tropicalis* isolates (13384, 13603) and one isolate (13606) with an intermediate genotype between *P. capsici* and *P. tropicalis*. These three isolates were inoculated onto and isolated from pickling cucumber fruits instead of pepper fruits, as they were not pathogenic on pepper when retrieved from long-term storage. Isolates were inoculated onto and isolated from host fruits (either green pepper or pickling cucumber, see above) prior to the virulence experiments to minimize differences in isolate virulence caused by variation in long-term storage conditions. Isolates were thereafter transferred to new UCV8A weekly. The mating type (MT), and sensitivity to mefenoxam of isolates was determined as previously described (Lamour and Hausbeck 2000), and *P. capsici* was confirmed using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (1963). Population subdivision was assessed for a group of 236 *P. capsici sensu stricto* isolates (Quesada-Ocampo et al. 2011), 126 of which are included in this study, with the model-based Bayesian clustering algorithm implemented in Structure 2.3X (Pritchard et al. 2000). Population structure figures for *P. capsici sensu stricto* with virulence information were sorted by proportionate membership (Q) of an isolate in a genetic cluster and were generated using the Population Sorting Tool (PST) (J. J. Morrice, unpublished data), a graphic editing program created in R (R-Development-Core-Team 2008). For statistical analysis, each isolate was assigned to the Kth genetic cluster to which the isolate predominantly belonged. An isolate was assumed to belong equally to two genetic clusters if its membership was not >0.1 (proportion membership) higher in one genetic cluster than another; these isolates (n=8) were deemed highly admixed and were excluded from statistical analyses, but not from population structure figures.

Characterization of virulence on fruits. The virulence of each isolate was characterized on pickling cucumber, zucchini, green bell pepper, and green tomato fruits. Pickling cucumber ('Vlaspik') and tomato ('Mountain Fresh') fruits were grown at the Michigan State University (MSU) Plant Pathology Farm, East Lansing, MI, a site without a history of *P. capsici*. Pickling cucumber fruits were hand-harvested when 2 to 3 cm in diameter, and green tomato fruits were harvested when 4 to 5 cm in diameter. Mature green bell pepper and zucchini fruits were purchased from a local source.

For each isolate, one (zucchini, pepper) or two (cucumber, tomato) detached fruits were surface disinfested for 5 min in a 10% sodium hypochlorite solution, rinsed with distilled water, and air-dried. Agar plugs (6-mm-diameter) of actively growing *P. capsici* were used for inoculations. For the experiments examining virulence on pickling cucumber or tomato fruits, one agar plug was placed in the center of each of two unwounded fruits. For the zucchini experiments, the stem and blossom end of the fruit were inoculated with the same isolate, and one unwounded zucchini fruit was used per isolate. A shallow wound (~1-2 mm deep) was created in the center of each side of a pepper fruit using a sterilized 1-mm-diameter dissecting needle prior to inoculation with an agar plug over each wound (two plugs per fruit). As a control, two sterile 6-mm-diameter

UCV8A plugs were inoculated onto one (pepper, zucchini) or two (cucumber, tomato) fruits for each experiment to ensure that fruits were not infected with *P. capsici* at the onset of the experiment. For all experiments, the agar plug was covered with a 12 mm diameter screw cap (Axygen Scientific, Union City, CA) that was affixed to the fruit surface using petroleum jelly. Fruits were placed in clear polystyrene boxes with lids (23 x 10 x 32 cm, Potomac Display, Hampstead, MD), which contained wet paper towels to maintain approximately 100% relative humidity and incubated at room temperature (~25 ± 2°C) for three (cucumber, zucchini) or four (tomato, pepper) days under constant fluorescent lighting before measuring isolate virulence. The experiment was conducted twice for each host type.

To estimate virulence, two perpendicular measurements were made for each fruit for the both the lesion diameter and visible pathogen growth diameter, and the sporulation density was visually rated (0=none, 1=light, 2=heavy sporulation) using stereomicroscopy to visualize individual sporangia (Leica M165C, Wetzlar, Germany). Three tissue sections were excised from under the point of inoculation on control fruits and plated onto BARP-amended UCV8A to confirm that fruits were not infected with *P. capsici* at the onset of the experiment. For statistical analyses, isolates were grouped by predominant genetic cluster membership, host family of origin, and geographic origin, resulting in an unequal number of replicates per group (see Tables 3, 4, 5).

Data analysis. All statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Inc., Cary, NC). *Phytophthora tropicalis* and isolates with an intermediate genotype were removed from the dataset prior to statistical analysis leaving only *P. capsici sensu stricto* isolates. For each host (cucumber, zucchini, tomato, and pepper), data were subjected to analysis of variance ($P \leq 0.05$) using PROC MIXED, and Tukey's HSD was used for separation of means ($P \leq 0.05$) to account for unequal sample sizes. Isolates were grouped by predominant genetic cluster membership, host family of origin, and geographic origin; these were the main factors in ANOVA analyses. Isolate and run were considered random factors. The contrast option in PROC MIXED was used to compare vegetable (Cucurbitaceae, Fabaceae and Solanaceae) versus tropical (Sterculiaceae, Proteaceae, and Piperaceae) host family of origin ($P \leq 0.05$). Eight isolates that were highly admixed (did not have predominant membership in any one genetic cluster) were removed prior to statistical analyses with genetic cluster as the main effect, but these isolates were included in the other analyses. Due to unequal and sometimes small sample sizes in the various categories, the KENWARDROGER option was specified in the model to calculate appropriate error degrees of freedom (Kenward and Roger 1997).

