Survival and Spread of *Phytophthora capsici* in Coastal Peru

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Accepted for publication 11 February 2008.

ABSTRACT

Hurtado-Gonzales, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R., and Lamour, K. 2008. Survival and spread of *Phytophthora capsici* in coastal Peru. *Phytopathology* 98:688-694.

Phytophthora capsici is a soilborne pathogen that causes significant losses to pepper production in Peru. Our objective was to investigate the mechanisms by which *P. capsici* is able to survive and spread. During 2005 to 2007, 227 isolates of *P. capsici* were collected from four species of pepper (*Capsicum annum*, *C. baccatum*, *C. chinense*, and *C. pubescens*) and tomato (*Solanum lycopersicum*) at 33 field sites in 13 provinces across coastal Peru. All 227 isolates were of the A2 mating type and

amplified fragment length polymorphism (AFLP) analysis indicates that 221 of the isolates had the same genotype. Analyses of six polymorphic single nucleotide polymorphism (SNP) loci showed fixed heterozygosity suggesting a single clonal lineage is widely dispersed. Members of the same clonal lineage were recovered during 2005 to 2007 from geographically separate locations from each of the host types sampled. Our results indicate that clonal reproduction drives the population structure of *P. capsici* in Peru. The impact of continuous cropping and irrigation from common river sources on the population structure in Barranca Valley are discussed.

Phytophthora capsici Leonian is a soilborne plant pathogen which causes fruit, crown, and root rot in a wide range of vegetable hosts including peppers, tomato, squash, melons, and most recently green and lima beans (5,9,30,35,60). *P. capsici* was first described as a pathogen of pepper in New Mexico (42). Since the initial species description, *P. capsici* has been reported on additional hosts (18,28,29,32–34,44,46,50,58,68,72). *P. capsici* can produce massive amounts of asexual deciduous sporangia on infected host and large scale epidemics can be initiated from a limited number of infected plants (62). Unlike many other *Phytophthora* diseases that are favored by cooler temperatures, disease is favored by warm (25 to 28°C) and wet conditions (9). *P. capsici* is heterothallic requiring the interaction of two mating types (A1 and A2) to complete the sexual stage and produce thick-walled sexual oospores. Oospores are capable of surviving in the soil for several years, germinating in favorable environmental conditions (9,15,49,61). In the United States, both mating types are found at most locations and oospores are thought to play an important role in the epidemiology (37–40,58). In addition, *P. capsici* has been isolated from creeks used for irrigation of vegetables in Michigan and it has been shown to infect diverse weeds (16,19,56). In many cases crop rotation has not reduced the residual inoculum sufficiently to provide effective protection (41).

In Peru, *P. capsici* was first reported as the causal agent for wilt and root rot infecting several *Capsicum* spp. in 1971 (10). Since then, no other investigation has been conducted despite increasing losses due to *P. capsici* for pepper growers in Peru. Various practices are applied to control the epidemics, such as moderate irrigation, the use of well-drained fields, crop rotation, and fungicide treatments (3,48). The Andean region of Peru-Bolivia is considered one of the centers of origin for species within the genus *Capsicum*. Archeological studies in coastal Peru have revealed evidence for the presence of *Capsicum* spp. dating back

4,000 years (54). It is not known how long *P. capsici* has been present in and around the *Capsicum* center of origin, nor is it known whether *P. capsici* infected other ancient local crops, such as cucurbits, prior to *Capsicum* (6). Several varieties of *C. annum*, as well as other species of *Capsicum* such as *C. baccatum*, *C. chinense*, and *C. pubescens* are cultivated across the coastal area of the country and several other *Capsicum* spp. are grown in the Amazon (55). Thus, a high diversity of the host may impact the pathogen population structure and evolution (73). A better understanding of the mechanisms by which *P. capsici* is surviving and spreading will assist in developing effective management and breeding strategies.

Our primary objective was to characterize the survival and spread of *P. capsici* in pepper production areas of coastal Peru. We report on the absence of diversity in *P. capsici* recovered from peppers and tomatoes from across coastal Peru for mating type, amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNP) markers, and we present a fine scale genotypic analysis of *P. capsici* recovered from pepper in the Barranca valley.

