

# *Phytophthora infestans* Populations from Tomato and Potato in North Carolina Differ in Genetic Diversity and Structure

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## ABSTRACT

Wangsomboondee, T., Groves, C. T., Shoemaker, P. B., Cubeta, M. A., and Ristaino, J. B. 2002. *Phytophthora infestans* populations from tomato and potato in North Carolina differ in genetic diversity and structure. *Phytopathology* 92:1189-1195.

*Phytophthora infestans* causes a destructive disease on tomato and potato. In North Carolina (NC) potatoes are mostly grown in the east, whereas tomatoes are grown in the mountainous areas in the western part of the state. Five genotypes of *P. infestans* were identified from 93 and 157 isolates collected from tomato and potato over a 5 year period between 1993 and 1998. All isolates collected from potato in eastern NC were the US-8 genotype, whereas only a single isolate was the US-1 genotype. Tuber blight was found on immature daughter tubers in a single field in 1997, however infection on mature tubers was not observed.

Within potato fields, a range of sensitivity to metalaxyl was observed among isolates but all were either intermediate or highly resistant to the fungicide. In contrast, isolates from tomatoes included previously reported US-7 and US-8 genotypes and two new genotypes called US-18 and US-19 (A2 mating type, allozyme genotype *Gpi* 100/100 and *Pep* 92/100). These genotypes had unique restriction fragment length polymorphism banding patterns, were sensitive to metalaxyl, and have not been reported elsewhere. All genotypes, with the exception of the US-1, were the 1a mitochondrial haplotype. Thus, isolates of *P. infestans* from tomato were more genetically diverse over time in NC than those from potato and include two new genotypes that are sensitive to metalaxyl.

*Additional keywords:* epidemiology, Irish potato famine, population genetics.

Late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, has re-emerged as a serious disease of tomato and potato in the United States and Canada during the last decade (12). Disease increases have been attributed to increased resistance to the fungicide metalaxyl and associated with genetic changes in populations of *P. infestans* (3,5,19,20,22,29). The migration of *P. infestans* via infected potato and tomato plants from Mexico and sexual reproduction of the pathogen have contributed to genetic changes in recent populations of the pathogen (8,13,19,21). Comparison of mating type, allozyme genotype at the *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) loci, and DNA fingerprinting with the RG57 probe have confirmed that populations composed of new genotypes are rapidly replacing the former genotypes (13,14). Data also indicate that new genotypes of *P. infestans* may exhibit greater fitness than old genotypes (27).

Studies of populations of *P. infestans* in the tomato and potato growing regions in North Carolina (NC) began in the early 1990s (11,19). Large-scale production of potato is geographically isolated in the eastern part of the state, whereas large-scale tomato production occurs in the western part of the state. Epidemics of the disease have occurred yearly in tomato growing areas of NC between 1991 and 1998. Tomatoes can only be produced in western NC if multiple fungicide applications are made for early and late blight control. In contrast, the disease was uncommon prior to 1993 in potato growing areas of NC, but since then, epidemics have occurred sporadically in different fields, and in those fields, severe disease has occurred (11).

In a previous study, isolates of *P. infestans* collected in NC between 1993 and 1995, primarily from tomato, were characterized

using allozymes at *Gpi* and *Pep* loci, mating type, and metalaxyl sensitivity (11). Four different allozyme genotypes (US-1, US-7, US-8, and US-18) were identified in NC (11). The US-18 genotype was first isolated in eastern Tennessee in 1994. DNA fingerprinting of the isolates was not conducted in the previous study (11). The dynamics of changes in genotypes of *P. infestans* have been examined in many areas of the United States (12,13,19,21,22), but only a single study has been conducted in NC (11).

The occurrence of metalaxyl resistant isolates in populations of *P. infestans* has played a major role in the increased severity of late blight epidemics observed in recent years (3,5,22). It is believed that resistance in the United States and Canada originated from migration of resistant isolates rather than by mutation and selection after migration (22). In contrast, resistance to metalaxyl in Europe may have evolved from selection of metalaxyl resistant mutants within clonal lineages (22). Likewise, in Africa, metalaxyl resistant isolates have been found within the US-1 clonal lineage (35). A dominant locus responsible for resistance has been shown to have different chromosomal locations in different isolates (10). It is likely that development of metalaxyl resistance in *P. infestans* may have arisen independently in different areas of the world. Variation in the level of sensitivity to metalaxyl within the US-7 and US-8 genotypes of *P. infestans* has also been observed (11). In NC, US-7 and US-8 genotypes consisted of both intermediate and highly resistant isolates and there was not a complete correlation between genotype and metalaxyl sensitivity (11).

The present study was conducted to more thoroughly examine the genetic structure and diversity among isolates of *P. infestans* sampled from tomato and potato in NC between 1993 and 1998. Because the two host crops are geographically isolated in NC, we were interested in determining whether the same inoculum sources were responsible for epidemics. We used additional geno-

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typic markers including mitochondrial DNA (mtDNA) haplotyping and DNA fingerprinting with the probe RG57 to further examine the genetic structure of populations from potato and tomato. We expanded our sample of isolates on potato and evaluated within-field variation in sensitivity to metalaxyl. A preliminary report of a portion of this work has been presented (36).