Results

When the entire collection of isolates was used, most of the isolates were pathogenic to cucumber (137 of 140), tomato (131 of 140), and zucchini (131 of 140) and all of the isolates were pathogenic to pepper (Table 2). Only one or two of 126 *P. capsici sensu stricto* isolates were nonpathogenic to a particular host (Table 2). The type culture for *P. capsici*, 13692 (ATCC.15399, New Mexico), was pathogenic to all fruits tested (data not shown). All nine *P. tropicalis* isolates (13377, 13384, 13385, 13398, 13602, 13603, 13607, 13608, 13609) included in this study were able to cause disease on cucumber and pepper, but only ~50% were able to cause disease on tomato or zucchini. Of the five isolates with an intermediate genotype (13366, 13367, 13368, 13606, 13614), 2 or 3 isolates were nonpathogenic to cucumber, tomato, or zucchini (Table 2). Water-soaked lesions were the main symptom observed; these expanded over time,

Table 2 Differences in pathogenicity between *Phytophthora capsici sensu stricto* and other isolates included in this study

Phytophthora spp.	n[b]	Number of non-pathogenic isolates (% of total)			
		Cucumber	Tomato	Zucchini	Pepper
P. capsici	126	1 (1)	1 (1)	2 (2)	0 (0)
Intermediate[a]	5	2 (40)	3 (60)	3 (60)	0 (0)
P. tropicalis	9	0 (0)	5 (56)	4 (44)	0 (0)

[a] Isolates that were identified as *P. capsici* in some genes and *P. tropicalis* in others

[b] n=total number of isolates used in this study.

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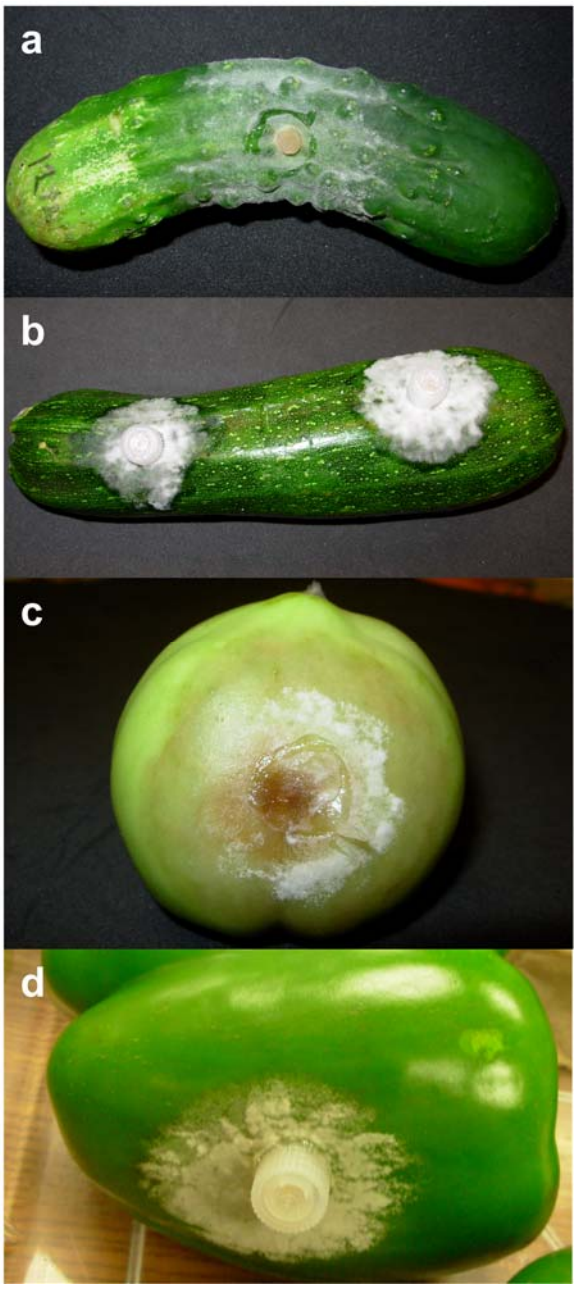

Fig. 1. Symptoms of Phytophthora fruit rot of **a,** pickling cucumber, **b,** zucchini, **c,** tomato, and **d,** pepper caused by *Phytophthora capsici*

and mycelia and sporangia were produced in the lesion (Fig. 1). Differences in virulence were observed between different *P. capsici sensu stricto* isolates with some isolates causing large lesions to form (Fig. 2) and others being nonpathogenic to a particular host (Table 2).