MATERIALS AND METHODS

Isolate recovery and sampling scheme. Samples collected in 2005 (n = 23) were received as standard diagnostic specimens and processed by the diagnostic clinic at the National Agricultural University, La Molina (Lima, Peru). Isolates were recovered from seven different hosts at 23 field sites (Fig. 1 and Table 1). During 2006 to 2007 a more intensive sampling was performed at 10 pepper fields located in Barranca, a coastal province 120 m north of the city of Lima. The fields spanned four districts: Barranca, Supe, Caral, and Minas (Fig. 2; Table 2). Fields were previously cultivated with the following hosts: tomato, potato, paprika, and/or artichoke. Fields varied in size from 3 to 10 ha and isolates were recovered from the following *Capsicum* species: *C. annum*, *C. baccatum*, *C. chinense*, and *C. pubescens*. Plants varied in age from 3 weeks-old to 3 months old and wilting was the primary symptom of infected plants. Adjacent plants within a field were avoided. All of the isolates were recovered from the crown region

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doi:10.1094/PHYTO-98-6-0688

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of infected plants. A minimum of 12 symptomatic plants were collected per field and transported to Lima for pathogen isolation at the diagnostic clinic (National Agricultural University, La Molina). *P. capsici* culture collection isolates CBS121656 (iso-

lated from infected cucumbers in Michigan 1997) and CBS121657 (isolated from infected pumpkins in Tennessee 2004) were also included in the study.

For pathogen isolation, infected plants were thoroughly washed with tap and sterile distilled water. Small sections of tissue were then excised from the edge of expanding lesion at the crown level and transferred to potato dextrose agar plates amended with PARP (100 ppm of pimaricin, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene). Plates were incubated for 2 days at room temperature. Hyphal tips were sub-cultured from actively expanding mycelium and transferred to V8-juice agar amended with PARP. For long-term storage, agar plugs of mycelium were stored in 2 ml screw cap tubes with 1 ml of sterile distilled water and three sterile hemp seeds.

To determine the mating type of *P. capsici* isolates, plugs of actively expanding mycelium were placed at the center of V8-juice agar plates approximately 2 cm away from "tester" isolate CBS121656 (mating type A1) or CBS121657 (mating type A2). Plates were wrapped with Parafilm and incubated in the dark at room temperature for at least 1 week, after which observations were made for the production of oospores at the interface using a light microscope. Isolates able to produce oospores when crossed against CBS121656 were determined as A2 mating type. Conversely, isolates that were able to form oospores when crossed against CBS121657 were determined as A1 mating type.

DNA isolation and AFLP analyses. To determine genetic similarity among *P. capsici* isolates, AFLP profiles were generated for all 227 isolates. Isolates were grown and treated as previously described for DNA isolation (36). AFLP genotyping was done using *EcoRI* and *MseI* restriction endonucleases, adaptors, and primers as described by Vos et al. (70). Preselective amplification was done using no selective nucleotides (Eco+0/Mse+0) and selective amplification was conducted with selective primer pair (Eco+CG/Mse+CG). Selective amplifications were diluted and labeled in a separate reaction according to Habera et al. (25). AFLP fragments were resolved and analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter) following the manufacturer's protocols. A 600 bp DNA size standard was used to resolve AFLP fragments (Beckman Coulter). AFLP marker sizes ranged from 70 to 500 bp. AFLP profiles were generated twice for each isolate using independent DNA extractions. A binary matrix was constructed using only clearly resolved,

Fig. 1. Geographic locations for *Phytophthora capsici* isolates collected in Peru. Superscript *a* following the location identifier indicates that isolates from clonal lineage PcPE-1 were present, *b* indicates isolates from PcPE-2 were present, and *c* indicates the location of LT2145.

TABLE 1. *Phytophthora capsici* isolates collected during 2005 from pepper and tomato in Peru

Isolate	Isolation date	MT ^a	Origin (Province, State)	Host
LT2135	01/24/05	A2	Viru, La Libertad	<i>Capsicum annuum</i>
LT2136	01/25/05	A2	Huaral, Lima	<i>Solanum lycopersicum</i>
LT2137	01/25/05	A2	Carabayllo, Lima	<i>Solanum lycopersicum</i>
LT2138	01/27/05	A2	Huaral, Lima	<i>Solanum lycopersicum</i>
LT2139	01/27/05	A2	Santa Rosa Irrigation, Lima	<i>Capsicum annuum</i>
LT2140	02/02/05	A2	Santiago Valley, Ica	<i>Solanum lycopersicum</i>
LT2141	02/14/05	A2	Santa Rosa Irrigation, Lima	<i>Capsicum annuum</i>
LT2142	03/08/05	A2	Pampas de Villacuri, Ica	<i>Capsicum annuum</i>
LT2143	04/27/05	A2	Nepeña, Ancash	<i>Capsicum annuum</i>
LT2144	05/04/05	A2	Barranca, Lima	<i>Capsicum chinense</i>
LT2145	05/10/05	A2	Viru, La Libertad	<i>Capsicum annuum</i>
LT2146	05/12/05	A2	Mala, Lima	<i>Capsicum baccatum</i>
LT2147	05/12/05	A2	Mala, Lima	<i>Capsicum baccatum</i>
LT2148	05/31/05	A2	Chinchua, Ica	<i>Capsicum annuum</i>
LT2149	05/31/05	A2	Chinchua, Ica	<i>Capsicum annuum</i>
LT2150	08/17/05	A2	Ica Valley, Ica	<i>Capsicum annuum</i>
LT2151	08/17/05	A2	Ica Valley, Ica	<i>Capsicum annuum</i>
LT2152	09/07/05	A2	Viru, La Libertad	<i>Capsicum annuum</i>
LT2153	10/23/05	A2	Ica Valley, Ica	<i>Solanum lycopersicum</i>
LT2847	06/27/05	A2	Curahuasi, Abancay	<i>Capsicum annuum</i>
LT2848	06/27/05	A2	Curahuasi, Abancay	<i>Capsicum annuum</i>
LT2849	06/27/05	A2	Curahuasi, Abancay	<i>Capsicum annuum</i>
LT2850	09/19/05	A2	Supe, Lima	<i>Capsicum annuum</i>

