European Journal of Plant Pathology **104:** 583–594, 1998.

French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype

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Accepted 15 May 1998

Key words: allozymes, DNA fingerprinting, host specificity, late blight, mating type, virulence

Abstract

Prior to 1996, the A2 mating type of *Phytophthora infestans* was not detected on potato in France, but was found at one site on tomato in 1995. This finding lead to the question of the extent of differences and relationships existing between the populations of *P. infestans* present on each host. A collection of 76 isolates collected in France, mainly in 1996, from potato and tomato was characterised for mating type, allozyme genotype at the *Gpi* and *Pep* loci, and mitochondrial DNA haplotype; 74 of these isolates were also characterised for multilocus RFLP fingerprint, and 62 for virulence. All isolates except four showed allozyme genotypes (*Gpi* 90/100 or 100/100, *Pep* 83/100 or 100/100) and mtDNA haplotypes (Ia or IIa) characteristic of the populations introduced into Europe in the late 1970s. The four exceptions were isolates collected from tomato in Southern France in 1988-1991, which showed some characteristics of the former European populations (*Gpi* 86/100, *Pep* 92/100, mtDNA Ib). Both mating types were present among the collections from both hosts, but isolates with the A2 mating type were found on potato only in one garden crop, adjacent to tomato. Nine different RG57 fingerprints were observed, with a greater diversity among tomato isolates. Furthermore, tomato and potato collections differed markedly in the frequencies of genotypes present. Finally, tomato isolates generally had a lower virulence complexity than potato isolates. These data suggest that *P. infestans* populations on tomato and potato are largely separated, despite the occurrence of limited gene flow.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of potato and tomato world-wide. The first major migration of *P. infestans* to the United States and Europe, either directly from its homeland in central Mexico (Fry et al., 1992, 1993; Goodwin et al., 1994) or from a secondary, South American centre (Tooley et al., 1989; Andrivon, 1996), was responsible for the Great Irish Famine of 1846–1849 and for dramatic crop losses in a number of European countries during the second half of the 19th century. Since this period, migrations, probably within exported seed tubers, allowed the late blight pathogen to spread throughout the world (Fry et al., 1993). As a consequence of the latest of these movements, populations of *P. infestans* have changed in several North-European countries during the 1980s. Comparison of mating types, physiological races, genotypes at the glucose phosphate isomerase (*Gpi*) and peptidase (*Pep*) allozyme loci, mitochondrial DNA haplotypes, and RFLP fingerprints showed that the 'old' European populations of the fungus were rapidly being replaced by 'new' populations, genetically different and probably originating from central Mexico (Spielman et al., 1991; Fry et al., 1992, 1993). Unlike the former populations, which were made up exclusively of isolates belonging to the A1 mating type, 'new' genotypes comprised isolates of both mating types (Fry et al., 1991; Spielman et al., 1991), providing the fungus with the opportunity to undergo sexual reproduction. The displacement mechanism of the 'old' by the 'new' population is not yet understood, although fitness differences between 'old' and 'new'

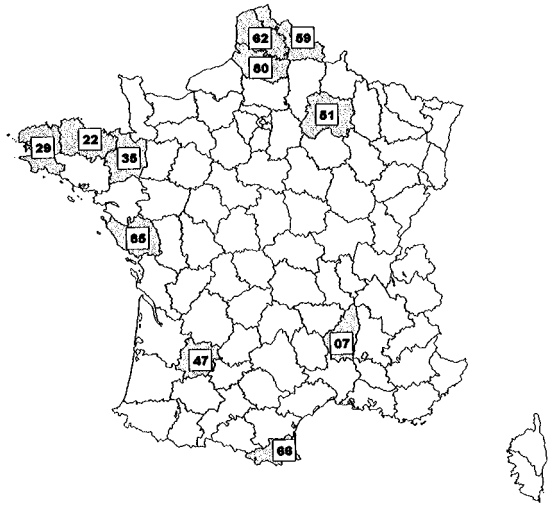

Figure 1. Location of sampling sites for the *Phytophthora infestans* collections. Numbers on the map correspond to the administrative (ZIP) numbers of the departments from which samples were collected.

genotypes (Fry et al., 1992, 1993; Kato and Fry, 1995; Kato et al., 1995, Day and Shattock, 1997) and frequent demographic bottlenecks, resulting in genetic drift and founder effects (Fry et al., 1992; Andrivon, 1994 a, 1994b), are probable contributing factors.

Contrasting with the situation in populations from northern Europe, only A1 isolates have been reported from potatoes in France (Andrivon et al., 1994; Lebreton and Andrivon, 1995). However, both A1 and A2 isolates were recovered in 1995 on blighted tomatoes from a single private garden in northern France (Lebreton et al., 1996). Evidence for some host specialisation in *P. infestans* has been provided in the past, both in 'old' and 'new' populations. Population surveys in the USA (Goodwin et al., 1995), South America (Fry et al., 1992; Forbes et al., 1997; Oyarzun et al., 1998) and Southeast Asia (Koh et al., 1994) showed host-related differences in the nature and frequency of *P. infestans* clones present. Furthermore, a significantly higher frequency of A2 isolates and of rare allozyme genotypes (such as *Pep* 100/100) in allotment gardens than in commercial potato fields has also been observed in the Netherlands (Drenth et al., 1993b). A higher fitness of A2 genotypes on tomato than on potato cultivars under European conditions provides a possible explanation for this situation. Legard et al. (1995) showed that US-7 isolates are more pathogenic to tomato than to potato. This genotype happens to be of the A2 mating type, but differs from European A2 genotypes by a number of features, such as alleles at the *Gpi* and *Pep* loci.