When isolates were grouped by host family of origin, differences in mean lesion diameter were not observed on cucumber (P=0.0757), zucchini (P=0.1858), and pepper (P=0.9995), but differences were noted on tomato (P=0.0041) with fabaceous and solanaceous isolates resulting in larger lesions than the isolate from a sterculiaceous host (Table 3).

When isolates were incubated at 32°C for five days on UCV8A, the sterculiaceous isolate grew to the edge of a 4.6 cm diameter Petri dish; isolates from fabaceous and solanaceous hosts had growth diameters ranging from 1.2 to 4.6 cm (data not shown). Differences in mean pathogen growth diameter were observed on zucchini (P=0.0310), but not on the other host fruits (P>0.0493) when isolates were grouped by host family of origin (Table 3). Differences in mean sporulation density were apparent on cucumber (P=0.0026) and pepper (P=0.0282) fruits, but not on the other host fruits (P>0.0269) by host family. Differences in sporulation density in culture were not apparent by host family for these isolates (Granke et al. 2011), but sporulation density in culture was correlated with sporulation density on host fruits in this study (data not shown, Pearson's correlation, P=0.05). On cucumber, solanaceous isolates resulted in less sporulation than fabaceous isolates (Table 3). On pepper, isolates from cucurbitaceous and solanaceous hosts sporulated better than isolates from piperaceous hosts (Table 3). When isolates from vegetable hosts were compared with isolates from tropical hosts, isolates from vegetable hosts resulted in significantly larger lesions, pathogen growth diameter, and sporulation density on tomato (P<0.0321). Isolates from tropical hosts sporulated less in culture than isolates from vegetable crops (data not shown). Vegetable isolates resulted in greater sporulation on zucchini (P=0.0333), but not in larger lesions (P=0.2100) or more pathogen growth (P=0.0808). Differences between vegetable and tropical isolates were not apparent on cucumber (P>0.0727) and pepper (P=0.0503).

When isolates were grouped by continent of origin, differences in mean lesion diameter were observed on tomato (P=0.0075), with Asian and S. American isolates causing larger lesions than African isolates (Table 4). No differences in mean lesion diameter were observed on the other host fruits

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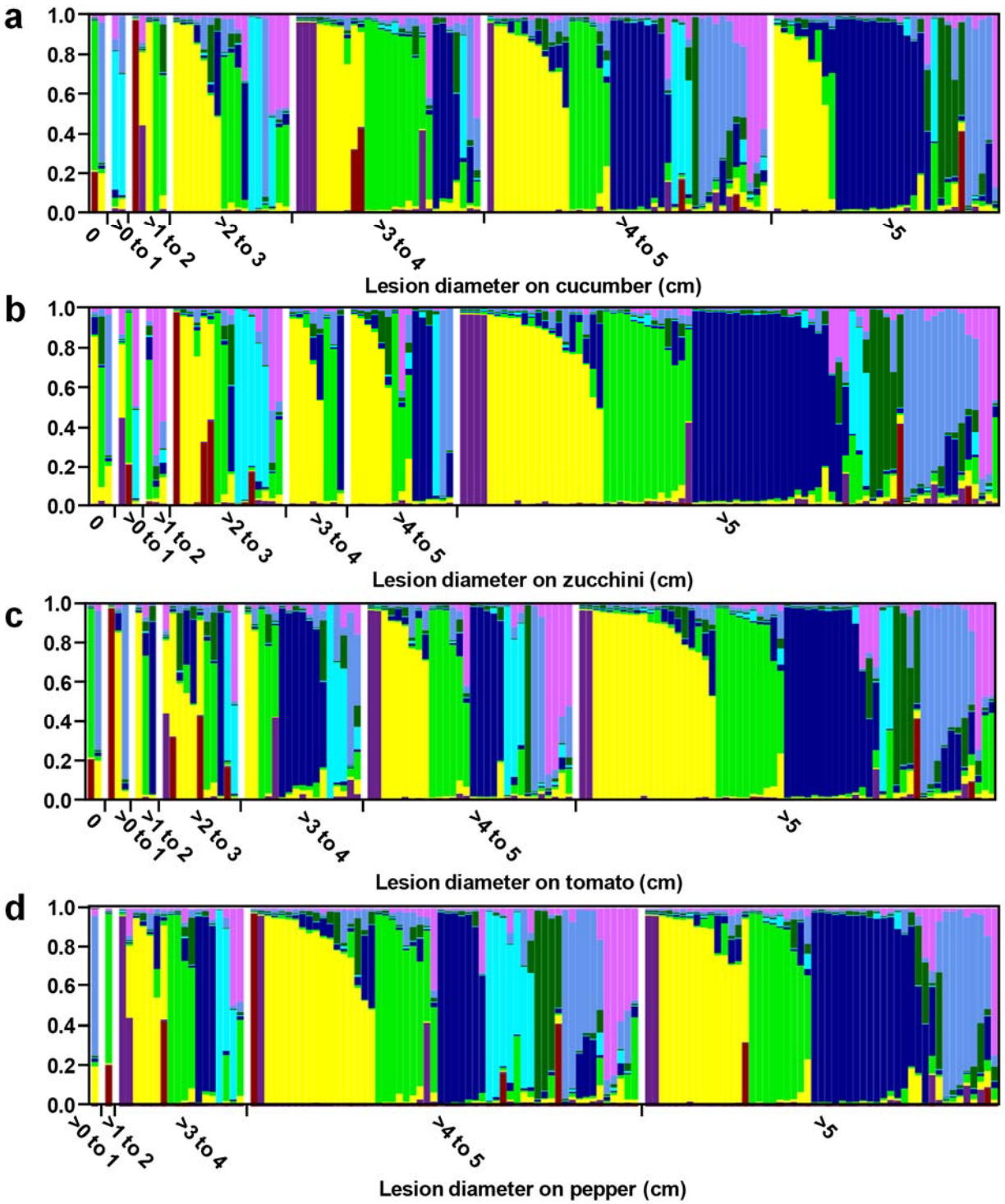