## MATERIALS AND METHODS

**Fungal isolates and cultures.** Late blight-infected tomato and potato samples were collected from various locations in NC between 1993 and 1998 (Table 1). Two hundred fifty isolates of *P. infestans* from 54 fields in NC were examined over the course of this study (Table 1). Samples were taken to maximize the number of different field locations sampled. Multiple samples from infected potato were collected from multiple foci within a field early in epidemic development in Washington County in 1997, and in 1998 from two different fields in Sampson and Greene counties (Table 1). Samples from four potato fields in Pasquotank County in 1998 were also collected. Leaves were sampled in most fields, but isolates from the field in Washington County in 1997 were obtained from tubers, leaves, and stems.

Pure cultures were obtained by placing infected plant tissue in plastic bags or petri dishes lined with moistened filter paper and incubating at 18°C for 1 to 2 days until sporulation occurred. A small piece of rye B agar on the tip of a hypodermic needle was touched to sporangia and transferred to rye B agar (22). For tissue without obvious sporulation, the pathogen was isolated after

inoculating a potato tuber bait. Surface-disinfected tuber slices (5 mm thick) were placed on top of infected tissue in a petri dish. The petri dishes were put in a moist chamber and incubated at 18°C for 5 to 7 days. Once sporulation was observed, isolation was made as described previously. Pea broth (120 g of frozen peas per liter of sterile distilled water) was used to culture mycelia of *P. infestans* for allozyme analysis, mtDNA haplotyping, and restriction fragment length polymorphism analysis. Mycelia was removed from the broth by vacuum filtration on Whatman No. 1 filter paper and kept frozen at -20°C for future use.

**Mating type.** Mating type was determined by placing an unknown isolate between (2.5 cm) known A1 (94-1, US-1 genotype) and A2 (93-4, US-8 genotype) tester isolates of *P. infestans* on rye B agar media. Hyphal interaction zones were observed microscopically after 7 to 10 days of incubation at 18°C in dark. Oospores were produced in the margins of opposite mating types. Isolates that produced oospores with the known A1 tester isolate were designated the A2 mating type and isolates that produced oospores with the known A2 tester isolate were designated the A1 mating type. Positive controls consisted of pairings between the opposite mating type of tester isolates, and negative controls consisted of pairing between isolates of the same mating type.

**Allozyme analysis.** Mycelia obtained from pea broth culture as described previously was cut into small pieces (0.5 to 1 cm<sup>2</sup>) and placed in sterile 1.5-ml microcentrifuge tubes. The mycelia was centrifuged at 8,000 × g to remove excess water. Fifty microliters of extraction buffer (20% sucrose, 2% Triton X-100, 0.01% bromophenol blue, and 9.8 ml of H<sub>2</sub>O) and 10 mg of glass beads (Sigma Chemical, St. Louis) were added to each tube and the mycelia was ground for 30 s with a hand drill equipped with a separate, sterile Konte pestle for each isolate. Samples were centrifuged for 3 min at 8,000 × g, and the supernatant was collected for allozyme analysis.

Allozyme genotypes were determined on all isolates at the *Gpi* locus and for some isolates at the *Pep* locus by polyacrylamide gel electrophoresis using the Phastsystem (Amersham Biosciences, Piscataway, NJ) according to methods described previously (11, 18). Precast homogeneous polyacrylamide gels (12.5%) and standard native buffer strips were used for the *Gpi* assay, while homogeneous polyacrylamide gels (12.5%) with agarose buffer strips made with Tris-citrate buffer (0.155 M Tris and 0.043 M citric acid, pH 7.0) were used for the *Pep* assay. The agarose buffer strips were prepared with 3% agarose using buffer strip molds (Amersham Biosciences) (11).

Isolates of the four clonal genotypes US-1, US-6, US-7, and US-8 were used as standards on each gel (18). Migration distances of proteins from the unknown isolates were compared with migration distances of proteins from the known genotypes. Alleles in individual isolates were scored based on the migration of their proteins relative to the protein produced at the 100 allele (18).

**mtDNA haplotyping.** DNA from a subset of isolates of *P. infestans* was extracted according to the cetyltrimethylammonium bromide (CTAB) extraction method (34). mtDNA haplotyping was performed via polymerase chain reaction (PCR) (23). Two primer sets were used that amplify the P2 and P4 (2F/2R and 4F/4R) regions of mtDNA of *P. infestans* (23). The final PCR reaction volume of 30 µl consisted of 1 µl (1 to 10 ng) of template DNA, 3 µl of 10× buffer (100 mM Tris-HCl, 15 mM MgCl<sub>2</sub>, and 500 mM KCl, pH 8.3), 14.01 µl of sterile distilled H<sub>2</sub>O, 200 µM each dNTPs, 2.75 mM MgCl<sub>2</sub>, 0.325 µM forward and reverse primers of either 2F (5'-TTCCCTTTGTCTCTACCGAT-3') and 2R (5'-GCTTATGCTTCAGTTGCTCAT-3') or 4F (5'-TGGTCA-TCCAGAGGTTT-ATGTT-3') and 4R (5'-CCGATACCGATAAC-AGCACCAA-3') (23), 160 µg of bovine serum albumin per ml, and 0.1 unit of *Taq* polymerase. All reactions were overlaid with sterile mineral oil prior to thermal cycling. Thermal cycling parameters were an initial denaturation at 94°C for 90 s followed by 40 cycles consisting of denaturation at 94°C for 40 s, annealing at