The observation that both mating types were present on tomato in France, but that only A1 isolates were recovered from potato raised questions about the characteristics and relationships between *P. infestans* populations present on each host plant. Unfortunately, the number of isolates obtained from tomato in 1995 was very limited, and did not allow this comparison to be made. Blighted tomatoes were therefore sampled again in 1996, both in the garden where A2 isolates were recovered in 1995, in neighbouring gardens and in commercial greenhouses. Isolates from potatoes were also obtained from the same gardens and from commercial crops from the same areas. The aim of this paper is to compare the genotypic and phenotypic characteristics of French *P. infestans* isolates collected from potato and tomato, in an attempt to better understand the structure and possible interactions between populations present on these hosts.

Materials and methods

Fungal isolates

Blighted plant material was collected in western and northern France, by officers of the French Plant Protection Service, from potato fields and from tomato plants grown in private gardens or in commercial greenhouses, and sent by mail or brought directly to the laboratory. Usually, only one isolate was kept from a given crop and sampling time; however, in a few instances, isolations were made from different plants from a single stand, in order to assess variation within a crop.

Isolations were carried out from typical symptoms on potato tubers, stems and leaves or on tomato foliage or fruits as previously described (Andrivon et al., 1994), except that pea agar supplemented with nystatin (80 $mg.l^{-1}$) and ampicillin (160 $mg.l^{-1}$), two antibiotics highly effective for isolating *P. infestans* (Sato and Kato, 1993), was used as the isolation medium. Each isolate was derived from a single lesion (stems and leaves) or from a single organ (potato tuber or tomato fruit). All isolates were transferred to and routinely maintained on clarified V8 agar at 18 °C during characterisation, and most were preserved in liquid nitrogen.

The 76 isolates retained for the analysis (Table 1) were chosen to represent as much variability as possible, on the basis of sampling sites (Figure 1), types

Table 1. Characteristics of the *Phytophthora infestans* isolates used in this study

Isolate number	Origin[1]	MT[2]	*Gpi*	*Pep*	mtDNA	RG57	Virulence
Tomato isolates							
PN01[3]	47 - u	1	86/100	92/100	Ib	nd[4]	nd
PN02[3]	07 - u	1	86/100	92/100	Ib	nd	nd
PN03[3]	66 - u	1	86/100	92/100	Ib	FR-02	nd
PN04[3]	Spain -u	1	86/100	92/100	Ib	FR-03	nd
48.96	29 - C	1	100/100	100/100	Ia	FR-01	1. 3.4 7.
49.96	29 - C	1	90/100	83/100	Ia	FR-01	4. 7.
50.96	29 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
51.96	29 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
148a.96[5]	29 - C	1	100/100	100/100	Ia	FR-01	nd
148b.96	29 - C	1	100/100	100/100	Ia	FR-01	nd
148c.96	29 - C	1	100/100	100/100	Ia	FR-01	nd
149a.96	29 - C	1	100/100	100/100	Ia	FR-01	nd
149b.96	29 - C	1	100/100	100/100	Ia	FR-01	nd
149c.96	29 - C	1	100/100	100/100	Ia	FR-01	nd
164a.96	62 - G	2	100/100	100/100	IIa	FR-04	1. 3.4. 7
164b.96	62 - G	2	100/100	100/100	IIa	FR-04	1. 3.4. 7
164c.96	62 - G	2	100/100	100/100	Ia	FR-04	1. 3.4. 7
166a.96	62 - G	1	100/100	100/100	Ia	FR-05	1. 3.4. 7
166b.96	62 - G	1	100/100	100/100	Ia	FR-05	1. 3.4. 7
166c.96	62 - G	1	100/100	100/100	Ia	FR-05	1. 3.4. 7
167a.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
167b.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
167c.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
168a.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
168b.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
168c.96	62 - G	1	100/100	100/100	Ia	FR-01	1. 3.4. 7
179a.96	35 - C	2	90/100	83/100	Ia	FR-06	1. 3.4. 7.
179b.96	35 - C	2	90/100	83/100	Ia	FR-06	1. 3.4. 7.
179c.96	35 - C	2	90/100	83/100	Ia	FR-06	1. 3.4. 7.
192.96	62 - G	1	100/100	100/100	Ia	FR-07	1. 3.4.
193.96	62 - G	1	100/100	100/100	Ia	FR-07	1. 3.4.
194.96	62 - G	1	100/100	100/100	Ia	FR-07	1. 3.4.
195.96	62 - G	1	100/100	100/100	Ia	FR-07	1. 3.4.
197.96	62 - G	1	100/100	100/100	IIa	FR-07	1. 4.
198.96	62 - G	1	100/100	100/100	IIa	FR-07	1. 4.
199.96	62 - G	1	100/100	100/100	Ia	FR-07	1. 3.4. 7.
Potato isolates							
7.95	85 - C	1	90/100	100/100	Ia	FR-01	1. 3.4. 7. 10.11.
53.95	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 11.
57.95	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4.7. 10.11.
72.95	22 - C	1	90/100	83/100	Ia	FR-01	1. 4. 11.
73.95	62 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 10.11.
104.95	51 - C	1	90/100	83/100	Ia	FR-01	1. 4. 7
108.95	80 - C	1	90/100	83/100	Ia	FR-01	1. 3.4.7. 11.
113.95	35 - G	1	100/100	100/100	IIa	FR-06	1. 4. 10.11.
114.95	29 - V	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 10.11.