Fig. 2 Population structure of *Phytophthora capsici* isolates grouped by virulence (mean lesion diameter) on **a,** cucumber, b, zucchini, **c,** tomato, and **d,** pepper. Each bar represents a single *P. capsici* isolate, which may be partitioned into colored segments representing an individual's 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

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Table 3 Differences in virulence between *Phytophthora capsici* isolates with diverse host families of origin

Host family	Cucumber	Zucchini	Tomato	Pepper
Mean lesion diameter (cm ± standard deviation)[a]				
Fabaceae (12)	5.0 ± 0.2	5.7 ± 1.2	4.9 ± 1.5 a	5.4 ± 0.7
Cucurbitaceae (46)	4.3 ± 0.2	5.1 ± 1.7	4.6 ± 1.8 ab	4.8 ± 0.9
Solanaceae (64)	3.7 ± 0.2	4.6 ± 2.2	4.8 ± 1.7 a	4.7 ± 1.0
Piperaceae (2)	3.2 ± 0.6	2.6 ± 3.1	2.1 ± 1.7 ab	4.5 ± 2.2
Proteaceae (1)	4.6 ± 0.4	2.7 ± 3.9	2.7 ± 0.4 ab	4.5 ± 1.3
Sterculiaceae (1)	1.6 ± 1.6	2.9 ± 4.1	0.7 ± 1.0 b	4.4 ± 0.7
P-value	0.0757	0.1858	0.0041	0.9995
Pathogen growth diameter (cm ± standard deviation)[a]				
Fabaceae (12)	2.5 ± 1.6	4.7 ± 1.1 a	3.1 ± 1.8	3.7 ± 1.0
Cucurbitaceae (46)	2.1 ± 1.6	4.1 ± 1.7 ab	2.5 ± 1.9	3.4 ± 1.3
Solanaceae (64)	1.5 ± 1.5	3.7 ± 2.0 ab	2.8 ± 1.8	3.6 ± 1.1
Piperaceae (2)	0.8 ± 1.5	1.2 ± 1.8 b	0.9 ± 1.1	2.8 ± 1.9
Proteaceae (1)	2.5 ± 1.1	1.9 ± 2.6 ab	0.0 ± 0.0	1.9 ± 0.9
Sterculiaceae (1)	0.0 ± 0.0	2.0 ± 2.8 ab	0.0 ± 0.0	2.0 ± 0.3
P-value	0.0683	0.0310	0.0493	0.2789
Sporulation density (0 to 2 scale)[ab]				
Fabaceae (12)	1.6 ± 0.6 a	0.9 ± 0.8	1.3 ± 0.7	1.5 ± 0.6 ab
Cucurbitaceae (46)	1.3 ± 0.8 ab	0.8 ± 0.7	1.1 ± 0.8	1.5 ± 0.6 a
Solanaceae (64)	0.9 ± 0.8 b	0.6 ± 0.7	1.2 ± 0.8	1.6 ± 0.7 a
Piperaceae (2)	0.5 ± 1.0 ab	0.1 ± 0.3	0.3 ± 0.5	0.3 ± 0.3 b
Proteaceae (1)	0.8 ± 0.4 ab	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7 ab
Sterculiaceae (1)	0.0 ± 0.0 ab	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4 ab
P-value	0.0026	0.0839	0.0269	0.0282

[a] Means with no letter or that share letters are not significantly different (Tukey's HSD, $P<0.05$)
[b] Visual rating scale: 0=no sporulation, 1=light sporulation, 2=heavy sporulation

($P>0.0945$) when isolates were grouped by continent of origin. Differences in pathogen growth and sporulation density were observed on cucumber ($P<0.0012$), but not on the other host fruits ($P>0.0358$) when isolates were grouped by continent of origin. Isolates from N. America grew more and sporulated better on cucumber than isolates from S. America (Table 4). Isolates from N. America also sporulated better in culture than isolates from S. America in a previous study that used the same isolates (Granke et al. 2011).