TABLE 1. Isolates of *Phytophthora infestans* collected from potato and tomato fields in North Carolina between 1994 and 1998 and tested for mating type, genotype, and mitochondrial DNA (mtDNA) haplotype

Year	Location, County	No. of isolates	Mating type	Genotype <sup>a</sup>	mtDNA <sup>b</sup>
Potato isolates					
1994	Camden	1	A1	US-1	Ib
1994	Fletcher	1	A2	US-7	Ia
1994	Washington	5	A2	US-8	Ia
1994	Yancey	1	A2	US-8	Ia
1994	Fletcher	1	A2	US-7	Ia
1995	Washington	2	A2	US-8	Ia
1996	Pasquotank	6	A2	US-8	Ia
1997	Washington	39	A2	US-8	Ia
1998	Greene	46	A2	US-8	Ia
1998	Sampson	35	A2	US-8	Ia
1998	Pasquotank, field 1	5	A2	US-8	Ia
1998	Pasquotank, field 2	5	A2	US-8	Ia
1998	Pasquotank, field 3	5	A2	US-8	Ia
1998	Pasquotank, field 4	5	A2	US-8	Ia
Tomato isolates					
1993	Fletcher	1	A2	US-7	Ia
1993	Yancey	1	A2	US-7	Ia
1993	Alleghany, field 1	1	A2	US-8	Ia
1993	Alleghany, field 2	1	A2	US-7	Ia
1994	Charleston <sup>c</sup>	1	A2	US-7	Ia
1994	Macon, field 1	6	A2	US-7	Ia
1994	Macon, field 2	1	A2	US-8	Ia
1994	Yancey	1	A2	US-8	Ia
1994	Madison	1	A2	US-8	Ia
1994	Henderson	2	A2	US-7	Ia
1994	Fletcher	1	A2	US-7	Ia
1994	Haywood	1	A2	US-7	Ia
1995	Waynesville	3	A2	US-18	Ia
1996	Fletcher	8	A2	US-18	Ia
1996	Waynesville	3	A2	US-18	Ia
1996	Yadkin	3	A2	US-18	Ia
1997	Fletcher	5	A2	US-19	Ia
1998	Waynesville	3	A2	US-18	Ia

<sup>a</sup> Genotype was determined by DNA fingerprinting with the RG57 probe (19).

<sup>b</sup> mtDNA haplotype was determined by methods of Griffith and Shaw (23).

<sup>c</sup> Isolate from South Carolina.

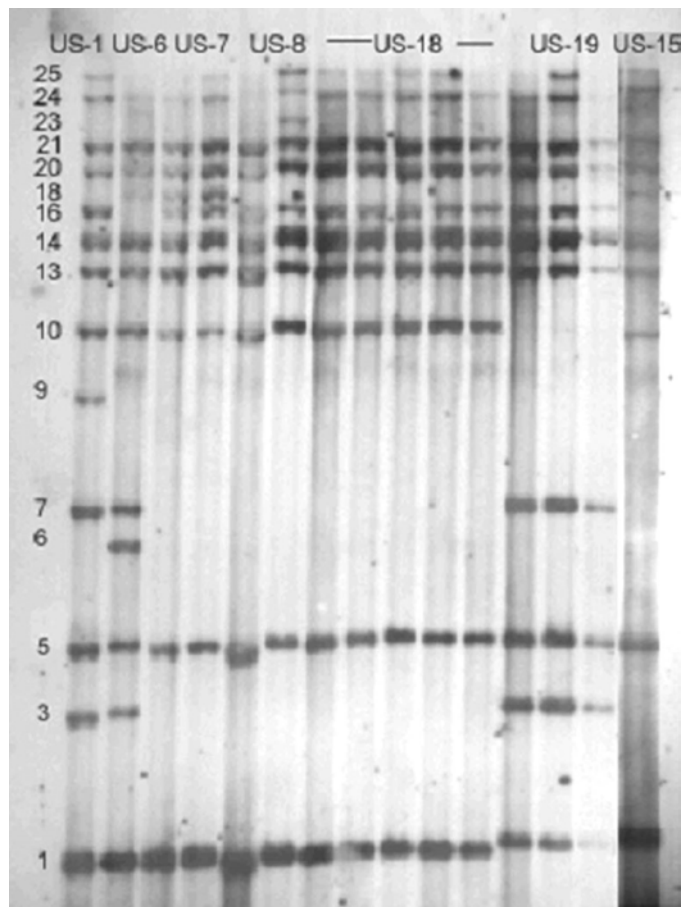
55°C for 60 s, and extension at 72°C for 90 s. A final extension at 72°C for 10 min followed.

PCR products from the P2 (1,240 bp) and P4 (957 bp) regions of mtDNA were digested with *MspI* and *EcoRI*, respectively, at 37°C for 2 h. Digested products were subject to electrophoresis on 1.6% agarose gels containing 0.5 µg of ethidium bromide per ml with 1× Tris-borate-EDTA running buffer. A 100-bp DNA ladder was included on each gel as a molecular size standard.