Table 1. Continued

Isolate number	Origin[1]	MT[2]	*Gpi*	*Pep*	mtDNA	RG57	Virulence
35.96	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4.7. 11.
37.96	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 10.11.
38.96	22 - C	1	90/100	100/100	IIa	FR-08	1. 4.
43.96	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
54.96	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 10.11.
63.96	22 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.8.10.11.
74.96	29 - C	1	100/100	100/100	IIa	FR-07	1. 4.
80.96	29 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 10.11.
88b.96	29 - B	1	100/100	100/100	IIa	FR-07	1. 3.4. 7. 10.11.
90a.96	29..- B	1	90/100	83/100	Ia	FR-01	nd
90b.96	29 - B	1	90/100	83/100	Ia	FR-01	nd
92a.96	29 - B	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 11.
106.96	29 - B	1	90/100	83/100	Ia	FR-01	nd
109. 96	29 - B	1	90/100	83/100	Ia	FR-01	nd
129.96	35 - G	1	90/100	83/100	Ia	FR-01	1. 4. 7.
138.96	29 - B	1	100/100	100/100	IIa	FR-06	1. 4. 11.
139.96	29 - B	1	90/100	83/100	Ia	FR-01	1.2.3.4.6.7.
150.96	62 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
153.96	62 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
154.96	62 - G	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 11.
156.96	62 - G	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
157.96	62 - G	1	90/100	83/100	Ia	FR-01	1. 3.4. 7.
158b.96	29 - B	1	90/100	100/100	IIa	FR-09	1.2.3.4.6.7.
158d.96	29 - B	1	90/100	100/100	IIa	FR-09	1.2.3.4.6.7.
161.96	62 - C	1	90/100	83/100	Ia	FR-04	1. 3. 7.8.10.11.
162.96	62 - V	1	90/100	83/100	Ia	FR-04	1. 4. 7. 11.
165a.96	62 - G	2	100/100	100/100	IIa	FR-04	1. 3.4. 7.
165b.96	62 - G	2	100/100	100/100	IIa	FR-04	1. 3.4. 7.
165c.96	62 - G	2	100/100	100/100	IIa	FR-04	1. 3.4. 7.
200.96	29 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. .11.
203.96	59 - C	1	90/100	83/100	Ia	FR-01	1. 3.4. 7. 10.11.

[1] Numbers in this column correspond to the administrative number of the department of origin of the isolate; letters correspond to the type of stand (C: commercial crop; G: garden; B: Breeding nursery; V: volunteers or refuse piles; u: unknown).
[2] 1=A1; 2=A2.
[3] Isolates PN01 to PN04, kindly provided by Dr P. Nicot, INRA Montfavet, had been collected from tomato before 1992.
[4] nd: not determined.
[5] Isolates with same number and a, b, c postscripts are from different plants of a single site.

of stands (commercial crops, home gardens, refuse piles), and time of collection during the epidemic. Thirty-six of these isolates, including 32 isolates collected in France in 1996 and four isolates (three from southern France and one from Spain) recovered before 1992 and maintained in culture collections since then, were collected on tomato. The remaining 40 isolates, collected on potato, included i) 7 isolates from the private gardens where most of the tomato isolates were sampled, ii) a selection of 23 isolates from commercial fields in Brittany (western France) and in northern France, and representing the different allozyme genotypes, virulence combinations and metalaxyl sensitivities detected in a larger survey of French populations of *P. infestans* (Lebreton et al., in press), and iii) 10 isolates collected from the highly susceptible cultivar Bintje at a breeding site in western Brittany.

Mating type determination

The mating type of each isolate was determined by pairing with A1 and A2 testers on 10% clarified V8

agar at 18 °C in the dark for 6–8 days, and examining the plates under the microscope for the presence of oospores (Andrivon et al., 1994).

Virulence tests

Virulence patterns were determined as described by Andrivon (1994a), using the potato clones of the international *P. infestans* differential host set. The set included the clones containing the R1 to R11 race-specific resistance genes separately, except for the R9-carrying line which was not available. Fourteen of the 76 isolates, including the four older isolates collected from tomato (PN01 to PN04), isolates 148a-c.96 and 149a-c.96 from tomato greenhouses, and isolates 90a-b.96, 106.96 and 109.96 from the potato breeding site at Ploudaniel, were not tested for this trait.

Identification of allozyme genotypes, mitochondrial DNA haplotypes and RG57 phenotypes

Genotypes at two polymorphic allozyme loci, *Gpi* (glucose phosphate isomerase, EC 5.3.1.9) and *Pep* (peptidase, EC 3.4.3.1), were determined using the protocols of Spielman et al. (1989, 1990) modified as previously described (Lebreton et al., in press).

