

Characterization of *Phytophthora infestans* Populations in Western Washington

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ABSTRACT

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The first detection in the United States of isolates of *Phytophthora infestans* having metalaxyl insensitivity and complex pathotypes occurred in western Washington during the early 1990s. To determine the genetic structure of the current population in western Washington, a total of 115 isolates of *P. infestans* were obtained during 1996 from infected tubers or foliage of potato, tomato, nightshade, and bittersweet throughout the region. An additional 45 isolates were collected from a single field. Based on mating type, metalaxyl-insensitivity, and molecular markers (allozymes of glucose-6-phosphate isomerase, peptidase, and RG57 DNA fingerprint), all of the isolates were A1 mating type and had the US-11 multilocus genotype. Analyses of an additional 120 isolates collected during 1997 from potato, tomato, and nightshade were performed. As in 1996, US-11 was the predominant genotype detected on all three hosts. However, three additional A2 mating type genotypes were also detected: US-7, US-8, and US-14. These three genotypes represent the first A2 mating type isolates detected in western Washington. Most of a subset of 60 isolates infected 4 to 7 of the 10 potato differentials tested. This included 90% of the isolates collected in 1996 (all US-11), plus 72% of the US-11 and 100% of the US-8 and US-14 isolates collected during 1997. Virulence phenotypes in this region are complex even without the selection pressure of R-genes in the local commercial cultivars. The apparent increase in genetic variation observed in populations of *P. infestans* in western Washington from 1996 to 1997 most likely occurred by migration rather than by sexual recombination.

Additional keywords: late blight

Late blight of potato, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, has been a production constraint in western Washington since 1990, when the first metalaxyl-insensitive isolates of *P. infestans* were detected (5,7). Some of the most complex virulence pathotypes among isolates of *P. infestans* in North America outside Mexico also have been reported from this region (7,19). Moreover, parts of western Washington adjoin British Columbia, Canada, where the A2 mating type was first reported in 1989 (2,4,5). Evidence that isolates collected in northwestern Washington and British Columbia, Canada, originated by sexual recombination has subsequently been presented (18).

Potatoes are well-adapted to this coastal region west of the Cascade Mountains,

which is distinguished by a mild marine climate (83 cm annual precipitation and 15.2/1.7°C average annual max/min temperature). This climate can also be highly conducive to late blight, which occurs annually (9). Red and white potatoes for fresh market predominate production, although some russet and chipping types are also produced. Despite annual outbreaks of late blight, commercial production in northwestern counties of Washington has increased from 1,520 ha in 1990 (41) to 3,838 ha total production in 1997 (D. Havens, *personal communication*). In southwestern Washington and the Olympic Peninsula, only a few commercial potato fields exist and typically are grown under organic production systems. As a consequence of a large urban population, tomatoes are widely grown in home gardens and nurseries throughout the entire region and are often in close proximity to commercial production fields. Western Washington contrasts with the semiarid region of the Columbia Basin and eastern Washington, where mainly Russet-type potatoes for processing are produced commercially under irrigation on approximately 57,600 ha (42).

Prior to 1989, the US-1 genotype of *P. infestans* was probably the predominant genotype in this area, as it was throughout the world (13). Goodwin et al. (12) reported a number of genotypes among isolates collected from northwestern Washington and British Columbia during 1990 and 1991, including US-6, US-6.2, US-6.3, US-6.4, US-6.5, CDA-2, and CDA-3, among which only CDA-3 from British Columbia was of the A2 mating type. In 1992, the isolates from western Washington that were analyzed for allozyme and DNA fingerprint were all US-6 (18). Samples collected from blighted foliage from northwestern Washington during 1994 consisted of a previously undescribed genotype, US-11, which is A1 mating type (10,16,23).