**DNA fingerprinting.** DNA was extracted from frozen mycelia according to a modification of Goodwin et al. (17). Two to four plates of mycelia from pea broth cultures of each isolate were vacuum-filtered through Whatman No. 1 filters and lyophilized at -5°C for 12 h. Lyophilized mycelia was ground in liquid nitrogen, suspended in 4 ml of prewarmed (65°C) extraction buffer (50 mM Tris, pH 8.0, 150 mM EDTA, and 1% Sarkosyl), vortexed for 60 s, and incubated for 20 min at 65°C. Four milliliters of cold 5 M NH<sub>4</sub>OAc was added to each tube, which were then mixed by inversion, placed on ice for 20 min, and centrifuged for 10 min at 8,240 × g. The supernatant was transferred to a new tube, precipitated with a two-thirds volume of isopropanol, and centrifuged for 5 min at 8,240 × g. The pellets were dried and resuspended in 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris, pH 8.0, and 1 mM EDTA). The DNA suspension was transferred to a new 1.5-ml microcentrifuge tube and RNase A (Boehringer Mannheim, IN) was added to a final concentration of 0.1 mg/ml. The tubes were incubated for 20 min at 37°C and extracted twice with 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated with 0.1 volume of 3 M NaOAc and 2 volumes of absolute ethanol. The tubes were centrifuged for 15 min to pellet the DNA. Pellets were washed with 70% ethanol, air dried, and resuspended in 100 to 300 µl of TE buffer. Two micrograms of DNA was digested with *EcoRI* and subjected to electrophoresis on 0.8% agarose gels at 50 V for 18 h. Southern transfer was performed by depurination in 300 ml of 0.25 M HCl for 10 min, and denaturation in 1× Southern base solution (10 g of NaOH and 87.63 g of NaCl per liter) twice for 15 min each. DNA was transferred to nylon membranes (Hybond-N+, Amersham Biosciences) by capillary transfer overnight. The membrane was neutralized in 200 ml of 1 M Tris, pH 7.5, and 1.5 M NaCl for 2 to 3 min. DNA was fixed on the membrane by UV cross-linking. Hybridization with the RG57 probe (17) was performed following the protocol of the random primer fluorescein labeling kit with antiluorescein-AP (NEN Life Science Products, Boston). The method of purification of the fluorescein-labeled probe was modified from the protocol by precipitating the labeled DNA probe in 2 volumes of absolute ethanol and 0.1 volume of 3 M NaOAc for 12 h, washing with 70% alcohol, and resuspending in 50 to 100 µl of TE buffer. Known US-1, US-6, US-7, and US-8 genotypes were used as standards.

**Metalaxyl sensitivity of isolates.** Metalaxyl sensitivity of isolates collected from 1996 to 1998 was examined on rye B agar

amended with 0, 5, and 100 µg of metalaxyl per ml (Ridomil 2E, Syngenta, Research Triangle Park, NC). A single disk (0.5 cm) of mycelia from each isolate was transferred to rye B agar amended with metalaxyl as described previously and incubated at 18°C for 10 to 14 days. Two replications were conducted per treatment for each isolate, and the experiment was repeated at least twice. Radial growth of each colony was measured after 10 to 14 days, and the relative growth rate of each isolate at each metalaxyl concentration was calculated as a percentage of the growth on



**Fig. 1.** DNA fingerprint patterns with RG57 probe of five genotypes (US-1, US-7, US-8, US-18, and US-19) of *Phytophthora infestans* found in North Carolina between 1993 and 1998 compared with US-6 and US-15 genotypes. Genotype names are indicated above each lane. RG57 fingerprint band numbers are indicated on the left. Isolates numbers from lanes 1 to 15 are 94-1 (US-1), 94-52 (US-6), 93-1 (US-7), 93-5 (US-7), 95-4-2 (US-8), 97-24 (US-8), 96-6-2 (US-18), 96-7-4 (US-18), 96-9-3 (US-18), 95-9-1 (US-18), 98-104 (US-18), 97-1-1 (US-19), 97-1-2 (US-19), 97-1-3 (US-19), and 80787-94L (US-15), respectively.

**TABLE 2.** Allozyme genotypes of isolates of *Phytophthora infestans* collected from potato and tomato fields in North Carolina between 1993 and 1998

Allozyme genotype <sup>a</sup>			Number of isolates						
<i>Gpi</i>	<i>Pep</i>	Genotype	1993	1994	1995	1996	1997	1998	Total
<b>Potato</b>									
86/100	92/100	US-1	0	1	0	0	0	0	1
100/111	100/100	US-7	0	2	0	0	0	0	2
100/111/122	100/100	US-8	0	6	3	6	39	100	154
									157
<b>Tomato</b>									
100/111	100/100	US-7	4	48	1	0	0	0	53
100/111/122	100/100	US-8	1	14	0	0	0	0	15
100/100	92/100	US-18	0	0	3	14	0	3	20
100/100	92/100	US-19	0	0	0	0	5	0	5
									93

<sup>a</sup> Genotype was identified by allozyme analysis at *glucose-6-phosphate isomerase (Gpi)* and *peptidase (Pep)* loci.

nonamended rye B media (control plate). Isolates with a colony diameter less than 40% of the nonamended control at 5 and 100 µg/ml were classified as metalaxyl sensitive. Isolates with a colony diameter greater than 40% of the nonamended control at 5 µg/ml but less than 40% of the nonamended control at 100 µg/ml were classified as intermediate. Isolates with a colony diameter greater than 40% of the nonamended control at both 5 and 100 µg/ml were classified as metalaxyl resistant (6).