^a MT = mating type.

replicated markers. Using the program NTSYSpc 2.11a (Exeter Software, Setauket, NY), the combined data matrix was used to construct a genetic similarity matrix of all possible pairwise comparisons of individuals using Jaccard's similarity coefficient: $GS(ij) = a/(a + b + c)$. $GS(ij)$ is the measure of genetic similarity between individuals i and j , where a is the number of polymorphic bands shared by i and j , b is the number of bands present in i and absent in j , and c is the number of bands present in j but absent in i . A dendrogram constructed by employing an unweighted pair-group method with arithmetic average (UPGMA) cluster analysis. Bootstraps values were generated in phylogenetic analysis using parsimony (PAUP) version 4.0b10 with 1,000 replicates (66).

SNP genotyping. Twenty isolates from the Barranca valley (2006 to 2007) and 23 isolates from the diagnostic clinic in 2005 were selected for subsequent analyses using six nuclear SNP

markers (Table 3). The SNP markers were heterozygous in at least one of the samples tested. Custom TaqMan SNP Genotyping Assays (Foster City, CA) were designed according to the manufacturer's instructions (Table 3). SNP assays were performed using 7.5 μ l of iQ Supermix (Bio-Rad), 0.325 μ l of Taqman probe/primers allelic discrimination cocktail (40 $\times$), 2 μ l of DNA ($\approx$ 15 to 20 η g) and 3.2 μ l of molecular biology grade H₂O. Polymerase chain reactions (PCR) were carried out in 96-well plates (Bio-Rad) in triplicates for each isolate on an iQ5 real-time thermal cycler (Bio-Rad) using the following parameters: 95°C for 10 min, 40 cycles of 92°C for 15 s and 60°C for 1 min. Each assay was standardized for their optimal cycling PCR conditions. Results were analyzed with the accompanying iQ5 optical system software 1.0 (Bio-Rad) using the allelic discrimination option, adjusting parameters for cycle threshold (Ct) values and relative fluorescent units (RFU) according to the manufacturer's instructions. Results were scored as allele 1 = 11 (FAM), allele 2 = 22 (VIC), and heterozygous = 12. A concatenated similarity tree with bootstraps (AFLP and SNP) was built as described above.

Fig. 2. Close-up depicting the location of 10 fields sampled in the Barranca Valley. Black circles indicate specific fields.

RESULTS

AFLP genotyping and mating type. AFLP using the Eco+CG/Mse+CG primer pair generated 50 reproducible markers. Four of the 50 alleles identified were only found in the United State's isolates (CBS121656 and CBS121657). Isolates recovered from the Barranca valley in 2005 ($n = 2$) and 2006 to 2007 ($n = 204$, represented by isolate LT3738) were found to exhibit a single AFLP genotype with 35 AFLP markers. Seventeen of the twenty-three isolates submitted to the clinic in 2005 had the LT3738 genotype. Of the remaining clinic isolates, all except one had a different AFLP genotype composed of 45 AFLP markers (represented by isolate LT2137). Representative isolates LT3738 and LT2137 had 34 AFLP markers in common. The last isolate (LT2145) had a unique AFLP genotype consisting of 37 AFLP markers. AFLP genotypes LT3738 and LT2137 were identified as clonal types and are referred to as PcPE-1 and PcPE-2.

TABLE 2. *Phytophthora capsici* isolates collected from peppers at 10 locations in Lima, Barranca province, in 2006 to 2007

Field	No. of isolates	Year	Origin	Host
1	19	2006	Barranca, Lima	<i>Capsicum pubescens</i>
2	18	2006	Supe, Lima	<i>Capsicum chinense</i>
3	12	2006	Supe, Lima	<i>C. baccatum</i> var. <i>pendulum</i>
4	13	2006	Supe, Lima	<i>C. baccatum</i> var. <i>pendulum</i>
5	18	2006	Barranca, Lima	<i>Capsicum annum</i>
6	36	2006	Barranca, Lima	<i>Capsicum annum</i>
7	36	2007	Caral, Lima	<i>Capsicum annum</i>
8	18	2007	Minas, Lima	<i>Capsicum annum</i>
9	14	2007	Minas, Lima	<i>Capsicum annum</i>
10	20	2007	Minas, Lima	<i>Capsicum annum</i>