Two previous studies have assessed pathotype diversity among US-6 isolates from the northwestern Washington population of *P. infestans* (7,19). Deahl et al. (7) were the first to identify complex races: 4 out of 32 (12.5%) evaluated had six or more virulence factors. Goodwin et al. (19) evaluated 77 isolates of *P. infestans* for their pathogenicity phenotype, including eight isolates from northwestern Washington. Six of the isolates had virulence to seven, one isolate to four, and one isolate had virulence to three potato resistance genes. All of these isolates were pathogenic on tomato without R-genes and on the two tomato differential cultivars tested. In addition, hairy nightshade (*Solanum sarachoides* Sendtner ex. Mart.) was identified as another host for *P. infestans* in northwestern Washington (6). Isolates from hairy nightshade have also proved to be complex, with six (6) or seven virulence factors (19). The pathotype diversity among US-11 isolates from this region has not yet been assessed.

Several hypotheses have been proposed to predict the consequences of new genotypes of *P. infestans* migrating into commercial potato production regions of the United States and Canada. Goodwin et al. (19) noticed a rapid loss of pathogenicity within the US-6 clonal lineage and proposed that pathogenicity to many resistance genes may be lost within clonal pathogen populations. Vanderplank (40) proposed a similar hypothesis called

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“stabilizing selection against unnecessary virulence genes.” Some possible results from the establishment of sexually reproducing populations of *P. infestans* throughout the United States and Canada have been discussed (12), but they never have been tested experimentally. Western Washington represents a unique natural laboratory for evaluating some of these hypotheses because (i) it is the first location in North America in which insensitivity to metalaxyl was detected (7); (ii) highly complex pathotypes of *P. infestans* have been detected since the early 1990s (7,19); (iii) the first detection of probable sexual reproduction in North America outside Mexico was immediately north, in British Columbia during 1992 (18); (iv) the mild climate favors consecutive epidemics and interaction among new or introduced genotypes; (v) previous studies (4–7,12,16, 18,19,23) provide an initial baseline for estimating changes in recent populations; and (vi) assorted susceptible hosts including hairy nightshade, volunteer and commercial potatoes, garden tomatoes, and bittersweet (*Solanum dulcamara* L.) are juxtaposed in the region.

The purpose of this study was to use neutral genetic markers to characterize the western Washington *P. infestans* population and to establish baseline data of virulence pathotypes.

MATERIALS AND METHODS

Sources of isolates and culture of *P. infestans*. Plant tissue infected with *P. infestans* was obtained from farm managers, potato producers, WSU-Puyallup Plant Disease Clinic, master gardeners, private consultants, and home gardeners in the Washington counties west of the Cascade Mountains during 1996 and 1997. In addition, foliar lesions (45 samples total) were collected approximately every 10 m from late blight field plots where *Solanum* germ plasm was evaluated for resistance during

1996 at WSU-Mount Vernon. Samples were received as infected foliage, tubers, or fruits of potato, tomato, hairy nightshade, and bittersweet. Depending on the quality of the sample, one to eight distinct foliar, tuber, or fruit lesions caused by *P. infestans* were isolated into pure culture using two methods. In the first method, infected tissues from tubers and fruit were rinsed under tap water, surface-sterilized in 0.5% sodium hypochlorite for 30 s, and rinsed twice in distilled water. Potato tuber pieces were dipped in a benomyl suspension (50 mg 50% Benlate WP per 100 ml) (in order to retard *Fusarium* spp. development) prior to placement onto a selective medium (rye agar amended with 20 mg of rifamycin, 200 mg of vancomycin, 200 mg of ampicillin, 68 mg of PCNB, and 50 mg 50% Benlate WP per liter) (29,37,38). Plates were incubated for 4 to 5 days at 20°C, then sporangia were transferred to rye agar. For the second method, foliage and stem tissues were placed in moist chambers for 24 to 48 h; sporangia were then transferred via a 5 to 7 mm³ piece of healthy tuber tissue cut from a surface-disinfested tuber and placed directly onto rye agar selective medium. Cultures were incubated at 18 to 20°C for 4 days; sporangia were collected from the tuber piece and transferred to rye agar amended with sucrose (1.0 g/liter) to obtain pure cultures. Several isolations were made per site. However, only one isolate per lesion on a leaf or tuber was cultured. Only one lesion per leaf was sampled, but one to five lesions per sample or location were sampled. Long-term maintenance of isolates was on slants of rye agar plus sucrose at 10°C under sterile mineral oil (39).