## RESULTS

**Mating type.** All isolates from potato and tomato were the A2 mating type with the exception of a single A1 isolate collected from a potato field in Camden County in 1994 and reported previously (11) (Table 1). Oospores were produced on the control plates inoculated with tester isolates of the opposite mating types and were not produced on plates inoculated with tester isolates of the same mating type.

**Allozyme genotypes.** Four different allozyme genotypes were identified among isolates of *P. infestans* collected between 1993 and 1998 from tomato and potato. The US-8 genotype (A2, *Gpi* 100/111/122 and *Pep* 100/100) was the only genotype identified from potato in eastern NC after 1994 (Table 2). Fifty-three isolates collected from tomato prior to 1996 were the US-7 genotype (A2, *Gpi* 100/111 and *Pep* 100/100) (Table 2). No US-7 genotypes were found among isolates from tomato or potato after 1995 (Table 2). The remaining 25 isolates collected from tomato between 1995 and 1998 were *Gpi* 100/100 and *Pep* 92/100 (Table 2). The allozyme genotype resembled US-6 but isolates were A2 mating type and sensitive to metalaxyl unlike previously identified US-6 genotypes (19). Three of these isolates collected in 1995 were identified as novel and named the US-18 genotype by allozyme genotyping in previous work (11).

**DNA fingerprinting.** The DNA fingerprint patterns of the three genotypes identified in NC (US-1, US-7, and US-8) and two other genotypes, not observed in NC, US-6 and US-15, were compared with the DNA fingerprints of the US-18 genotype found in western NC on tomatoes (Fig. 1; Table 3). Although US-18 had an allozyme genotype that was identical to US-6 at both the *Gpi* and *Pep* loci (Table 3), the DNA fingerprint patterns of this group indicated two distinct genotypes (Fig. 1). The DNA fingerprint of the US-18 genotype was most similar to the US-8 genotype with the exception that band 23 was not present in any isolates of the US-18 genotype tested. A novel genotype named US-19, collected in 1997 in Fletcher, was also A2, *Gpi* 100/100 and *Pep* 92/100, however the DNA fingerprint pattern was clearly different from US-18 (Fig. 1). The DNA fingerprint pattern of the US-19 genotype was similar to the US-1 and US-6 genotypes with the exception that bands 9 and 10 from US-1 and bands 6, 10, and 18 from

US-6 were not present (Fig. 1; Table 3). The DNA fingerprint patterns of US-18 and US-19 were different from each other and from US-15 (Fig. 1). The isolate of the US-15 genotype obtained from S. Kim in Pennsylvania (*personal communication*) is also A2, *Gpi* 100/100 and *Pep* 92/100, but has a unique DNA fingerprint that was not reported previously (19) and is shown here (Fig. 1; Table 3). Cultures of the US-15, US-18, and US-19 genotypes have been deposited into the *Phytophthora* collection at the University of California, Riverside, and the American Type Culture Collection, Rockville, MD.

**mtDNA haplotyping.** Four mtDNA haplotypes (Ia, Ib, IIa, and IIb) have been reported in *P. infestans* (23) (Fig. 2). Little variation was observed in mtDNA haplotypes among isolates in NC. All isolates of the US-7, US-8, US-18, and US-19 genotypes were the Ia mtDNA haplotype (Table 1). The US-1 genotype was the Ib mtDNA haplotype (Fig. 2). The IIa and IIb mtDNA haplotypes were not found among isolates of *P. infestans* on either potato or tomato in NC.

**Metalaxyl sensitivity.** Twenty-six (12.2%) isolates of *P. infestans* collected from tomato and potato between 1993 and 1998 were sensitive, 57 isolates (26.8%) were intermediate, and 130 isolates (61%) were resistant to metalaxyl (Table 4). Variation for metalaxyl sensitivity was observed within genotypes of these isolates. Isolates that were sensitive to metalaxyl were identified among isolates of the US-7, US-18, and US-19 genotypes from tomato (Table 4), but no metalaxyl sensitive isolates were identified among isolates of the US-8 genotype from either host (Table 4). Isolates with intermediate sensitivity to metalaxyl were found among US-7, US-8, US-18, and US-19 genotypes from both tomato and potato.