The PCR-RFLP method of Day & Shattock (1997) was used with several modifications to identify mitochondrial DNA haplotypes. DNA from mycelium plugs (1–2 cm^2) collected from 8–10 day old cultures on clarified V8 agar medium was extracted once with 1:1 phenol/chloroform and twice with 24:1 chloroform/isoamyl alcohol, and precipitated with 20 μl 3M NaOAc and 750 μl isopropanol (Lebreton et al., in press). The pellets were dried, washed with 50 μl of 70% ethanol and resuspended in 100 μl Tris-EDTA buffer. DNA quality and concentration were checked by measuring ODs at 260 and 280 nm on a spectrophotometer. Extracted DNA was amplified with two pairs of oligonucleotide primers (PiMtP2F/PiMtP2R and PiMtP4F/PiMtP4R) synthetized by Appligene, Illkirch, France, according to sequences provided by Dr G. Griffith and Dr D.S. Shaw, University of Wales, Bangor (pers. comm.). Amplification reactions were performed in 100 μl total volume using a Perkin Elmer 9600 thermocycler. Each reaction was prepared with 200 μM of each dNTP, 2.75 mM $MgCl_2$, oligonucleotide primers (0.325 μM each), 1 X Thermo buffer, 1U Taq DNA polymerase and 100 ng total DNA. The amplification programme consisted of one denaturation cycle of 94 °C for 90s, followed by 40 cycles of 94 °C for 40s, 60 °C for 60s, 72 °C for 90s. Mitochondrial DNA from each sample was amplified using both the PiMtP2F/PiMtP2R primers, yielding a 1243 bp amplification product (P2), and the PiMtP4F/PiMtP4R primers, yielding a 957 bp amplification product (P4). Enzymatic digestion of 8 μl amplified DNA in a 20 μl restriction digest at 37 °C for 60 min was performed using *Msp*I for the P2 amplification product and *Eco*RI for the P4 product. Digestion patterns were revealed after electrophoresis of 20 μl of digested DNA samples through a 2.5% agarose gel in a 1 X TBE buffer and staining with ethidium bromide. DNA haplotypes of unknown strains were determined by comparing their PCR-RFLP patterns with those of four reference isolates, representing the four major mitochondrial haplotypes of *P. infestans* (designated Ia, Ib, IIa, and IIb) characterised so far in world collections of the pathogen (Carter et al., 1991). Haplotypes Ia and IIa are characteristic of the genotypes introduced in Europe since the mid 1970s, while the 'old' European genotypes belonged to the Ib type; type IIb is common in western USA but very rare in Europe (D.S. Shaw, pers. comm.).

All isolates but two (PN01 and PN02) were fingerprinted using the moderately repetitive probe RG57 (Goodwin et al., 1992). Polymorphism in the restriction patterns was revealed using chemoluminescence, as described by Pipe and Shaw (1997). Profiles were scored for presence/absence of the various bands revealed by the probe, using reference isolates as controls. Bands were numbered according to Goodwin et al. (1992). Band 4 was not revealed under these conditions (ND Pipe, pers. comm.). Only a faint signal was obtained with band 19, which could therefore not be reliably scored. A multilocus genotype was derived for each isolate on the basis of the presence or absence of fragments revealed by the probe; each fragment was assumed to represent a single RFLP locus. The Shannon diversity index (Groth and Roelfs, 1987; Andrivon, 1994 b) was used to measure genotypic diversity (Drenth et al., 1993a) among isolates collected from tomatoes and potatoes. Genotypic diversity was calculated as: $H_S = -\sum_j (p_j . \ln p_j)$, j = 1.....N, where p_j is the frequency of isolates with the j^{th} genotype from tomato or potato and N is the number of genotypes present.

Data analysis

Patterns of the 74 isolates collected from potatoes and tomatoes were obtained based on the combinations

Table 2. RG57 fingerprint profiles found among French isolates of *Phytophthora infestans*

Profile	Bands [1]																							
	1	2	3	5	6	7	8	9	10	11	12	13	14	14a	15	16	17	18	20	21	22	23	24	25
F-01	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
F-02	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	0	1	1
F-03	1	0	1	1	0	1	0	1	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
F-04	1	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	0	1	1	1	0	1	1
F-05	1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	1	0	0	1	1	1	0	0	1
F-06	1	0	0	1	0	0	0	1	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
F-07	1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
F-08	1	1	0	1	1	0	1	0	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
F-09	1	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	0	1	1	1	0	1	1
US-1 [2]	1	0	1	1	0	1	0	1	1	0	0	1	1	–	0	1	0	0	1	1	0	0	1	1

[1] Bands were scored for presence (coded 1) or absence (coded 0). Band numbers are those of Goodwin et al. (1992). Band 4 was not revealed in our testing conditions. Band 19 only gave a faint signal and was therefore not scored.

[2] The US-1 profile (Goodwin et al., 1995) is included as a reference for comparison with FR-02 and FR-03, since they all correspond to isolates with the same mating type (A1) and allozyme genotype (*Gpi* 86/100 *Pep* 92/100).

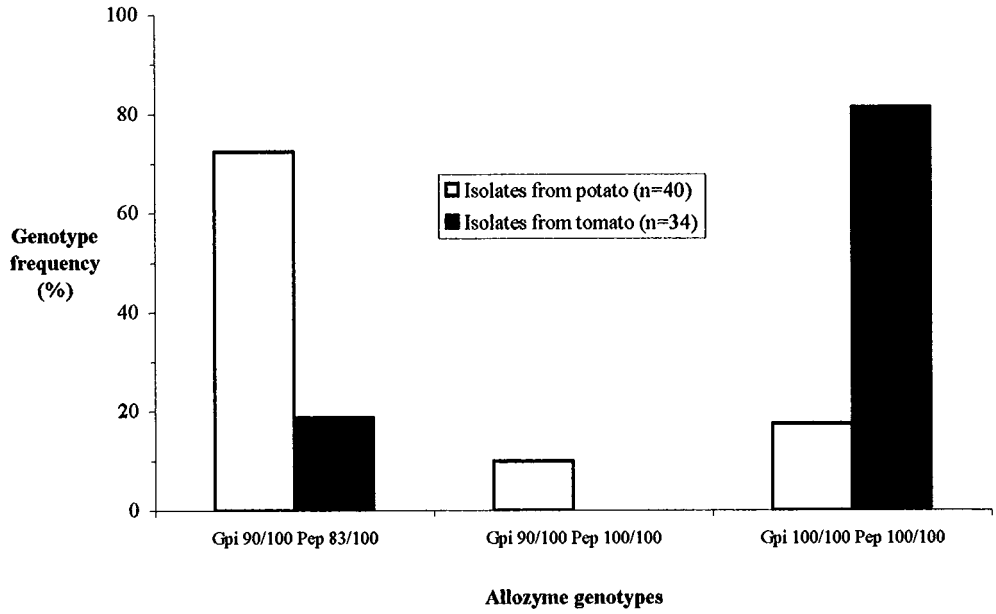

Figure 2. Frequencies of allozyme genotypes among *Phytophthora infestans* isolates collected from potatoes and tomatoes in France in 1995 and 1996.