TABLE 3. Primers and probe sequences used for single nucleotide polymorphism (SNP) genotyping with Taqman assays^a

Locus name	Primer sequence	Internal reporter oligonucleotide (probe) VIC	Internal reporter oligonucleotide (probe) FAM
SNP11	F_5'-AGATTGAGAAGAAGGAACCTGGTCATG-3' R_5'-CCTTCTCACCTGTAACCTTGCTAAT-3'	5'-ACGCACAGT <u>G</u> CCGTAG-3'	5'-ACGCACAGT <u>A</u> CCGTAG-3'
SNP14	F_5'-CGCTGTGTGTCGATAGTGGGAATG-3' R_5'-TCTTCTCTCTGTGTTGCGACTACCA-3'	5'-CTTGAGCT <u>A</u> ATTCTCT-3'	5'-TTGAGCT <u>C</u> ATTCTCT-3'
SNP15	F_5'-TTCCGACGACGAACGAACA-3' R_5'-GTACCGTGCCACGCAGAT-3'	5'-TCTTGCATAC <u>A</u> TTCTGG-3'	5'-TCTTGCATAC <u>G</u> TTCTGG-3'
SNP16	F_5'-GCCAGCATGCTCGATATTGG-3' R_5'-GCAGTCAGGGCCAAGCA-3'	5'-CAATTGCAAC <u>A</u> AGGCAA-3'	5'-CCAATTGCAAC <u>T</u> AGGCAA-3'
SNP20	F_5'-CTCCGCGCGACAACCTC-3' R_5'-GATGATGCTAAGATCGTACCAAAGC-3'	5'-TACTCT <u>G</u> TGGAAATG-3'	5'-CTACTCT <u>A</u> TGGAAATG-3'
URA3	F_5'-CGAAGGACAACGCGAACTTG-3' R_5'-CTGCGTGACGCCATCAAC-3'	5'-TGCTGCG <u>C</u> TCGACTG-3'	5'-TGCTGCA <u>T</u> TCGACTG-3'

^a Underlined nucleotide refers to the assessed SNP. F = forward and R = reverse.

Isolates belonging to the clonal lineage PcPE-1 were recovered from widely dispersed geographic fields (e.g., Ica and Trujillo, Fig. 1) whereas isolates from PcPE-2 were confined to Lima and Ica (Fig. 1). Mating type analysis revealed that all 227 isolates were the A2 mating type.

SNP and genotyping. Analysis of six SNP markers showed a strong correlation with the AFLP analysis. Thirty-four of the thirty-seven isolates identified as the PcPE-1 type using AFLP had SNP profiles identical to PcPE-1, five isolates with the PcPE-

TABLE 4. Summary of single nucleotide polymorphism (SNP) multilocus genotypes for 43 representative isolates of the PcPE-1 and PcPE-2 clonal lineages and the unique isolate LT2145

Locus name/ isolate ID	Clonal lineage				
	PcPE-1			PcPE-2	Unique isolate
	LT3738	LT3726	LT3753	LT2137	LT2145
SNP11	A/A	A/A	A/A	G/A	G/A
SNP14	A/A	A/A	A/A	A/C	A/C
SNP15	A/G	A/A	A/A	A/A	A/G
SNP16	A/T	A/T	A/T	A/T	T/T
SNP20	A/G	A/G	A/G	A/G	A/A
URA3	A/G	A/G	G/G	A/G	A/G
Genotype frequency ^a	0.79	0.05	0.02	0.12	0.02
	N = 34	N = 2	N = 1	N = 5	N = 1

^a N represents the number of isolates identified with a particular SNP genotype configuration.

2 AFLP profile had SNP profiles identical to PcPE-2, and LT2145, which had a unique AFLP profile, also had a unique multi-locus SNP profile (Table 4). Two isolates identified as the PcPE-1 type using AFLP (LT3726 and LT3762) had a single SNP marker, SNP 15, changed from AG to AA (Table 4). Additionally, LT3753, identified as PcPE-1 with AFLP, had two SNP markers changed from hetero- to homozygosity; URA3 changed from AG to GG, and SNP 15 changed from AG to AA (Table 4). Clone correction of the 227 isolates reduced the total number of unique multi-locus genotypes to five, and for all six SNP markers, only two of the three possible genotypes were observed. Due to the small size of the clone corrected data set, Hardy-Weinberg and other population genetic metrics that are based on allele frequencies were not calculated. A UPGMA cluster analysis combining AFLP and SNP markers revealed that the two distinct clusters (PcPE-1 and PcPE-2) shared approximately 70% of the markers (Fig. 3).