Characterization of isolates. Isolates were analyzed for mating type, sensitivity to metalaxyl, and genotype at the two allozyme loci, glucose-6-phosphate isomerase (*Gpi*, E.C. 5.3.1.9) and peptidase (*Pep*, E.C. 3.4.3.1). Isolates were paired

with known A1 and A2 tester strains on rye agar or rye agar with sucrose. Isolates that produced oospores when paired with the A1 tester strain were designated A2, and isolates that produced oospores with the A2 tester strain were designated A1 when scored after 2 to 3 weeks.

Radial growth on metalaxyl-amended rye agar, as described by others (7,20, 29,30), was used to estimate metalaxyl sensitivity. A 5-mm colonized plug from a 7- to 14-day-old colony was placed on rye agar amended with 0, 5, or 100 µg of metalaxyl per ml. Two stock solutions of technical-grade metalaxyl were prepared as 5 and 100 mg/ml in pure dimethylsulfoxide (DMSO). Control plates were amended with the same concentrations of DMSO as metalaxyl-amended plates, and there were two replicates of each metalaxyl concentration for each isolate. Plates were scored after 7 to 10 days by measuring the diameter of the colony and subtracting 5 mm to correct for the plug. The mean colony diameters at 5 and 100 µg of metalaxyl per ml were divided by the mean colony diameter on the control plates. Isolates that grew more than 40% of the control on metalaxyl medium of 5 µg/ml were considered to be resistant (20).

Mycelia from 7- to 14-day-old rye agar plus sucrose plates were used to analyze the two allozyme loci, *Gpi* and *Pep*, using cellulose-acetate electrophoresis as described by Goodwin et al. (15). Allozyme alleles were designated by the numbers representing their percent mobility relative to previously established standards (10,12,17).

A subset of 45 isolates collected during 1996 was further analyzed with DNA probe RG57 as described by Goodwin et al. (14). Mycelia were grown on rye agar with sucrose for 14 to 21 days at 20°C. Mycelia were collected from 8 to 10 cultures and ground in liquid nitrogen, vortexed with 4 ml of prewarmed (65°C) extraction buffer (50mM Tris, pH 8.0; 150 mM EDTA; 1% sarkosyl) for 30 to 60 s, and incubated for 20 min at 65°C. Next, 4 ml of cold 5 M NH₄OAC was added to each tube, then mixed by inversion, placed on ice for 20 min, and centrifuged for 20 min at 8,240 × g. The supernatant was transferred to a glass tube with 5 ml of cold isopropanol and centrifuged for 15 min at 10,000 × g. The supernatant was decanted and the pellets were dried briefly, then resuspended in 0.5 ml of TE plus RNase (0.1 mg/ml). This was incubated at 37°C for 20 min, then extracted twice with 25:24:1 phenol:chloroform:isoamyl alcohol. The DNA was precipitated with one-tenth volume of 3 M NaOAC and one volume of isopropanol. The tubes were centrifuged for 2 min to collect the DNA; then the pellets were dried briefly, washed in cold 70% ethanol, and resuspended in 100 to 300 µl of TE. For each isolate, approximately 2 µg of DNA was digested

Table 1. Genotypes of *Phytophthora infestans* detected in western Washington during 1996 and 1997

Host ^a	Genotype ^b	Mating type	<i>Gpi</i> ^c	<i>Pep</i> ^d	No. of isolates
1996					
Potato	US-11	A1	100/100/111	100/100	90
Potato ^e	US-11	A1	100/100/111	100/100	45
Tomato	US-11	A1	100/100/111	100/100	19
Nightshade	US-11	A1	100/100/111	100/100	5
Bittersweet	US-11	A1	100/100/111	100/100	1
Total					160
1997					
Potato	US-11	A1	100/100/111	100/100	51
Potato	US-8	A2	100/111/122	100/100	25
Potato	US-14	A2	100/122	100/100	6
Tomato	US-11	A1	100/100/111	100/100	30
Tomato	US-7	A2	100/111	100/100	6
Nightshade	US-11	A1	100/100/111	100/100	2
Total					120

^a Nightshade = *Solanum sarachoides*; bittersweet = *S. dulcamara*.