When isolates were grouped by predominant genetic cluster, differences in mean lesion diameter were observed on cucumber ($P=0.0018$) and zucchini fruits ($P=0.0212$), but not on tomato ($P=0.4542$) and pepper fruits ($P=0.5888$) (Table 5). Differences in growth diameter of isolates on UCV8A after five days incubation at 32°C were not apparent by genetic cluster in a previous study using the same isolates (Granke et al. 2011). Additionally, differences in pathogen growth ($P<0.0001$) and sporulation ($P<0.0001$) were noted on cucumber, but not on the other hosts ($P>0.1538$). On zucchini, isolates from cluster five resulted in larger lesions than isolates from cluster six. On cucumber, isolates from clusters five and seven resulted in larger lesions than isolates from clusters four and six. Isolates from clusters five, seven, and eight had larger mean pathogen growth diameters than isolates from cluster four on cucumber. In addition, isolates from cluster five sporulated more heavily on cucumbers than isolates from clusters two and four (Table 5).

Some population structure was apparent by virulence, with isolates from some genetic clusters resulting more frequently in large lesions, but isolate membership in a particular genetic cluster did not directly correspond with isolate virulence (Fig. 2). The only isolate belonging predominantly to cluster

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Table 4 Differences in mean lesion diameter (cm, ± standard error) between *Phytophthora capsici* isolates with diverse geographic origins

Continent	Cucumber[a]	Zucchini[a]	Tomato[a]	Pepper[a]
Mean lesion diameter (cm ± standard deviation)[a]				
N. America (93)	4.2 ± 1.7	4.9 ± 2.0	4.5 ± 1.8 ab	4.8 ± 0.9
Asia (18)	3.8 ± 1.4	4.6 ± 2.2	5.3 ± 1.6 a	4.9 ± 1.1
S. America (6)	3.1 ± 0.7	5.3 ± 1.0	5.4 ± 0.8 a	5.4 ± 0.8
Europe (8)	3.1 ± 1.9	3.8 ± 2.5	4.6 ± 1.5 ab	4.2 ± 1.2
Africa (1)	1.6 ± 2.3	2.9 ± 4.1	0.7 ± 1.0 b	4.4 ± 0.7
P-value	0.0945	0.3872	0.0075	0.1851
Pathogen growth diameter (cm ± standard deviation)[a]				
N. America (93)	2.0 ± 1.6 a	4.0 ± 1.8	2.5 ± 1.9	3.4 ± 1.1
Asia (18)	1.4 ± 1.4 ab	3.5 ± 2.1	3.4 ± 1.6	3.9 ± 1.2
S. America (6)	0.0 ± 0.0 b	4.0 ± 1.8	3.2 ± 1.5	4.0 ± 1.0
Europe (8)	1.1 ± 1.5 ab	2.9 ± 2.2	2.4 ± 1.9	3.3 ± 1.2
Africa (1)	0.0 ± 0.0 ab	2.0 ± 2.8	0.0 ± 0.0	2.0 ± 0.3
P-value	0.0012	0.2282	0.0358	0.1035
Sporulation density (0 to 2 scale)[ab]				
N. America (93)	1.2 ± 0.8 a	0.7 ± 0.7	1.0 ± 0.8	1.5 ± 0.6
Asia (18)	0.9 ± 0.8 ab	0.5 ± 0.6	1.4 ± 0.7	1.5 ± 0.7
S. America (6)	0.0 ± 0.1 b	0.7 ± 0.7	1.2 ± 0.8	1.5 ± 0.8
Europe (8)	0.8 ± 0.9 ab	0.5 ± 0.7	1.0 ± 0.8	1.5 ± 0.8
Africa (1)	0.0 ± 0.0 ab	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4
P-value	0.0001	0.2092	0.0408	0.7277

[a] Means with no letter or that share letters are not significantly different (Tukey's HSD, $P<0.05$)
[b] Visual rating scale: 0=no sporulation, 1=light sporulation, 2=heavy sporulation

one was weakly virulent, causing lesions with an average diameter ≤2 cm on cucumber and tomato, and was moderately virulent on zucchini and pepper. The cluster one isolate did not sporulate on host fruits. Isolates belonging predominantly to cluster two were moderately virulent on cucumber, moderately to highly virulent on tomato, highly virulent on zucchini, and low, moderately, and highly virulent to pepper. Cluster three isolates were distributed fairly uniformly, resulting in small to large lesions on host fruits, depending on the isolate (Fig. 2). Isolates belonging to cluster four were low to moderately virulent on cucumber, variable on tomato, and moderately to highly virulent on zucchini and pepper. Isolates from cluster five were moderately to highly virulent on all host fruits tested. Cluster six isolates were moderately to highly virulent on zucchini, tomato, and pepper, and variable on cucumber. Isolates belonging to clusters seven and eight were moderately to highly virulent on all hosts (Fig. 2). Cluster nine isolates were variable on all of the host fruits, with some isolates being weakly virulent and others highly virulent.