DISCUSSION

This is the first large-scale spatiotemporal population study of *P. capsici* infecting peppers and tomatoes in Peru using molecular tools. *P. capsici* is a major biological threat to peppers in Peru and our objective was to better understand how *P. capsici* is surviving and spreading. There are few population studies characterizing the genotypic diversity of *P. capsici* outside of the United States, and a limited number of studies indicating the frequency of the

Fig. 3. Unweighted pair-group method with arithmetic average cluster analysis of *Phytophthora capsici* isolates from 13 provinces across Peru during 2005 to 2007 using 50 amplified fragment length polymorphism markers (AFLP) and six single nucleotide polymorphism (SNP) loci. LT3738* represents 200 *P. capsici* isolates with identical AFLP profiles from Barranca province in Lima from 2006 to 2007. LT number refers to isolate identification for isolates that have not been deposited into a collection. Bootstrap values are based on 1,000 replicates and the major clonal lineages are indicated by the designators PcPE-1 and PcPE-2.

A1 or A2 mating types at specific sites. In Northwest Spain, 16 isolates from 11 locations had a low level of genetic variation based on random amplified polymorphism DNA (RAPD) analysis and all were a single (A1) mating type (63). Additional reports of only a single mating type include reports from Bulgaria (45 isolates, all A1), southern Italy (60 isolates, all A2), and south-eastern Spain (3 isolates, all A1) (27,31,52). Both mating types have been reported from Brazil, Canada, Mexico, and northern Italy (1,12,45,67).

AFLP and SNP analysis of 227 *P. capsici* isolates recovered from 2005 to 2007 from four different *Capsicum* spp. and tomato at 33 locations throughout Peru revealed a surprisingly homogeneous population with much lower genotypic and overall genetic diversity compared to populations analyzed in the United States (26,38,39). A single clonal lineage (PcPE-1) accounts for 221 of the isolates and this clonal type was recovered in all of the locations sampled except for three isolates from the Ica Valley (Figs. 1 and 3). Also surprising, but consistent with the molecular data, was the finding that all of the isolates had the A2 mating type. For the United States' populations that have been analyzed in detail, the overall picture is significantly different. In the United States, both the A1 and A2 mating types have been recovered from many different locations and the genetic structure clearly reflects the impact of sexual recombination on the overall population biology (4,11,26,29,33,37,39,41,51,58). In Michigan, the expansion of clonal lineages is common within single fields during a single year; but spread of clonal lineages over long distances or survival over the winter has not been detected (40,41).

Although AFLP data showed no new AFLP genotypes in the Barranca valley from 2005 to 2007, SNP genotyping of a subset of isolates revealed changes from heterozygosity to homozygosity at two loci; SNP15 and URA3 (Table 4). Since the overall AFLP profile is identical to the PcPE-1 clonal type there is no evidence that sex has occurred and most likely these changes are due to some kind of mitotic effect (e.g., gene conversion) (20). Mitotic recombination as well as mutation and gene conversion are thought to be sources of variation for *Phytophthora* spp. that spread as large clonal lineages such as *P. infestans*, *P. cinnamomi*, and *P. ramorum* (7,21,57).

Currently it is not clear how a single clonal lineage has spread throughout coastal Peru. In the United States, *P. capsici* does not appear to be spread long distances aerially similar to *P. infestans* and movement is most likely via infested water, soil, or plant parts (59). The finding of a limited number of widely dispersed clonal lineages parallels the situation with *P. infestans* in Peru where clonal lineages define the population structure. For both *P. capsici* and *P. infestans* migration of the opposite mating type (A1 for *P. capsici* and A2 for *P. infestans*) could significantly impact the overall diversity of populations and may contribute to increased crop losses and pathogen survival (2,8,13,14,17,21–24,53,64,65,69).

Coastal Peru is a very dry agricultural system with very little annual rainfall (less than 50 mm/year) and above-ground infection and sporulation is generally not observed. The geography at many of the sites sampled precludes infested irrigation water accounting for the observed population structure as they are separate valleys and rely on separate water sources. Further investigations of movement on seedlings and/or seed are warranted. For the isolates collected from the ten fields in the Barranca valley the situation may be easier to explain. The pepper cropping season in the Barranca coastal areas starts in October and plants are mature in April. Further up the valley, at a higher elevation (Caral and Minas districts), the climate allows and farmers practice year-round pepper cultivation. Irrigation water in the Barranca valley comes from the Supe River, which runs west from the slope Andean mountains to the Pacific Ocean (Fig. 2) and it is possible that *P. capsici* outbreaks occurring in higher areas of the valley

contribute to the incidence of pepper root rot and crown rot in lower parts of the valley. In addition, alternative explanations such as movement of plant seedling, tools, and survival of latent mycelium or sporangia in plant debris should not be excluded. Current methods to control *P. capsici* include avoiding excess water in the plant rhizosphere through conservative irrigation and planting on raised beds. Drip irrigation is employed in some cases, but not all farmers have access to this technology. In light of our findings it will be important to test the above-ground irrigation water for the presence of *P. capsici*.