^b US genotype designations sensu Goodwin et al. (16,18).

^c Glucose-6-phosphate isomerase.

^d Peptidase.

^e *P. infestans* isolates collected from one field at WSU-Mount Vernon REU.

with the restriction enzyme *EcoRI* according to the manufacturer's directions. Gel electrophoresis in 0.8% agarose gels, alkaline blotting to Hybond N+ nylon membrane, hybridization with ³²P random primed RG57 probe, and autoradiography were all according to the manufacturer's instructions (Amersham Life Science, Arlington Heights, IL) and standard protocols (28).

A subset of 60 isolates collected in 1996 and 1997 was analyzed for virulence phenotype on detached leaflets in inverted water agar (1.5%) plates as described by Tooley et al. (38). Ten single R-gene differential potato cultivars were obtained from the Potato Introduction Station, Sturgeon Bay, WI: PI 423651 (R1), PI 423652 (R2), PI 423653 (R3), PI 203900 (R4), PI 303146 (R5), PI 587059 (R6), PI 303148 (R7), PI 303149 (R8), LB 1 (R9), and PI 423656 (R10). Two detached leaflets of each differential and a susceptible (R0) cultivar Red LaSoda, Russet Norkotah, or Shepody were placed abaxial side up, two replicates per isolate, for a total of four leaflets. A 50- μ l droplet containing a suspension of sporangia (1.0 to 2.5 \times 10⁴ sporangia per ml) was placed on each leaf. Plates were incubated at 18°C with a 12-h light:dark cycle. After 7 days, leaflets were scored for the presence or absence of a sporulating lesion. Every isolate was assayed at least twice, with the most consistent reactions reported here.

RESULTS

1996 collection. A total of 115 isolates of *P. infestans* from single lesions on infected tubers and/or foliage from four different hosts in 40 separate fields and gardens throughout western Washington were collected in 1996. An additional 45 isolates were collected from *Solanum* late blight resistance evaluation plots at WSU-Mount Vernon. All 160 isolates collected were the US-11 genotype (Table 1), which is A1 mating type, *Gpi* 100/100/111, *Pep* 100/100, and resistant to metalaxyl. Isolates were regarded as highly resistant to metalaxyl by mycelial growth of more than 40% of the control on agar plates amended with 5 μ g of metalaxyl per ml (Fig. 1). DNA fingerprint analyses of a subset of 45 isolates from 23 locations revealed a clonal population, because all isolates had the same DNA fingerprint (Fig. 2). One isolate from the intensively sampled research plots was missing fingerprint band number 20.

Virulence phenotypes (Table 2) were quite variable for isolates collected in 1996, in contrast to the uniformity of the multi-locus genotype determined with neutral genetic markers (Table 1). Previously, US-6 from northwestern Washington had very complex pathotypes present in the population as measured on nine potato differentials (19). In this study, the majority of isolates (85.7%) infected four to six potato differentials. Only one isolate in-

fectured seven differentials. However, the seventh differential was R10, which produced a very slow-growing lesion.

1997 collection. A total of 120 isolates of *P. infestans* were collected from single lesions on infected tubers and/or foliage on three hosts from 45 locations in 1997. Four genotypes of *P. infestans* were detected: US-7, US-8, US-11, and US-14 (Table 1). Genotypes US-7, US-8, and US-14 are the A2 mating type (Table 1). All except two US-8 isolates were highly resistant to metalaxyl (Fig. 1) and represented complex pathotypes (Table 2). The US-8 genotype had an average of 9.0 virulence factors compared with 4.8 for US-11 and 7.0 for US-14.

Differentials R2, R4, and R10 have been reported to have an incomplete hypersensitive response with slowly expanding lesions (3). The differentials were transplanted into the WSU-Mount Vernon research field plots in 1997; the R2 differen-

tial did not survive, and all but R8 and R9 had large sporulating lesions and eventually died by the end of the epidemic. These results indicate that a population of *P. infestans* with a highly complex virulence phenotype occurred in the region again in 1997.