Discussion

P. capsici continues to threaten vegetable production in major growing regions of the United States and elsewhere. Challenges to management of *P. capsici* include long-term survival of the pathogen as oospores in the soil (Lamour and Hausbeck 2003), a limited selection of fungicides that provide effective control (Hausbeck and Lamour 2004), the presence of fungicide resistant pathogen populations (Lamour and Hausbeck 2000), and a lack of commercially acceptable resistant varieties (Thabuis et al. 2004, Thabuis et al. 2003). To develop host varieties that are effective in multiple regions, it is key to use a selection of *P. capsici* isolates that represent the phenotypic, genotypic, and virulence diversity of the pathogen. It has been previously observed that isolates of *P. capsici* may differ in

Table 5 Differences in mean lesion diameter (cm, ± standard error) between *Phytophthora capsici* isolates by predominant genetic cluster of membership

GC (n)[b]	Cucumber[a]	Zucchini[a]	Tomato[a]	Pepper[a]
Mean lesion diameter (cm ± standard deviation)[a]				
1 (1)	1.6 ± 2.3 ab	2.9 ± 4.1 ab	0.7 ± 1.0	1.6 ± 2.3
2 (4)	3.9 ± 1.1 ab	5.3 ± 1.1 ab	5.0 ± 0.9	3.9 ± 1.1
3 (33)	4.2 ± 1.6 ab	4.7 ± 2.1 ab	4.8 ± 1.8	4.2 ± 1.6
4 (20)	3.3 ± 1.5 b	4.6 ± 2.2 ab	4.4 ± 2.0	3.3 ± 1.5
5 (26)	4.9 ± 1.3 a	5.5 ± 1.5 a	4.7 ± 1.6	4.9 ± 1.3
6 (9)	3.2 ± 1.8 b	3.6 ± 2.4 b	4.4 ± 1.4	3.2 ± 1.8
7 (4)	5.5 ± 0.8 a	5.8 ± 0.8 ab	5.4 ± 0.5	5.5 ± 0.8
8 (13)	4.7 ± 1.2 ab	5.2 ± 1.8 ab	4.4 ± 2.3	4.7 ± 1.2
9 (8)	3.7 ± 1.8 ab	3.5 ± 2.6 ab	4.7 ± 1.5	3.7 ± 1.8
P-value	0.0018	0.0212	0.4542	0.5888
Pathogen growth diameter (cm ± standard deviation)[a]				
1 (1)	0.0 ± 0.0 ab	2.0 ± 2.8	0.0 ± 0.0	0.0 ± 0.0
2 (4)	0.9 ± 1.5 ab	4.2 ± 0.7	2.6 ± 1.7	0.9 ± 1.5
3 (33)	1.9 ± 1.6 ab	3.7 ± 2.0	2.9 ± 1.8	1.9 ± 1.6
4 (20)	0.9 ± 1.1 b	3.6 ± 1.9	2.4 ± 2.0	0.9 ± 1.1
5 (26)	2.5 ± 1.6 a	4.5 ± 1.5	2.6 ± 1.9	2.5 ± 1.6
6 (9)	1.3 ± 1.4 ab	2.9 ± 2.0	2.2 ± 1.6	1.3 ± 1.4
7 (4)	3.6 ± 0.9 a	4.7 ± 0.6	3.5 ± 0.9	3.6 ± 0.9
8 (13)	2.5 ± 1.3 a	4.3 ± 1.5	2.9 ± 2.0	2.5 ± 1.3
9 (8)	1.3 ± 1.4 ab	2.8 ± 2.4	2.2 ± 1.9	1.3 ± 1.4
P-value	<0.0001	0.0138	0.4983	0.8289
Sporulation density (0 to 2 scale)[ab]				
1 (1)	0.0 ± 0.0 abc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2 (4)	0.4 ± 0.5 c	0.6 ± 0.5	1.2 ± 0.9	0.4 ± 0.5
3 (33)	1.2 ± 0.8 abc	0.6 ± 0.6	1.2 ± 0.8	1.2 ± 0.8
4 (20)	0.8 ± 0.7 c	0.8 ± 0.8	0.9 ± 0.9	0.8 ± 0.7
5 (26)	1.5 ± 0.7 a	0.7 ± 0.7	1.1 ± 0.8	1.5 ± 0.7
6 (9)	0.8 ± 0.8 bc	0.5 ± 0.7	0.9 ± 0.8	0.8 ± 0.8
7 (4)	1.9 ± 0.4 ab	0.9 ± 0.7	1.4 ± 0.4	1.9 ± 0.4
8 (13)	1.5 ± 0.7 ab	0.9 ± 0.8	1.2 ± 0.8	1.5 ± 0.7
9 (8)	0.8 ± 0.8 abc	0.3 ± 0.5	0.9 ± 0.8	0.8 ± 0.8
P-value	<0.0001	0.1538	0.4157	0.8788