of markers presumed to be selectively neutral: mating type, *Gpi* and *Pep* allozymes, mtDNA haplotypes and RG 57 loci. Each *Gpi* band (86,90,100) and *Pep* band (83, 92,100), mtDNA restriction fragments and RG 57-hybridizing restriction fragments were scored either as present (1) or absent (0), providing 21 different scorable traits for each isolate. mtDNA fragments were considered as separate traits because they each correspond to one restriction site. Bands of the same electrophoretic mobility were assumed to be identical. Analyses were based on Jaccard's distance d= 1-C/(2N-C), which measures the proportion of common discrete data (C) between two patterns with a total number N of different bands (Jaccard, 1908). A dendrogram was derived from the distance matrix by the UPGMA (unweighted paired group-method,

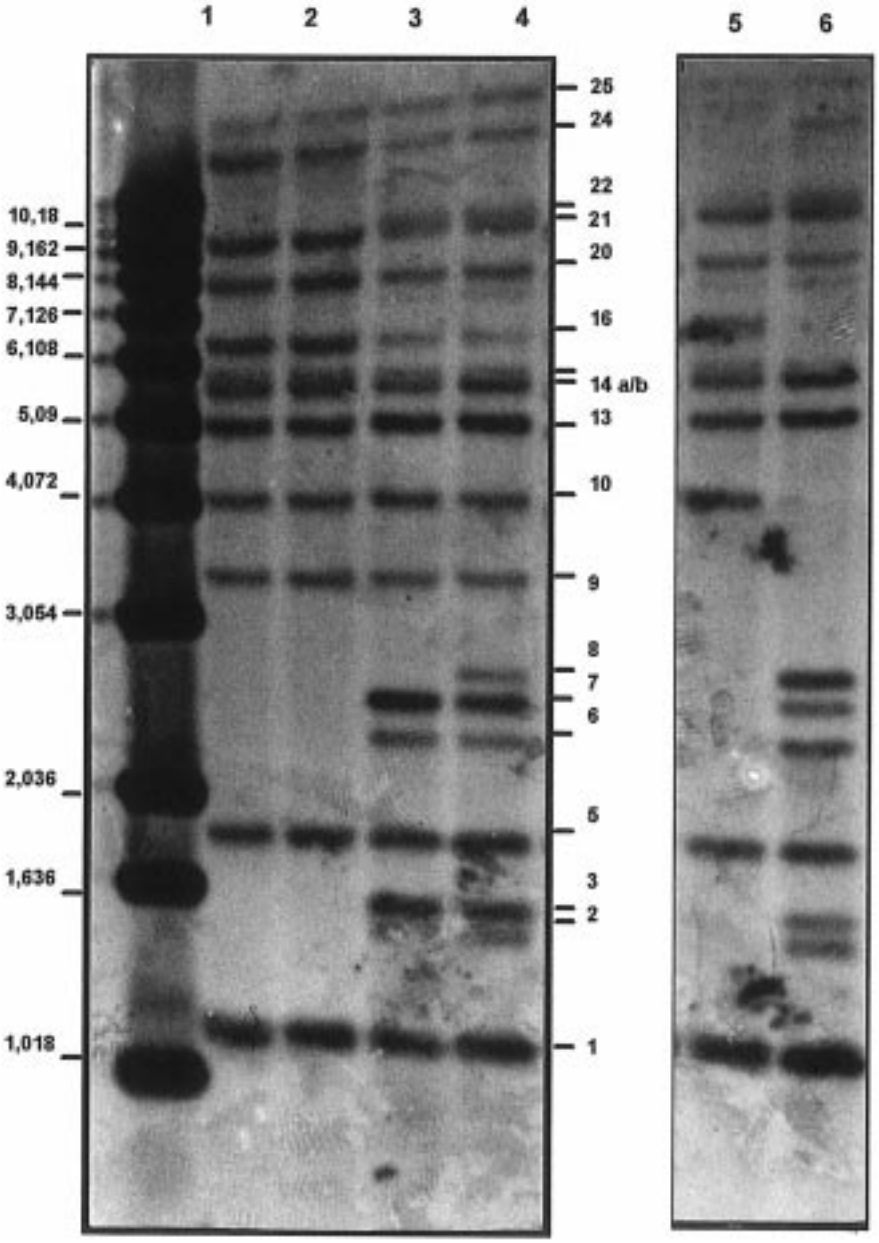

Figure 3. DNA fingerprint patterns of genotypes FR-01 (lane 4), FR-03 (lane 3), FR-04 (lane 6), FR-05 (lane 5) and FR-06 (lanes 1 and 2) of *Phytophthora infestans* found in France in 1996. Total genomic DNA of isolates 113.95 (lane 1), 138.96 (lane 2), PN03 (lane 3), 153.96 (lane 4), 166b.96 (lane 5) and 161.96 (lane 6) was digested with the restriction enzyme *Eco*RI and probed with digoxygenin-labeled RG57. Band numbers are indicated on the right; size markers in kilobases (DNA molecular weight marker X, Boehringer) are indicated on the left.

arithmetic mean) algorithm (Sneath and Sokal, 1973) contained in the computer program package Phylip 3.5 (developed by J. Felsenstein, Department of Genetics, University of Washington, Seattle). Bootstrap analyses were used (5000 replicates) to assess the degree of support for each group on the strict consensus tree.