Peppers, originally cultivated in South America, now have more than five cultivated species and over twenty wild species (27). Archeological studies revealed deposits of cultivated types of *Capsicum baccatum* dating back 4,000 years in coastal areas in Peru (54) and a major portion of *Capsicum* evolution appears to have occurred in the south-central Peruvian and Bolivian Andes (43). Our studies suggests that *P. capsici* in Peru has the profile of an introduced pathogen. Additional investigation into the population structure of *P. capsici* at other locations in Peru (e.g., *C. pubescences* cultivated in the Amazon, in the eastern side of the Andes) and other areas in South America may provide some clues to the current situation and may help identify current and past possible routes of dissemination.

Breeding for resistance against *P. capsici* has been challenging due to the high diversity of pathogen populations in the United States (47,71). If the genotypic clonality of *P. capsici* in Peru is reflective of fewer race types of *P. capsici*, then this could offer the advantage for pepper breeders to generate resistant pepper lines that protect against one or a few clonal lineages. In light of the mitotic genetic changes that may be occurring within the context of a clonal population, it will be important to include a panel of clonal isolates from different geographic locations in the screening program.

LITERATURE CITED

- Anderson, T. R., and Garton, R. 2000. First report of blight of field peppers caused by *Phytophthora capsici* in Ontario. Plant Dis. 84:705-705.
- Andrison, D. 1996. The origin of *Phytophthora infestans* populations present in Europe in the 1840s: A critical review of historical and scientific evidence. Plant Pathol. 45:1027-1035.
- Apaza, W., Quino, Y., Negishi, H., and Suyama, K. 2005. Control of *Phytophthora capsici* in pepper (*Capsicum annum* Leon) with *Trichoderma viride* in different substrates. J. ISSAAS 11:71-78.
- Biles, C. L., Liddell, C. M., and Faubion, G. F. 1991. *Phytophthora capsici* strain characterization in southern New Mexico. Phytopathology 81:1192.
- Davidson, C. R., Carroll, R. B., Evans, T. A., Mulrooney, R. P., and Kim, S. H. 2002. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatus*) in the Mid-Atlantic Region. Plant Dis. 86:1049-1049.
- Dillehay, T. D., Rossen, J., Andres, T. C., and Williams, D. E. 2007. Pre-ceramic adoption of peanut, squash, and cotton in northern Peru. Science 316:1890-1893.
- Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L., and O'Brien, P. A. 2003. Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. Phytopathology 93:695-704.
- Drenth, A., Tas, I. C. Q., and Govers, F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. Eur. J. Plant Pathol. 100:97-107.
- Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora Diseases Worldwide*. American Phytopathological Society, St. Paul, MN.
- Fernandez-Northcote, E. N. 1971. La marchitez y otras enfermedades de *Capsicum* spp. cultivadas en el Peru. Paper read at Primer Congreso Nacional de Investigadores Agrícolas y Pecuarios del Peru, at Lima, Peru.
- Fernandez-Pavia, S. P., Biles, C. L., Waugh, M. E., Onsurez, W., Rodriguez-Alvarado, G., and Liddell, C. M. 2004. Characterization of southern New Mexico *Phytophthora capsici* Leonian isolates from pepper (*Capsicum annum* L.). Rev. Mexicana de Fitopatol. 22:82-89.
- Fernandez-Pavia, S. P., Rodriguez-Alvarado, G., and Sanchez-Yanez, J. M. 2003. Buckeye Rot of tomato caused by *Phytophthora capsici* in Michoacan, Mexico. Plant Dis. 87:872-872.
- Flier, W. G., Grunwald, N. J., Kroon, L. P. N. M., Sturbaum, A. K., van den Bosch, T. B. M., Garay-Serrano, E., Lozoya-Saldana, H., Fry, W. E.,