Isolates of the US-8 genotype were collected from potato in eastern NC in 1997 and 1998 (Table 1). The pathogen was isolated from tubers, leaves, and stems of potato from a field in Washington County in 1997. To our knowledge, this was the first report of tuber blight from immature daughter tubers in NC. Little blight was observed on mature tubers in the field. In 1998, late blight on potato occurred for the first time in two fields in Greene and Sampson counties (Table 1). Variation in metalaxyl sensitivity was observed among isolates of the US-8 genotype obtained from these three fields (Fig. 3A). Seventy-five percent of isolates obtained in the Greene County field were intermediate in sensitivity to metalaxyl, whereas 22 and 48% were intermediate in sensitivity to metalaxyl in the fields in Washington and Sampson counties. Equal percentages of isolates that were resistant (52%) or intermediate (48%) in sensitivity to metalaxyl were identified within the field in Sampson County. There was a greater percentage of metalaxyl resistant (82%) than intermediate isolates (22%) in the field in Washington County (Fig. 3A). No metalaxyl sensitive isolates were identified among the US-8 isolates from any of these

TABLE 3. Description of genotypes of *Phytophthora infestans* identified in North Carolina between 1993 and 1998

Genotype <sup>a</sup>	<i>Gpi</i>	<i>Pep</i>	Mating type	Mitochondrial haplotype	Metalaxyl sensitivity <sup>b</sup>	RG57 fingerprint <sup>c</sup>
US-1	86/100	92/100	A1	Ib	S	1010101011001101000110011
US-6 <sup>d</sup>	100/100	92/100	A1	IIb	R	1010111001001100010110011
US-7	100/111	100/100	A2	Ia	S to R	1000100001001101010110011
US-8	100/111/122	100/100	A2	Ia	I to R	1000100001001101000110111
US-15 <sup>d</sup>	100/100	92/100	A2	Ia	nd <sup>e</sup>	1000100001001110010110011
US-18	100/100	92/100	A2	Ia	S to I	1000100001001101000110011
US-19	100/100	92/100	A2	Ia	S to I	1010101000001101000110011

<sup>a</sup> Genotype was identified by mating type and allozyme analysis at *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) loci. US-6 and US-15 were genotypes not found in North Carolina.

<sup>b</sup> Metalaxyl sensitivity was determined by plating on rye B agar media amended with 0, 5, and 100 µg of metalaxyl per ml. S = sensitive isolates = <40% growth at 5 µg of metalaxyl per ml. I = intermediate isolates = >40% growth at 5 µg/ml and <40% growth at 100 µg of metalaxyl per ml. R = resistant isolates = >40% growth at 100 µg of metalaxyl per ml.

<sup>c</sup> Presence (1) or absence (0) of RG57 fingerprint bands. Bands 1 to 25 are indicated from left to right.

<sup>d</sup> Genotypes not observed in North Carolina but used for analysis.

<sup>e</sup> Not determined.

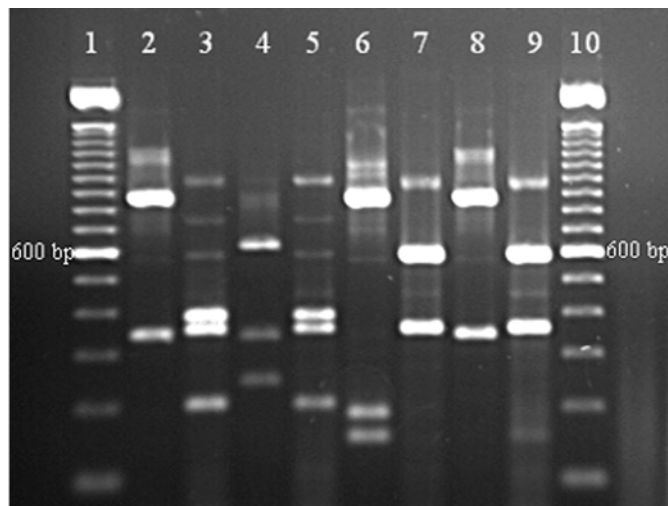
three potato fields (Fig. 3A). Metalaxyl sensitivity was similar among isolates from tubers, leaves, and stems of potato sampled in the field in Washington County in 1997. Seventy-five percent of isolates obtained from tubers were metalaxyl resistant, and 25% of isolates were intermediate in sensitivity to metalaxyl. Similarly, 70% of isolates from leaves or stems of plants were resistant to metalaxyl (Fig. 3B).

### DISCUSSION

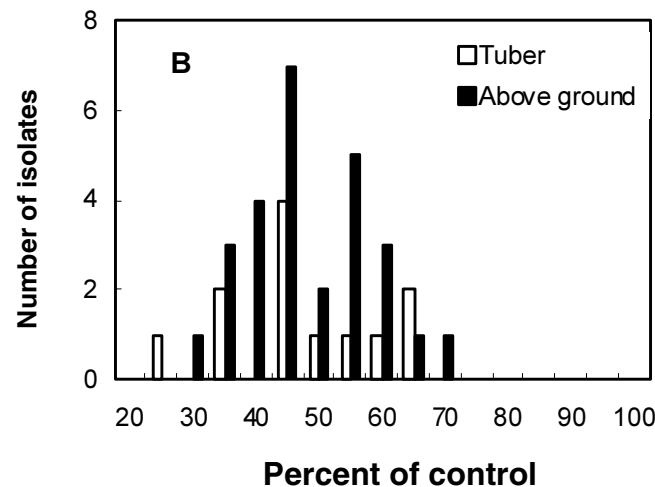
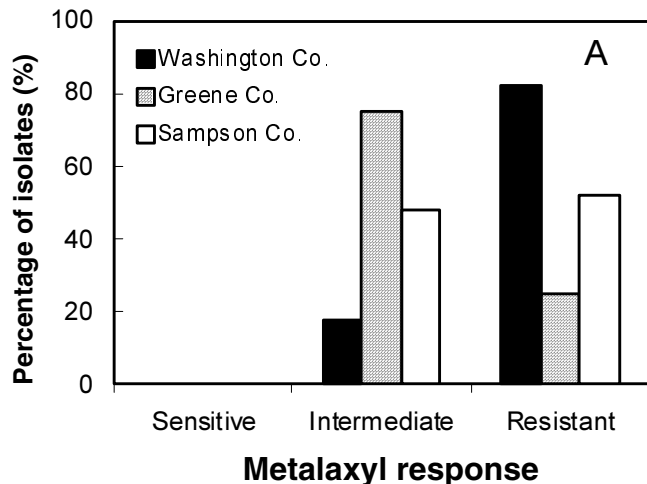
Genetic changes have occurred in the structure of late blight population on tomatoes in western NC over time. Late blight epidemics on tomato in NC in 1993 and 1994 were caused primarily by the US-7 genotype (11). The US-7 genotype was also the predominant genotype detected during 1993 throughout the United States on tomato and potato (21,22). US-7 isolates are tomato-aggressive (31). In contrast, after 1995, isolates of the US-7 genotype were not recovered from tomato fields sampled at Waynesville and Fletcher, NC, and it has been uncommon elsewhere in the United States (19). The US-18 and US-19 genotypes have been the only genotypes recovered each year since 1995 in tomato sampled in western NC and these genotypes have not been reported elsewhere in the United States (19). The US-17 genotype reported elsewhere on tomato has not been found in NC (19).