Results

Both mating types were present among *P. infestans* isolates from potato and tomato (Table 1). On tomato, A2 isolates were recovered from a commercial greenhouse crop in Brittany (isolates 179a-c.96) and from a garden in northern France (isolates 164a-c.96). In

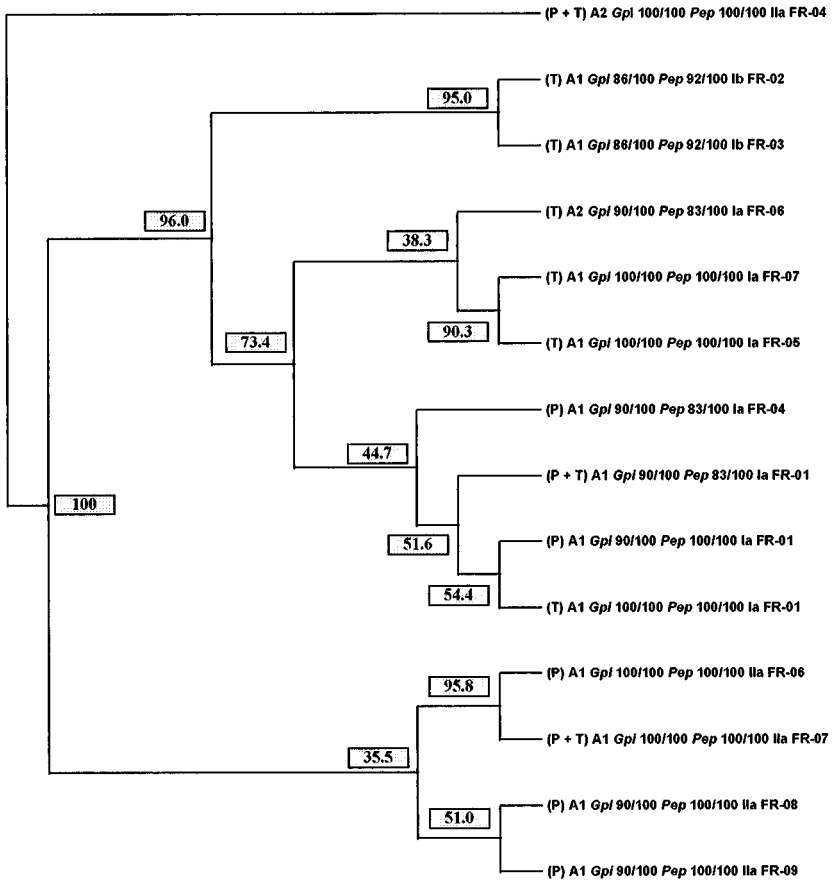

Figure 4. Dendrogram showing the relationships among *Phytophthora infestans* isolates collected from potato and from tomato. Cluster analysis was performed by the unweighted paired-group method with arithmetic mean (UPGMA) on a distance matrix calculated with the Jaccard similarity coefficient from a phenotypic description of each isolate (21 traits). The bootstraps values, noted as numbers on the main branches of the dendrogram, indicate the percentage of the 5000 iterations in which the major groups of isolates were formed.

this latter site, A2 isolates were also present on potato (isolates 165a-c.96).

Isolates collected from tomato in 1996 belonged to one of two allozyme combinations (*Gpi* 90/100 *Pep* 83/100; *Gpi* 100/100 *Pep* 100/100), the second being by far the most frequent (Figure 2). An additional genotype (*Gpi* 90/100 *Pep* 100/100) was found in four potato isolates from Brittany (isolates 7.95, 38.96, 158b.96 and 158d.96). The most common genotype among potato isolates was *Gpi* 90/100 *Pep* 83/100, contrasting with the dominance of the *Gpi* 100/100 *Pep* 100/100 genotype among tomato isolates (Figure 2). A fourth genotype (*Gpi* 86/100 *Pep* 92/100), characteristic of 'old' European populations (Spielman et al., 1991; Goodwin et al., 1994), was found in the four tomato isolates collected before 1992 (Table 1).

A total of nine different RG57 fingerprints, differing from one another by one to eight RFLP loci (Table 2), was observed among the 74 isolates tested. Three profiles were unique to tomato isolates: two (FR-02 and FR-03) were found in isolates PN03 and PN04, while the third (FR-05; Figure 3) corresponded to three isolates from a garden in northern France. Two profiles (FR-08, found only in isolate 38.96, and FR-09, in isolates 158b.96 and 158d.96) were restricted to potato isolates. Profile FR-01 (Figure 3) largely dominated among potato isolates, but had a low frequency among tomato isolates. Overall, the genotypic diversity was greater among tomato isolates (H_s = 1.41) than among potato isolates (H_s = 1.08).