DISCUSSION

During the 1990 through 1997 potato production seasons, populations of *P. infestans* in western Washington have been composed primarily of one or two genotypes with no evidence of sexual recombination. During or shortly before 1990, the US-1 genotype was displaced by US-6 (18), which was subsequently displaced by US-11 during 1994 (16,23). In this study, only the US-11 genotype was identified in 1996; whereas US-7, US-8, US-11, and US-14 were detected in 1997.

We, as well as others, have been able to document these population changes in this

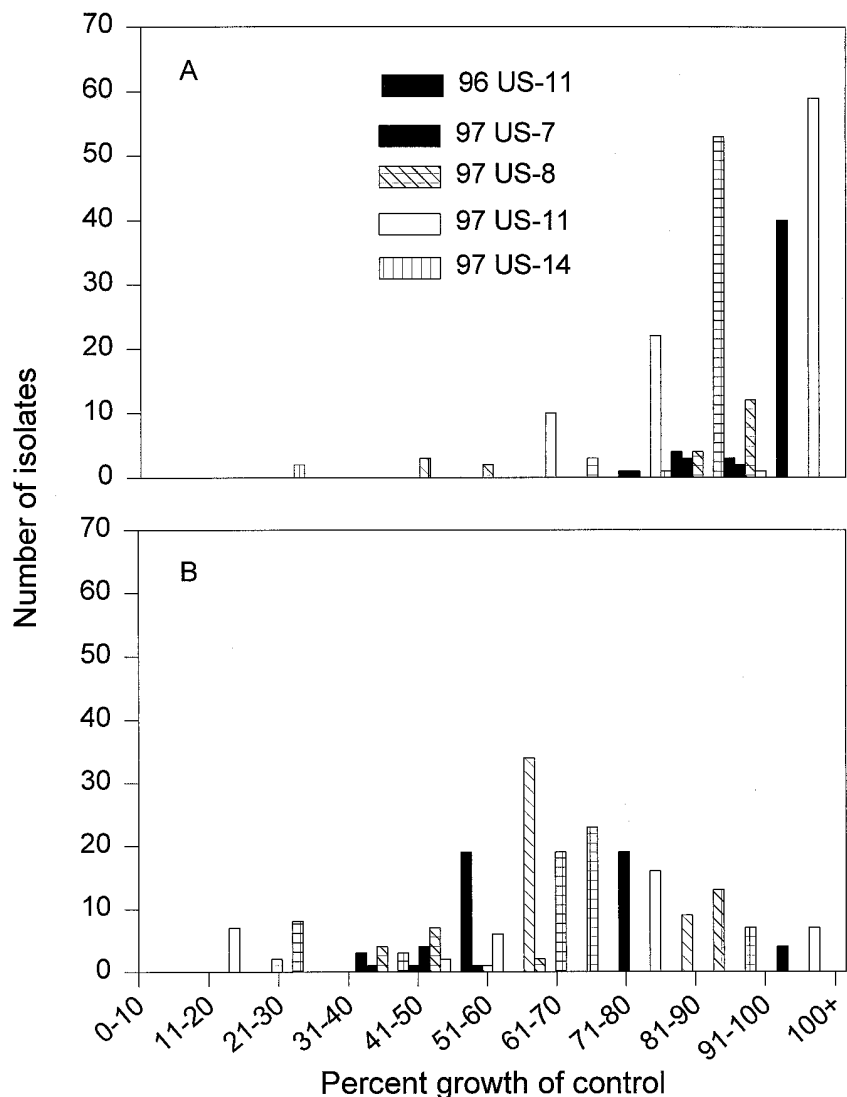


Fig. 1. Relative growth of 115 and 120 isolates of *Phytophthora infestans* collected from western Washington in 1996 and 1997, respectively. Metalaxyl at (A) 5 μ g/ml and (B) 100 μ g/ml. Radial growth on agar medium containing metalaxyl was expressed as a percentage of growth on agar medium containing 0 μ g of metalaxyl per ml.