[a] Means with no letter or that share letters are not significantly different (Tukey's HSD, $P<0.05$)
[b] Isolates that were highly admixed (n=8) were removed from data sets prior to analyses
[c] Visual rating scale: 0=no sporulation, 1=light sporulation, 2=heavy sporulation

virulence (Foster and Hausbeck 2010, Lee et al. 2001, Quesada-Ocampo and Hausbeck 2010, Ristaino 1990) and pathogenicity (Tamietti and Valentino 2001) on some, but not all (Quesada-Ocampo et al. 2009, Gevens et al. 2006) hosts. In our study, which used 126 *P. capsici* isolates originating from 12 countries and six host families including vegetable crops and tropical hosts, variations in virulence were noted between isolates on cucumber, tomato, zucchini, and pepper fruits. While isolates from some hosts of origin and genetic clusters were on average more virulent than others, the range of virulence of isolates within a category suggest that these characteristics cannot be used alone to guide isolate selection. Instead, isolates should be screened for virulence to the host of interest before choosing a subset for robust host resistance screening;

screenings should include isolates from diverse geographic locations and hosts.

In this study, the virulence of 126 *P. capsici sensu stricto* isolates were evaluated, and the pathogenicity of nine *P. tropicalis* and five isolates with an intermediate genotype were tested on cucumber, tomato, zucchini, and pepper fruits. All of the isolates included in this study, including those that were subsequently identified as *P. tropicalis* and intermediate genotypes, were pathogenic to wounded pepper fruits. Interestingly, the two *P. tropicalis* isolates and one intermediate isolate that were not pathogenic to wounded pepper fruits when retrieved from long-term storage were pathogenic after being inoculated onto and retrieved from cucumber fruits. This underscores the importance of passing isolates through host tissue prior to beginning a disease resistance screen, as isolate pathogenicity and virulence may be affected by repeated sub-culturing and storage. It has been previously suggested that other species such *Phytophthora parasitica* var. *nicotianae* lose virulence in culturing (Apple 1957), and it has been noted that some *P. capsici* isolates that have been sub-cultured for many years are nonpathogenic or weakly virulent (Aragaki and Uchida 2001, Kim and Hwang 1992, Lee et al. 2001, Polach and Webster 1972). Since at least some *P. tropicalis* and intermediate isolates were pathogenic to each of the hosts included in this study, pathogenicity to cucumber, tomato, zucchini, or pepper fruits should not be used to separate *P. tropicalis* from *P. capsici* isolates.

The *P. capsici* standard isolate 13692 (ATCC.15399), which was originally isolated from pepper in New Mexico, was included in our study. This isolate was moderately virulent to cucumber, zucchini and pepper fruits, causing on average lesions >2.5 cm diameter. On tomato fruits, isolate 13692 was highly virulent resulting in a mean lesion diameter >5 cm. When Polach and Webster tested the pathogenicity of this isolate in 1972, it was not pathogenic to any of the solanaceous and cucurbitaceous plants tested (Polach and Webster 1972). Aragaki and Uchida (2001) found this isolate to be nonpathogenic to moderately pathogenic on pepper plants in previous studies. In a study by Kim and Hwang (1992), 13692 was moderately virulent, with eight isolates tested being more virulent and four being less virulent than 13692 to pepper. Isolate 13692 was the second least virulent isolate in a screening by Ristaino (1990) that included 11 other isolates from pepper and cucurbit hosts. The differences in virulence observed for this isolate could be due to dissimilar storage conditions, inoculation technique, environmental conditions, or host type among experiments.

Isolates 13349 (SP98), 101 (OP97), and 12889 have been used as standard virulent isolates, and isolate 455 (SFF3) has been used as a weakly virulent standard in previous experiments in the Hausbeck lab on host fruits and roots (Foster and Hausbeck 2010, Quesada-Ocampo and Hausbeck 2010). In the current study, isolate 455 was the least virulent of these four isolates on all of the host fruits; it was moderately virulent on cucumber, zucchini, and pepper and weakly virulent on tomato (lesions<2 cm diameter). Isolate 12889 was highly virulent on cucumber, zucchini, and pepper and moderately virulent on tomato. Isolate 13349 was highly virulent to cucumber, zucchini, and tomato, and moderately virulent to pepper. Isolate 101 was highly virulent on zucchini and tomato fruits and moderately virulent on cucumber and pepper fruits. A previous study (Gevens et al. 2006) found no significant difference in virulence among isolates 101, 455, and 13349 on mature cucumber fruits, in disagreement with the results of our study with immature pickling cucumber fruits. Although the inoculation method was similar to that used in our study, the use of commercially mature fruits and multiple cultigens in the previous study versus the younger, more susceptible (Gevens et al. 2006) fruits in this study may account for the differences observed. While we observed similar lesion diameters on cucumbers inoculated with isolates 101 and 13349 as those reported by Gevens et al. (2006), the lesion diameter for isolate 455 was much smaller in our study than in previous work. It is possible that isolate 455 may have lost virulence in storage since it was isolated from cucumber in 1998. A previous study in our lab on pepper fruit (Foster and Hausbeck 2010) found that isolates 12289 and 13349 were more virulent to unwounded pepper fruit inoculated with a zoospore suspension than isolate 101. We saw the same general trend in our study.