- and Turkensteen, L. J. 2003. The population structure of *Phytophthora infestans* from the Toluca valley of central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum* spp. *Phytopathology* 93:382-390.
14. Forbes, G. A., Escobar, X. C., Ayala, C. C., Revelo, J., Ordonez, M. E., Fry, B. A., Doucett, K., and Fry, W. E. 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87:375-380.
15. French-Monar, R. D., Jones, J. B., Ozores-Hampton, M., and Roberts, P. D. 2007. Survival of inoculum of *Phytophthora capsici* in soil through time under different soil treatments. *Plant Dis.* 91:593-598.
16. French-Monar, R. D., Jones, J. B., and Roberts, P. D. 2006. Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. *Plant Dis.* 90:345-350.
17. Fry, W. E., Goodwin, S. B., Dyer, A. T., Matsuzak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Spielman, L. J., Deahl, K. L., Inglis, D. A., and Sandlan, K. P. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. *Plant Dis.* 77:653-661.
18. Galindo, J., and Zentmyer, G. A. 1967. Genetical and cytological studies of *Phytophthora* strain pathogenic to pepper plants. *Phytopathology* 57:1300-1304.
19. Gevens, A., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2006. Baiting *Phytophthora capsici* from Michigan surface irrigation water and characterization of isolates. *Phytopathology* 97:421-428.
20. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. *Phytopathology* 87:462-473.
21. Goodwin, S. B., Cohen, B. A., and Fry, W. E. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proc. Natl. Acad. Sci. USA* 91:11591-11595.
22. Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E. 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. *Phytopathology* 85:473-479.
23. Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1996. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology* 85:669-676.
24. Grunwald, N. J., Flier, W. G., Sturbaum, A. K., Garay-Serrano, E., van den Bosch, T. B. M., Smart, C. D., Matuszak, J. M., Lozoya-Saldana, H., Turkensteen, L. J., and Fry, W. E. 2001. Population structure of *Phytophthora infestans* in the Toluca valley region of central Mexico. *Phytopathology* 91:882-890.
25. Habera, L., Smith, N., Donahoo, R., and Lamour, K. H. 2004. Use of a single primer to fluorescently label selective amplified fragment length polymorphism reactions. *Biotechniques* 37:902-904.
26. Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. *Plant Dis.* 88:1292-1303.
27. Heiser, C. B., and Smith, P. G. 1953. The cultivated *Capsicum* pepper. *Econ. Bot.* 7:214-227.
28. Herrero, M. L., Blanco, R., Santos, M., and Tello, J. C. 2002. First report of *Phytophthora capsici* on cucumber and melon in southeastern Spain. *Plant Dis.* 86:558.
29. Hwang, B. K., de Cock, A. M., Bahnweg, G., Prell, H., and Heitefuss, R. 1991. Restriction fragment length polymorphisms of mitochondrial DNA among *Phytophthora capsici* isolates from pepper (*Capsicum annuum*). *Syst. Appl. Microbiol.* 14:111-116.
30. Hwang, B. K., and Kim, C. H. 1995. *Phytophthora* blight of pepper and its control in Korea. *Plant Dis.* 79:221-227.
31. Ilieva, S., and Vintanov, M. 1980. Cultural, morphological and physiological characteristics of *Phytophthora capsici* in sweet peppers. *Grdinarska-i-Lozarska-Nauka* 17:61-68.
32. Isakeit, T. 2007. *Phytophthora* Blight caused by *Phytophthora capsici* on pumpkin and winter squash in Texas. *Plant Dis.* 91:633-633.
33. Islam, S. Z., Babadoost, M., Lambert, K. N., Ndeme, A., and Fouly, H. M. 2005. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. *Plant Dis.* 89:191-197.
34. Kreutzer, W. A. 1937. *Phytophthora* rot of cucumber fruit. *Phytopathology* 27:955.
35. Kreutzer, W. A., Bodine, E. W., and Durrell, L. W. 1940. Cucurbit diseases and rot of tomato fruit caused by *Phytophthora capsici*. *Phytopathology* 30:972-976.
36. Lamour, K. H., and Finley, L. 2006. A strategy for recovering high quality genomic DNA from a large number of *Phytophthora* isolates. *Mycologia* 98:514-517.
37. 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.
38. 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.
39. Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatio-temporal genetic structure of *Phytophthora capsici* in Michigan. *Phytopathology* 91:973-980.
40. Lamour, K. H., and Hausbeck, M. K. 2002. The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. *Phytopathology* 92:681-684.
41. Lamour, K. H., and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* and sensitivity to mefenoxam. *Plant Dis.* 87:841-845.
42. Leonian, L. H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. *Phytopathology* 12:401-408.
43. MacLeod, M. J., Eshbaugh, W. H., and Guttman, S. I. 1979. A preliminary biochemical systematic study of the genus *Capsicum*-Solanaceae. Pages 701-714 in: *The Biology and Taxonomy of the Solanaceae*. J. G. Hawkes, R. N. Lester, and A. D. Skelding, eds. Academic Press, New York.
44. Marque, J. M., de Souza, N. L., and Cutolo-Filho, A. A. 2004. Mating type, mycelium growth and sporulation of *Phytophthora capsici* isolates obtained from pepper. *Summa Phytopathol.* 30:389-390.
45. Matsuoka, K., Luz, E. D. M. N., Faleiro, F. G., Cerqueira, A. O., Dantas, N. A., and Marques, J. R. B. 2003. Genetic diversity of *Phytophthora capsici* isolates from different hosts based on RAPD markers, pathogenicity and morphology. *Fitopatol. Bras.* 28:559-564.
46. Miller, S. A., Bhat, R. G., and Schmitthenner, A. F. 1994. Detection of *Phytophthora capsici* in pepper and cucurbit crops in Ohio with two commercial immunoassay kits. *Plant Dis.* 78:1042-1046.
47. Oelke, L. M., Bosland, P. W., and Steiner, R. 2003. Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. *J. Am. Soc. Hortic. Sci.* 128:213-218.
48. Pantoja-Garcia, N. R. 1994. *Phytophthora capsici* L.: Cultural and morphological characteristics on three commercial varieties of *Capsicum* and its chemical control using *Trichoderma viride* Pers. M.Sc., Plant Pathology, National Agricultural University, La Molina, Lima.
49. Papavizas, G. C., Bowers, J. H., and Johnston, S. A. 1981. Selective isolation of *Phytophthora capsici* from soils. *Phytopathology* 71:129-133.
50. Parra, G., and Ristaino, J. B. 1998. Insensitivity to Ridomil Gold (mefenoxam) found among field isolates of *Phytophthora capsici* causing *Phytophthora* blight on bell pepper in North Carolina and New Jersey. *Plant Dis.* 82:711.
51. Parra, G., and Ristaino, J. B. 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of bell pepper. *Plant Dis.* 85:1069-1075.
52. Pennisi, A. M., and Agosteo, G. 1998. Insensitivity to metalaxyl among isolates of *Phytophthora capsici* causing root and crown rot of pepper in southern Italy. *Plant Dis.* 82:1283.
53. Perez, W. G., Gamboa, J. S., Falcon, Y. V., Coca, M., Raymundo, R. M., and Nelson, R. J. 2001. Genetic structure of Peruvian populations of *Phytophthora infestans*. *Phytopathology* 91:956-965.
54. Pickersgill, B. 1969. The archaeological record of chili peppers (*Capsicum* spp.) and the sequence of plant domestication in Peru. *Am. Antiquity* 34:54-61.
55. Pickersgill, B. 1969. The domestication of chili peppers. Pages 443-446 in: *The Domestication and Exploitation of Plants and Animals*. P. J. Ucko and G. W. Dimbleby, eds. Aldine Publishing Company, Chicago, IL.
56. Ploetz, R. C., Heine, G., Haynes, J., and Watson, M. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. *Ann. Appl. Biol.* 140:61-67.
57. Prospero, S., Hansen, E. M., Grunwald, N. J., and Winton, L. M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. *Mol. Ecol.* 16:2958-2973.
58. Ristaino, J. B. 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* 80:1253-1259.
59. Ristaino, J. B., and Gumpertz, M. L. 2000. New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*. *Annu. Rev. Phytopathol.* 38:541-576.
60. Satour, M. M., and Butler, E. E. 1967. A root and crown rot of tomato caused by *Phytophthora capsici* and *P. parasitica*. *Phytopathology* 57:510-515.
61. Satour, M. M., and Butler, E. E. 1968. Comparative morphological and physiological studies of the progenies from intraspecific matings of *Phytophthora capsici*. *Phytopathology* 58:183-192.
62. Schlub, R. L. 1983. Epidemiology of *Phytophthora capsici* on bell pepper. *J. Agric. Sci. Camb.* 100:7-11.
63. Silvar, C., Merino, F., and Diaz, J. 2006. Diversity of *Phytophthora capsici* in northwest Spain: Analysis of virulence, metalaxyl response, and molecular characterization. *Plant Dis.* 90:1135-1142.

64. Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W., Tooley, P. W., and Fry, W. E. 1991. A second worldwide migration and population displacement of *Phytophthora infestans*. *Plant Pathol.* 40: 422-430.
65. Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology* 84:201-207.
66. Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA.
67. Tamietti, G., and Valentino, D. 2001. Physiological characterization of a population of *Phytophthora capsici* Leon. from northern Italy. *J. Plant Pathol.* 83:199-205.
68. Tian, D., and Babadoost, M. 2004. Host range of *Phytophthora capsici* from pumpkin and pathogenicity of isolates. *Plant Dis.* 88:485-489.
69. Tooley, P. W., Therrien, C. D., and Ritch, D. L. 1989. Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. *Phytopathology* 79:478-481.
70. Vos, P., Hogers, R., Bleeker, M., Reijmans, M., van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.
71. Walker, S. J., and Bosland, P. W. 1999. Inheritance of *Phytophthora* root rot and foliar blight resistance in pepper. *J. Am. Soc. Hortic. Sci.* 124:14-18.
72. Weber, G. F. 1932. Blight of peppers in Florida caused by *Phytophthora capsici*. *Phytopathology* 22:775-780.
73. Wolfe, M. S., and Finckh, M. R. 1997. Diversity of host resistance within the crop: Effects on host, pathogen and disease. Pages 378-400 in: Resistance of Crop Plants Against Fungi. H. Hartleb, R. Heitefuss, and H. H. Hoppe, eds. Gustav Fischer Verlag, Jena.