relatively small potato production region beginning in 1990 using mating type, the allozyme loci *Gpi* and *Pep* (18), and metalaxyl insensitivity (7). DNA fingerprint analyses confirmed that the *P. infestans* isolates collected during 1996 (US-11) were from a clonal population. The findings in 1997 indicate the *P. infestans* population was composed primarily of two genotypes, US-8 and US-11. The US-8 genotype has been predominant in many of the U.S. potato production regions since the mid-1990s (11,16,23) and is reported to have host specialization for potato (27), although it has been isolated occasionally from hairy nightshade. We similarly collected US-8 on potato but not on tomato. The US-11 genotype in this study has been reported from a few regions (16) but was first described in western Washington during 1994 (10,16,23). This genotype has

a very wide host range, including tomato, hairy nightshade, potato (6), and bitter-sweet. Both the US-8 and US-11 genotypes of *P. infestans* have complex pathotypes (Table 2; 19). The US-14 genotype also was detected during this study in 1997 from one field in western Washington but has been reported previously in New York (16) and eastern Washington (30). The US-7 genotype was recovered during 1997 from home garden tomatoes in several areas of western Washington in which transplants were purchased from garden centers that import tomato seedlings from outside of the production region. The US-14 genotype also had complex pathotypes (Table 2). It has been reported previously that US-7 has host specialization on tomato (27).

This is the first documented report of the A2 mating type and the *Gpi* 122 allele in

western Washington and is in contrast to other U.S. and Canadian potato production areas, including eastern Washington, where the A2 mating type has predominated for several years (2,11,16,18,30,32,33). Andrivon and de Vallavieille-Pope (1) stated that the different behavior of geographically distinct populations of the same fungus suggests that local factors are of the utmost importance in the determination of race diversity and complexity patterns. They further suggested that experience with one population of a pathogen is not sufficient to enable a reliable prediction of the structure and evolution of other populations of the same organism, even those that are geographically close. In British Columbia, both metalaxyl-sensitive and metalaxyl-insensitive strains of the A2 mating type were reported in 1992, 1993, and 1994 (2,18,20), but in 1995, the majority were A1 and metalaxyl-insensitive (2).

If there were earlier migrations of the A2 mating type from British Columbia into western Washington, they may not have been detected for two possible reasons. First, very few samples were collected in 1993, 1994, and 1995 from western Washington. In addition, 25 and 6% of the A2 isolates collected in British Columbia during 1993 and 1994, respectively, were metalaxyl-sensitive (2). Since potato producers in western Washington use metalaxyl in their fungicide spray program at the time of tuberization to manage storage rot diseases caused by *Pythium* spp. and pink rot (*Phytophthora erythroseptica* (Pethybridge)), they may inadvertently have been selecting for metalaxyl-insensitive strains which were predominantly A1 mating type.

The virulence pathotypes of *P. infestans* collected in this region in 1996 and 1997 were as complex as those reported previously (6,7,19). All differentials except R8 and R9 planted in Mount Vernon field plots in 1997 had large sporulating lesions caused by *P. infestans* and died before the end of the growing season (D. A. Inglis and A. E. Dorrance, unpublished data). The US-8 and US-14 genotypes on average contained more virulence factors than the "older" US-11 genotypes. These results indicate that the new migrants, US-8 and US-14, may have originated from a sexually reproducing population (37,38). A sexual population would be more likely to maintain virulence combinations than a population that is reproducing asexually if the observed mutation rate from virulence to avirulence is higher than from avirulence to virulence (19). While the number and types of potato cultivars produced in this region are quite diverse, few if any of these cultivars contain specific resistance genes to *P. infestans*; hence, selection of particular races by R-gene resistance is highly unlikely. In contrast, reports from Poland indicate that isolates prior to mi-