Currently, most host resistance screenings use isolates collected from cucurbitaceous and

solanaceous hosts (Foster and Hausbeck 2010, Quesada-Ocampo and Hausbeck 2010, Gevens et al. 2006, Lee et al. 2001). On tomato, differences in lesion diameter were observed by host family of origin in our study, and isolates from vegetable hosts were more virulent than isolates from tropical hosts. A gradation in isolate virulence was observed with some isolates causing larger lesions than others. Similarly, a previous study using 10 Korean tomato cultivars and seven *P. capsici* isolates found significant differences between isolate virulence; these isolates were either from pepper in Korea or from unknown hosts elsewhere (Kim and Hwang 1992). Furthermore, in a host resistance screen of tomato and wild relatives for Phytophthora root rot, differences in isolate virulence were observed between the four isolates used (Quesada-Ocampo and Hausbeck 2010). These same isolates were included in our study, and isolate 455 (SFF3) was the least virulent of the four in both the previous and current study.

Isolates within each host family of origin varied in virulence on zucchini and cucumber fruits, and categories that included more isolates (i.e. Cucurbitaceae and Solanaceae) showed the greatest range of virulence response. Lee et al. (2001) found that pumpkin isolates were generally more virulent on pumpkin than pepper isolates; however, the second most virulent isolate in their study was from pepper. Since differences in virulence on cucurbit fruits were observed by host of origin in our study, breeding programs for cucurbit fruit resistance should include isolates from diverse hosts of origin for resistance screening. Isolates collected from fabaceous hosts were highly virulent on all host fruits tested in this study, suggesting that isolates from fabaceous hosts should be incorporated into host resistance screenings. Some (5 of 12) of the isolates originating from fabaceous hosts were not as virulent on cucumber as the highly virulent isolates 12889 and 13349 (SP98), nor was isolate 101 (OP97). On zucchini and pepper, most of the bean isolates were as virulent as the standard isolates 101, 12889, and 13349. Most of the bean isolates (11 of 12) were as virulent to tomato as the standards as well. Interestingly, the Leonian isolate (13692) was as virulent as the standard highly virulent isolates (101, 12889, and 13349) on pepper and tomato, but not on cucumber and zucchini. While only five isolates originating from tropical hosts were included in this study, isolates collected from vegetable hosts were generally more virulent on zucchini and tomato fruits than isolates collected from tropical hosts. This may be due to sparse sampling of isolates from tropical hosts, or isolates recovered from tropical host tissue may be less virulent to vegetable host fruits generally. Future studies are needed to clarify if isolates retrieved from tropical hosts are generally less virulent than isolates retrieved from vegetable hosts.

Isolate membership in a particular genetic cluster did not directly correspond with isolate virulence on host fruits. A previous study by Islam et al. (2004) found a direct correspondence between 24 isolates grouped by virulence to processing pumpkin and RAPD groups. In our study, the relationship between genetic cluster membership and virulence was not as clear; this is likely due to the large number of isolates included, the continuum of lesion size over the collection of isolates, the susceptibility of cucumber, zucchini, tomato, and pepper fruits, and unequal sample sizes for each genetic cluster. However, some clusters were more likely to yield virulent isolates than others. For instance, all of the isolates from cluster two, five, and seven were moderately to highly virulent on cucurbit fruits. The only isolate from cluster one included in this study was weakly to moderately virulent on all of the hosts. Within clusters three, four, six, eight, and nine, a continuum of virulence was noted with some isolates causing small lesions to form and others causing large lesions to form on cucumber and zucchini fruits. Genetic clustering in this study was performed using neutral genes, and it is possible that a similar study using genes associated with virulence could result in clustering more predictive of isolate virulence. Since host selection is a strong evolutionary force shaping pathogen populations (Zhan et al. 2002, Montarry et al. 2006), it is likely that virulent isolates will contain allelic variants of virulence genes that allow them to cause disease in a particular host. Population studies that associate particular virulence genes to virulence and/or pathogenicity to a particular host, and take into account the population structure detected in *P. capsici* are needed to understand the genetic basis of virulence and pathogenicity. Furthermore, the

current study looked at virulence on host fruits. It is likely that virulence to other host tissue, especially root tissue, may show differences in isolate pathogenicity and virulence.

Thus, isolate genetic cluster membership may be used to guide initial isolate selection for host resistance screenings for cucumber and zucchini. Isolate host family of origin may also be used to guide isolate selection for host fruits. Isolates from fabaceous hosts have not been included in previous host screens to our knowledge, but may represent a source of highly virulent isolates for host fruit resistance screening. However, as variation exists among isolates within host family of origin and genetic cluster categories, preliminary virulence tests to the host of interest should be completed to guide final isolate selection for robust host resistance screening.

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