The origin of the US-18 and US-19 genotypes is still under investigation. The US-18 and US-19 genotypes have an allozyme genotype (*Gpi* 100/100 and *Pep* 92/100) and a mating type similar to the US-15 genotype found on tomato in Pennsylvania during

1994 (19). However, the DNA fingerprint pattern of the US-15 genotype is clearly different from both US-18 and US-19. In our study, the DNA fingerprint pattern of the US-18 genotype was very similar to the US-8 genotype, except band 23 did not appear in the US-18 genotype. The US-18 genotype most likely resulted from recombination between the US-1 and US-8 genotype because both genotypes have occurred in NC previously on tomato.



**Fig. 2.** Mitochondrial DNA haplotypes of *Phytophthora infestans*. DNA from every other lane was amplified with the P2 and P4 primer pairs. P2 products were digested with *MspI* and P4 products were digested with *EcoRI*. Type Ia, Ib, IIa, and IIb haplotypes are shown in lanes 2 to 3, 4 to 5, 6 to 7, and 8 to 9, respectively. Lanes 1 and 10, 100-bp DNA ladder.



**Fig. 3.** Variation in metalaxyl sensitivity among isolates of *Phytophthora infestans*. **A**, Percentage of isolates that were sensitive, intermediate, or resistant to metalaxyl within three different field locations in Washington, Greene, and Sampson counties. Total numbers from each field were 39, 16, and 29 isolates, respectively. **B**, Growth of isolates of *P. infestans* on rye B media amended with 100 µg of metalaxyl per ml expressed as a percentage of the nonamended control. Growth of the 12 isolates from tubers is compared to growth of 27 isolates from above ground portions of plants within the field in Washington County.

**TABLE 4.** Metalaxyl sensitivity and genotype of isolates of *Phytophthora infestans* collected from potato and tomato in North Carolina between 1993 and 1998

Response to metalaxyl <sup>a</sup>	Number of isolates							
	Tomatoes				Potatoes			
	US-7	US-8	US-18	US-19	US-1	US-7	US-8	Total
Sensitive	2	0	19	4	1	0	0	26
Intermediate	6	3	1	1	0	1	45	57
Resistant	44	11	0	0	0	1	74	130

<sup>a</sup> Response to metalaxyl was determined by comparing radial growth of colonies of isolates on rye B agar amended with metalaxyl at 5 and 100 µg/ml. Isolates growing less than 40% of nonamended control plate at 5 and 100 µg of metalaxyl per ml were sensitive. Isolates growing greater than 40% of nonamended control plate at 5 µg/ml but less than 40% of nonamended control plate at 100 µg/ml were metalaxyl intermediate. Isolates growing greater than 40% of nonamended control plate at both 5 and 100 µg/ml were metalaxyl resistant.

The hypothesis that the US-18 developed as a result of a mating type switch from an isolate of the US-6 genotype (11) is not supported by our genetic data or by the fact that the US-6 genotype has never been found in NC tomato fields. The US-6 genotype (*Gpi* 100/100 and *Pep* 92/100) was introduced into the United States in the late 1970s and was the most common and widespread genotype in the United States and Canada during 1987 and 1991 (14). The US-18 was first observed in fields in eastern Tennessee in 1994 (11), so there is a possibility that it migrated into NC from elsewhere.

The allozyme genotype of US-19 was identical to US-18, but DNA fingerprints were different. Unlike US-18, US-19 had additional bands 3 and 7 and lacked band 10 (Table 3; Fig. 1). DNA fingerprints of the US-19 genotype were most similar to the US-1 and US-6 genotypes with the exception that bands 9 and 10 from US-1 and bands 6, 10, and 18 from US-6 were not present in the US-19 genotype. This genotype could have arisen via recombination or have resulted from somatic variation in the US-6. Further studies are needed to support either hypothesis.