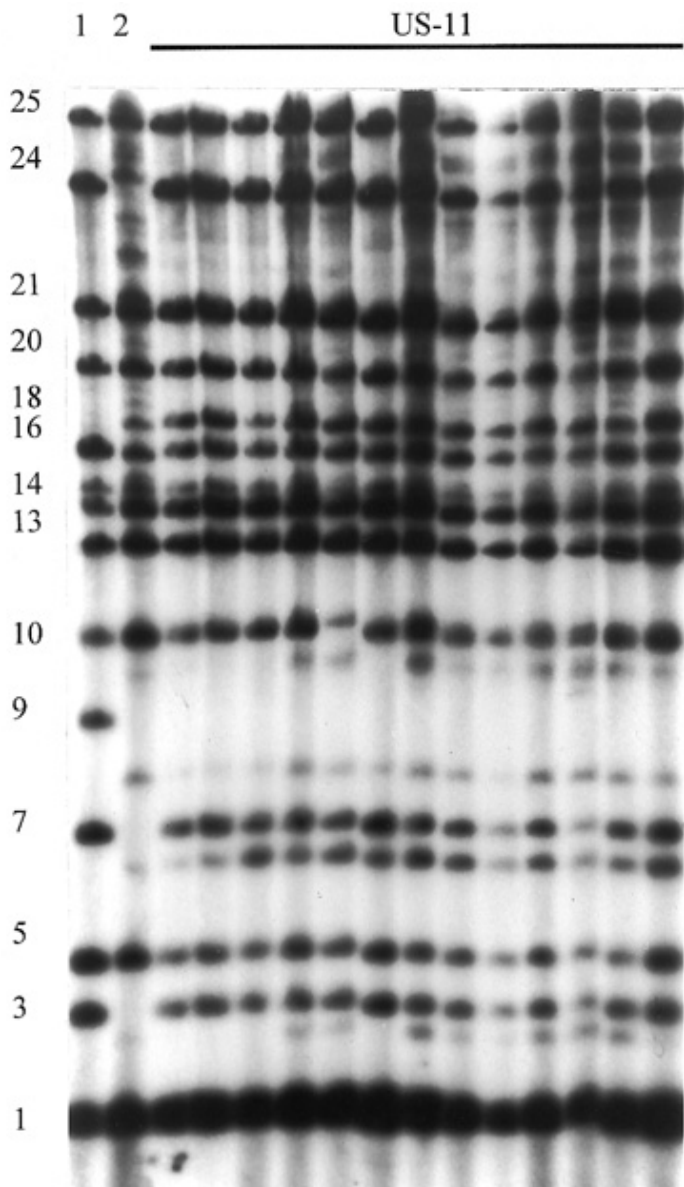


Fig. 2. DNA fingerprint patterns (probe RG57) for *Phytophthora infestans* standard isolates US-1 (lane 1), US-8 (lane 2), and representative *P. infestans* US-11 isolates (lanes 3 to 15) collected from throughout western Washington in 1996. Band numbers are indicated on the left.

grations had complex pathotypes, possibly because of the extensive utilization of resistance genes used in commercial cultivars (36).

Both Poland and the Netherlands have reported *P. infestans* populations that are reproducing sexually based on diverse RG57 fingerprints and detection of recombinant allozyme genotypes (8,35). In western Washington, a sexually reproducing population might be detected in the future by the appearance of isolates with homozygous *Gpi* genotypes (e.g., 100/100, 111/111) or by changes in combination of mating type with *Gpi* allozyme pattern that would result from crosses between US-11 (A1, *Gpi* 100/100/111) and US-8 (A2, *Gpi* 100/111/122). Genotypes US-11 (A1) and US-8 (A2) have an extra copy of at least one chromosome, as indicated by the unbalanced *Gpi* banding pattern (18). However, this should not inhibit formation and germination of oospores as a result of mating between these two genotypes (34; H. Judelson, *personal communication*). Previous crosses with US-8 as one parent yield normal numbers of progeny (16). If recombinant genotypes of *P. infestans* are found, this would indicate that oospores could have formed in plants, in fields, or in tubers in storage.

Previous migrations of new *P. infestans* genotypes into U.S. potato and tomato production regions have resulted in one clone predominating (11,12,16,18,30). In all of these previous migrations, the new migrants were more aggressive and more

fit than the resident population (25,26,31). The recovery of both A1 and A2 mating types from a few fields, and in one case from the same plant, may indicate that the population of *P. infestans* within western Washington someday may become more similar to potato production regions in the Netherlands and Poland where sexually reproducing populations exist.