Three mtDNA haplotypes were observed. Type Ib, characteristic of 'old' European populations of *P. infestans* (Carter et al., 1991), was found in the four older tomato isolates, while types Ia and IIa were re-

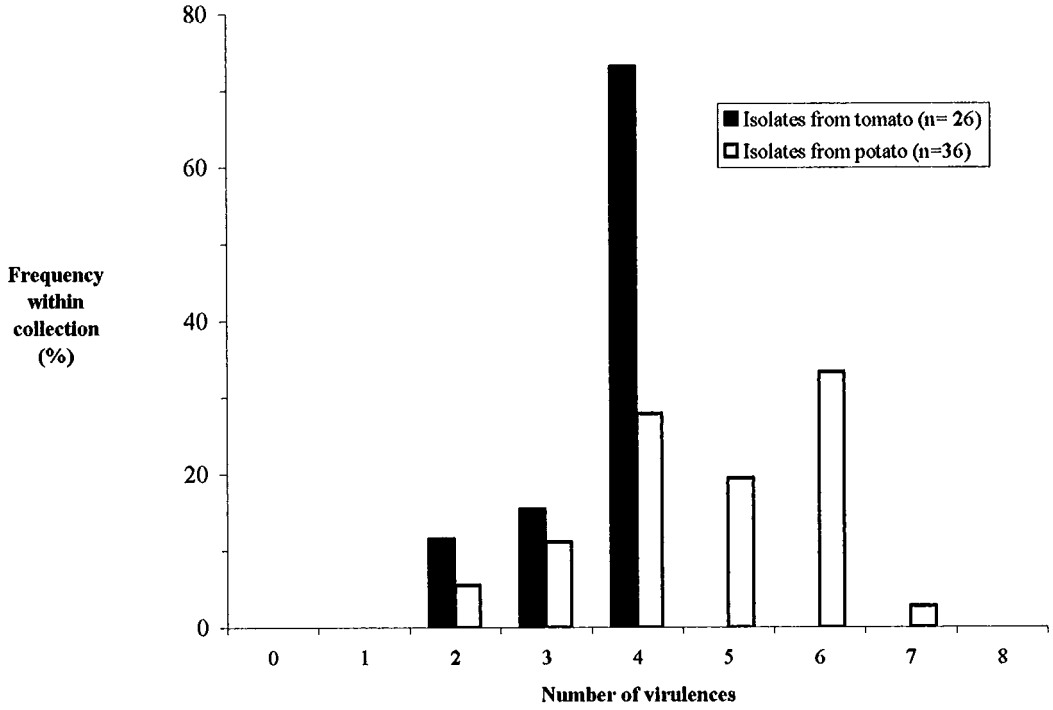

Figure 5. Distribution of isolates collected on potato and on tomato according to the number of virulence factors they carry.

covered from recent tomato and potato isolates. Type Ia largely predominated on both hosts.

A total of 14 multilocus phenotypes, based on combinations of markers presumed selectively neutral was identified. Several multilocus phenotypes were unique to one site or one isolate, particularly on potato for which four of the eight different phenotypes identified were restricted to a single site (Table 1). Only three phenotypes were found on both tomato and potato. However, the most common phenotype found on one host was infrequent on the other, reflecting the large discrepancies in the frequencies of allozyme genotypes between potato and tomato isolates. A very close association between mtDNA haplotype IIa and the 100/100 *Pep* genotype was observed among isolates collected on potato, but was not apparent among isolates sampled from tomato. A clear influence of the host of origin was evident in isolates collected in the same garden (164.96 to 168.96 and 192.96 to 198.96), although one phenotype was shared by isolates from the two hosts (164a-c.96 from tomato and 165a-c.96 from potato). UPGMA grouped the 14 multilocus phenotypes into five distinct clusters (Figure 4). Cluster 1, consisting of only one genotype combining rare traits (A2 mating type, *Gpi* 100/100 *Pep* 100/100, mDNA IIa), was clearly separated. Clusters 2 and 3 contained phenotypes isolated exclusively from tomatoes, while clusters 4 and 5 contained mainly phenotypes isolated from potatoes. The two 'tomato' isolates belonging to the 'old' European population (cluster 2) were clearly separated from the 'new' isolates collected from potatoes and tomatoes.

Four different, simple races (1.4., 4.7., 1.3.4 and 1.3.4.7) were identified among the 26 tomato isolates tested. By contrast, the 36 isolates from potato belonged to twelve, mainly complex races (Table 1). The average number of virulence factors per isolate was therefore greater among potato isolates (4.7 vs 3.6 for the tomato isolates), and the distribution of virulence complexity among tomato and potato collections was clearly different (Figure 5). No clear correlation was apparent between races and multilocus phenotypes based on neutral traits.

Discussion

Since the early 1980s, isolates of *P. infestans* belonging to the A2 mating type have been reported from most North European countries, but had not been found among the isolates collected from potatoes in

France so far (Andrivon et al., 1994; Lebreton and Andrivon, 1995). However, A2 isolates were detected for the first time in 1996 on blighted potato plants grown in gardens alongside tomatoes infected with these isolates.

While all isolates collected in 1995 and 1996 could be ascribed to the 'new' populations on the basis of their allozyme genotypes (*Gpi* 90/100 *Pep* 83/100, *Gpi* 90/100 *Pep* 100/100 or *Gpi* 100/100 *Pep* 100/100) and mtDNA haplotypes (Ia or IIa), the four tomato isolates collected in the late 1980s and early 1990s were clearly members of the 'old' population based on these criteria (*Gpi* 86/100, *Pep* 92/100, mtDNA Ib). The two isolates from this population that were tested for RG57 fingerprints yielded two profiles, clearly distinct from one another (five polymorphic bands). One of the profiles (FR-03), corresponding to an isolate collected in southern Spain in 1990, is very similar to US-1; this indicates that 'old' populations might have persisted on tomato in this area for more than 10 years after the major migration into Europe during the 1970s. The existence of a profile (FR-02) markedly different from US-1 in an isolate with traits considered characteristic of 'old' European populations (*Gpi* 86/100, *Pep* 92/100, mtDNA Ib) raises the question of the coexistence of several clones in these populations. A more thorough analysis of isolates belonging to this group -and particularly those from the Mediterranean area and/or collected from tomato- would be useful to address this issue.