Genetic changes in the structure of late blight populations on potato in NC occurred in the earlier 1990s when the US-1 clonal lineage was replaced by the US-8 genotype (11). Prior to 1984, US-1 was the predominant genotype in populations of *P. infestans* outside Mexico (12,13). The US-1 genotype was also reported in NC in 1994 by others (19). After the detection of A2 mating type outside Mexico in 1984, changes in populations of *P. infestans* occurred in Europe and subsequently worldwide (1,6,9,14). Since 1994, the US-8 genotype is the only genotype that has been found on potato in NC. The US-8 genotype has probably increased rapidly because it is more aggressive than the US-7 genotype on potato (19,22,28,31). Late blight on potato caused by the US-8 genotype still occurs in NC and elsewhere in the United States (19). The US-11 genotype (A1 mating type) reported in 1996 and 1997 in western Washington (7,15) has not yet been observed in potato fields in NC.

Our genetic data indicate that the source of inoculum of late blight epidemics on potato and tomato are distinct in NC. On potatoes, the US-8 genotype is most likely brought into potato growing regions in eastern NC by importation of infected seed tubers. Both the US-1 and US-8 genotypes have been found in the seed producing areas of the northeast and Canada where growers purchase seed (24,32). Tuber blight was found in an eastern NC potato field for the first time in 1997 and infected seed tubers were observed. Infected potato tubers used as seed may play an important role as source of initial inoculum in epidemics of late blight in the field. Because tuber blight on mature tubers has not been commonly observed in potato fields in eastern NC, new infections most probably result from the importation of infected seed each year and not from overwintering of infected daughter tubers in soil. Initial epidemics in the fields in Washington, Sampson, and Green counties also spread from distinct foci indicating potential seedborne inoculum.

The source of inoculum for late blight epidemics on tomato is clearly different than potato. Most tomato growers in NC produce their own tomato transplants that are generally free of disease. However, extensive tomato production in Florida during winter may provide a migration route for *P. infestans* up the East Coast (11). In addition, inoculum from unsprayed tomatoes planted in home gardens may initiate epidemics. Repeated recovery of the US-18 genotype suggests that it may be surviving from season to season on tomato debris in western NC. The possibility that isolates of the US-18 genotype may survive on weed hosts or on tomato fruit and plant debris left in fields needs further exploration (33). The cool weather and mild winters in tomato growing areas in western NC may support recovery of the pathogen after overwintering.

mtDNA haplotypes were identified for some of the isolates of *P. infestans* collected. All isolates were identified as the Ia haplotype

(US-7, US-8, US-18, and US-19 genotypes) with the exception of the US-1 genotype, which was the Ib haplotype. Recent isolates collected in western Europe were also predominately the Ia haplotype (4,29). The IIa and IIb haplotypes were not found in NC on either tomato or potato but were identified from isolates obtained from other regions in the United States, Canada, and Ireland (2). mtDNA in *P. infestans* is believed to be inherited maternally from parents to progeny without recombination during sexual reproduction (1,15). All the genotypes of *P. infestans* occurring in NC are from a common mtDNA lineage.

Our data indicate that variation in metalaxyl sensitivity occurred within the US-8 genotype from the potato fields sampled. Variation among isolates within genotypes collected from different geographic locations has been reported previously, but there are few reports of variation among isolates of the same genotype collected from the same field (11,22). Research on metalaxyl sensitivity of isolates of *P. infestans* from Mexico (20) and Israel (26,27) has shown that metalaxyl resistant isolates have a higher degree of fitness than metalaxyl sensitive isolates. Studies, including this one, have found that the level of resistance to metalaxyl is unrelated to the fungicide history of the field, suggesting migration of resistant isolates (11,16,22). Resistance to metalaxyl in *P. infestans* is associated with a single dominant gene and variation among isolates of the same clonal genotype may involve minor genes (10,30).

Isolates of the US-18 and US-19 genotypes collected from tomato fields remained sensitive to metalaxyl over a 4-year period. This suggests that fungicide sensitive isolates do not have reduced fitness compared with resistant isolates (27). However, further experimentation is needed to test this hypothesis. Metalaxyl is not used routinely on tomatoes in NC. Nevertheless, sensitivity to metalaxyl in these genotypes makes the use of the fungicide on tomatoes a possibility in some fields where known sensitive genotypes occur.

Knowledge of the genetic structure of populations of *P. infestans* within NC on tomato and potato will be useful for development of improved management strategies for the pathogen. Data from previous genotype surveys of pathogen populations on tomato may not be predictive of the genotypes in successive epidemics in subsequent years on tomato crops in the state. In contrast, genotypes on potatoes are genetically less heterogeneous and have predominately been US-8 since 1995 in NC. Information on genotypes that occur in potato seed production areas in Maine and Canada that ship potato seed tubers into NC will continue to be important for disease management in the future (19,24,32). One recent study indicated that fungicide treatments of seed pieces reduce sporulation of *P. infestans* on tuber surfaces and increase plant emergence (25). Integrated management techniques, including the use of pathogen-free seed potatoes and tomato transplants, use of PCR for development of clean seed (34,37), elimination of sources of inoculum, use of fungicides on both seed tubers and foliage, host resistance, and disease forecast modeling will help growers minimize losses caused by this devastating disease.

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