U.S. potato producers successfully manage late blight epidemics resulting from newly introduced genotypes of *P. infestans* provided they follow a protective fungicide spray program (21,22). In addition, potato producers in Mexico have been successful in managing late blight with preventive foliar fungicide spray programs in areas where sexually reproducing *P. infestans* populations predominate (21; H. Lozoya-Saldaña, *personal communication*). New studies indicate that fungicides for potato seed treatments can selectively impact the sporulation of *P. infestans* on tubers (24). If soilborne inoculum resulting from the formation of oospores becomes part of the etiology, the use of broad-spectrum fungicides applied to seed tubers and foliage will undoubtedly increase.

The genetic variation observed within populations of *P. infestans* in western Washington, the first area of the United States where metalaxyl insensitivity was detected and the first area in North America outside Mexico in which complex virulence pathotypes were identified, is probably the result of three separate migration events. It has become apparent that

tomatoes and potatoes produced in home gardens, weed hosts such as hairy nightshade, and volunteer potatoes may become very important in the epidemiology and maintenance of some *P. infestans* genotypes in western Washington. Specifically, the US-11 genotype (A1) which was found on these hosts, in contrast to the US-8 genotype (A2) which was only found associated with potatoes, may retain a possible fitness advantage and help maintain a clonal population structure. Future studies that characterize this western Washington population need to include late blight lesions from both home gardens and weed hosts.

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Table 2. Pathogenicity phenotypes of 60 isolates of *Phytophthora infestans* collected in western Washington during 1996 and 1997

1996				1997			
Host	No. of isolates	Race ^a	Octal ^b	Host	No. of isolates	Race	Octal
US-11 Genotype ^c				US-11 Genotype ^c			
Potato	1	7	0,0,4,0	Potato	1	2,5,7	2,2,4,0
Tomato	1	1,5,7	4,2,4,0	Potato	1	2,4,5,6,7	2,7,4,0
Potato	1	1,4,7	4,4,4,0	Tomato	2	1,7	4,0,4,0
Potato	6	1,2,5,7	6,2,4,0	Tomato	1	1,5,8	4,2,1,0
Potato	1	1,2,5,7,8	6,2,6,0	Tomato	2	1,5,7	4,2,4,0
Potato	1	1,2,5,6,7	6,3,4,0	Potato	1	1,4,5,7	4,6,4,0
Tomato	1	1,2,5,6,7,8	6,3,6,0	Tomato	1	1,3,5,7	5,2,4,0
Potato	3	1,2,4,5,7	6,6,4,0	Tomato	1	1,2,7,8	6,0,6,0
Potato	4	1,2,4,5,7	6,6,4,0	Potato	1	1,2,5,7,8	6,2,6,0
Tomato	3	1,2,4,5,7	6,6,4,0	Tomato	1	1,2,4,5,7	6,6,4,0
Potato	2	1,2,4,5,7,8	6,6,6,0	Potato	3	1,2,4,5,6,7,8	6,7,6,0
Hairy nightshade ^d	1	1,2,4,5,6,7	6,7,4,0	Potato	1	1,2,3,5,7	7,2,4,0
Potato	1	1,2,3,5,7	7,2,4,0	Potato	1	1,2,3,4,5,7,8	7,6,6,0
Potato	2	1,2,3,4,5,7	7,6,4,0	Tomato	2	1,2,3,4,5,7	7,6,4,0
Tomato	1	1,2,3,4,5,7	7,6,4,0	Potato	3	1,2,3,4,5,7	7,6,4,0
Hairy nightshade	1	1,2,3,4,5,7,10	7,6,4,1				
				US-8 Genotype ^c			
				Potato	2	1,2,3,4,5,6,7,10	7,7,4,4
				Potato	3	1,2,3,4,5,6,7,8,9,10	7,7,7,4
				Potato	1	1,2,3,4,5,6,8,10	7,7,2,4
				US-14 Genotype ^c			
				Potato	1	2,3,4,6,7,10	3,6,4,4
				Potato	1	1,2,3,4,5,6,7,10	7,7,4,4

^a Summary of two or sometimes three detached potato leaflet assays using inverted water agar dishes as described by Tooley et al. (37).

^b R5, R6, and R8 potato differentials were not available for each detached leaflet test; R11 was not tested; the corresponding octal digits were underlined to reflect the missing data.

^c US genotype designations sensu Goodwin et al. (16,18).

^d *Solanum sarachoides*.

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