Potato isolates showed a high level of similarity, and shared many features with populations from northwestern Europe, particularly the Netherlands (Fry et al., 1991; Drenth et al., 1993b, 1994). For instance, the most common allozyme phenotype was *Gpi* 90/100 *Pep* 83/100, and complex races predominated in all cases. The composition of *P. infestans* populations in western Europe can thus be regarded as quite homogeneous, but rather different from that of the former GDR or Poland, where allozyme genotypes rare in western Europe (e.g. *Gpi* 100/100 *Pep* 100/100) frequently predominated (Daggett et al., 1993; Sujkowski et al., 1994). The similarity of *P. infestans* populations on potato all across western Europe may reflect the extensive trade of seed potatoes between west-European countries and/or the lack of natural geographic barriers limiting the spread of the pathogen. However, RG57 fingerprints revealed a number of differences between French and other west European isolates of *P. infestans*. These differences, together with the high proportion of unique phenotypes in the collection of potato isolates tested, are further evidence for a metapopulation structure of the late blight pathogen in Europe (Fry et al., 1992; Andrivon, 1994a, 1994b).

While a single phenotype predominated among potato isolates, a much higher diversity was observed among tomato isolates. Furthermore, most isolates from tomato belonged to clones different from those recovered from potato, and included isolates of both mating types at the site most intensively sampled (Loos - 62). These peculiarities of tomato isolates therefore suggest the existence of host-related differences between *P. infestans* populations present on potato and tomato in France, with tomato populations being more diverse than their potato counterparts. These results confirm and extend the observations made on a small number of tomato isolates recovered in 1995 in a private garden (Lebreton et al., 1996). The six tomato isolates collected in 1995 split into 4 A1 and 2 A2; three of them shared the mtDNA IIa haplotype and *Gpi* 100/100 *Pep* 100/100 allozyme genotype (Lebreton et al., 1996). Unfortunately, these isolates were not maintained and tested for DNA fingerprints, precluding any possibility to compare them with those collected on the same site (Loos) in 1996.

A further interesting feature in French *P. infestans* populations recovered from potatoes is the very close association of the rare mtDNA IIa haplotype with the infrequent *Gpi* 100/100 *Pep* 100/100 genotype. Some of these isolates also belong to rare, simple races such as 1.4. (Table 1). Furthermore, isolates sharing the same multilocus phenotypes were found on tomato and potato plants grown adjacent to one another in the same garden (for instance isolates 164.96 and 165.96). These observations suggest that, although populations from potato and tomato appear clearly different, some limited gene flow may occur between tomato and potato populations. This gene flow could occur both ways, since several tomato isolates had all the characteristics most commonly found among potato strains (*Gpi* 90/100 *Pep* 83/100, A1, mtDNA Ia haplotype, RFLP fingerprint F-01).

The high diversity present among the isolates from tomatoes, together with the simultaneous presence in the same crop of A1 and A2 isolates, suggest that sexual reproduction of the fungus may be important on tomato plants as opposed to the situation in potato. However, although the poor preservation of the samples made observations difficult, microscopical examination of stained leaf and stem tissue of these plants did not reveal the presence of oospores. Furthermore, it should be noted that isolates collected

from a single plant always proved identical for the set of markers used, indicating that infection with more than one genotype was not frequent. Multilocus phenotypes among isolates originating from a single garden (e.g. 164–168.96 or 192–198.96) differed by one to 10 markers. While limited differences (such as between isolates 192.96 and 197.96) may be explained by the occurrence of single mutations, other explanations are required to account for the much larger distance between other pairs of isolates. Gene flow from neighbouring plots, as well as sexual reproduction, are possibilities, but the samples available do not allow to test the validity of these hypotheses.

The differences in population structures on potato and tomato may result from asexual vs sexual reproduction, but might also depend on the cultural calendar of the two crops. In regions such as Brittany, where potatoes are grown on early and short cycles (seed and early potatoes), outdoor crops of potato and tomato rarely overlap. This could favour a high influence of populations shifts from one host to the other in the course of the season, which may result in a large proportion of 'tomato adapted isolates' in the inoculum starting the epidemics on potato in the following year, and a final structure of populations quite similar on both hosts. Conversely, in areas such as Northern France, potatoes are grown during the spring, summer and autumn (ware and starch potatoes), meaning that the growing seasons of the two hosts largely overlap. This may favour the separation of populations adapted to one or the other host, with limited gene flow, particularly if the genotypic differences observed here are accompanied by pathogenicity differences. Unfortunately, the data available to date, particularly on population structures on tomato in these two regions, are too fragmentary to allow a thorough testing of this hypothesis. Further work is now needed to better quantify the influence of sex and of gene flow in those different situations.

Acknowledgements

We are much indebted to D.S. Shaw and N.D. Pipe, University of Wales, Bangor, for help with the mtDNA and RG57 technology, provision of reference isolates, and many helpful comments on the manuscript. Gift of four tomato isolates by Dr P.C. Nicot, INRA Montfavet is gratefully acknowledged. This work was funded by the Commission of European Communities, grant PL92-1363.

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