European Journal of Plant Pathology **109:** 277–281, 2003.

Short communication

Characteristics of *Phytophthora infestans* isolates from Uruguay

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Accepted 2 October 2002

Key words: allozymes, mating type, metalaxyl sensitivity, mitochondrial DNA haplotype, potato late blight

Abstract

Isolates of *Phytophthora infestans* were obtained from late blighted plants from several potato-growing regions of Uruguay in 1998 and 1999. Of these, 25 representative isolates (4 from 1998, 21 from 1999) from the main potato-growing areas of the country, were characterised in terms of mating type, metalaxyl resistance, allozyme genotype, mitochondrial haplotype, RG57 fingerprint (1999 isolates only) and pathotype. All isolates proved to be A2 mating type, monomorphic and homozygous at the loci coding for glucose-6-phosphate isomerase and peptidase (*Gpi 100/100*, *Pep 100/100*) and to possess mitochondrial haplotype IIa. Metalaxyl-resistant isolates constituted 92% of the total. All the 1999 isolates possessed the same RG57 fingerprint, which was that previously reported as associated with the clonal lineage BR-1 from Brazil and Bolivia, which is also A2, *Gpi 100/100*, *Pep 100/100*. Most of the isolates displayed broad-spectrum virulence and five carried virulence to 10 of the 11 R genes tested despite the absence of R genes in commercially grown potato cultivars. It was concluded that the Uruguayan *P. infestans* isolates resembled isolates from neighbouring South American countries, notably Brazil, and belong to the new populations of the pathogen now predominant in many countries.

Late blight, caused by the oomycete *Phytophthora infestans,* is the single greatest disease threat to commercial potato production. The Toluca Valley of Central Mexico is believed to represent the centre of origin of *P. infestans* (Niederhauser, 1991), from where it has migrated around the world. In the 1840s, the first known migration (Fry et al., 1993) indirectly caused the Irish Potato Famine. Subsequently, a single clonal lineage of the A1 mating type, known as the old population and designated US-1, became established worldwide (Goodwin et al., 1994). Late 20th century migrations of *P. infestans* from Mexico introduced new populations of the pathogen to Europe, North and South America and Asia (Fry et al., 1993). These are considered to be more aggressive, and may include both mating types and phenylamide fungicide-resistant strains, making disease control extremely difficult (Spielman et al., 1991; Drenth et al., 1994).

Late blight occurs in commercial potato and tomato crops in South America and can be very severe in the Andean region. Contemporary South American *P. infestans* populations have been investigated in several countries, but not in Uruguay (Ñústez, 1999). In the northern region of South America, the A2 mating type has not been found to date on either potato or tomato in Ecuador, the two hosts being attacked by different A1 clonal lineages of the pathogen (Forbes et al., 1997; Erselius et al., 2000). Similarly, only the A1 mating type has been reported in Colombia (Gonzalez Castano and Garcia, 1998; Ñústez, 1999) and Peru (Perez et al., 2001). Further south, Bolivian *P. infestans* populations belong predominantly to the A2 mating type (Forbes et al., 1998; Pérez et al., 1999), although the A1 and A2 mating types have been found together near the potato's presumed centre of origin (Ñústez, 1999). The A2 mating type has also been reported in Brazil

(Goodwin et al., 1994) and in Argentina (Forbes et al., 1998), but has not, so far, been identified in Chile (Rivera et al., 2002), while in Uruguay it appears that no previous testing of isolates for mating type has been carried out (F.L. Vilaro, unpubl.).

Late blight has been observed periodically in Uruguay for decades, but only became a major problem in the early 1990s (F.L. Vilaro, unpubl.). Significant losses have been recorded in spite of control programmes which have included combinations of protectant and systemic fungicides, notably metalaxyl. Infection of cultivars previously regarded as resistant has also been observed (M.C. Pagani, unpubl.). Such increased disease severity has elsewhere been associated with introduction of new genotypes of the pathogen. To determine if new *P. infestans* genotypes were associated with recent late blight epidemics in Uruguay, 25 single-sporangial isolates from foliage of eight different cultivars and two breeders' clones were selected from a collection obtained from potato crops during 1998 and 1999 and characterised using a range of markers. The crops sampled were from the main potato-growing areas of Uruguay (Table 1) and each isolate was from a different field.

Mating type was ascertained by pairing isolates with tester isolates of known mating type on agar plates and assessing oospore formation, each unknown isolate being inoculated with two A1 and two A2 reference isolates on separate plates. *In vitro* sensitivity to metalaxyl was assessed by comparing radial mycelial growth on rye agar amended with 10 mg metalaxyl l^{-1} to growth on metalaxyl-free controls (mean of three replicates); isolates were designated metalaxyl resistant (R), intermediate (I) or sensitive (S), using the criteria of Shattock (1988) and each isolate was tested at least twice. Genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase) and *Pep-1* (peptidase), were determined using the protocols of Goodwin et al. (1995) and mitochondrial DNA (mtDNA) haplotypes by PCR-RFLP using the method of Griffith and Shaw (1998), including standard isolates for comparison. DNA fingerprinting of selected isolates using probe RG57 was carried out essentially as described by Goodwin et al. (1992). Pathotype (virulence to overcome R genes) was determined by inoculation of detached leaflets of single and multiple differential potato genotypes (obtained from R. Young, West Virginia University, and the USDA Potato Introduction Station, Sturgeon Bay, Wisconsin). Two replicate leaflets per isolate were inoculated for each differential and for a susceptible R0 control and each isolate assayed at least twice; compatible reactions were defined as those in which a sporulating lesion developed.

Table 1. Collection information and characterisation of 25 isolates of *P. infestans* from Uruguay

Year	Host cultivar	Department[a] (number of isolates)	*In vitro* metalaxyl sensitivity[b]	Mating type	Allozyme genotype		mtDNA haplotype
					Gpi	*Pep*	
1998	Kennebec	Canelones (3)	R	A2	*100/100*	*100/100*	IIa
1998	Red Pontiac	Canelones (1)	R	A2	*100/100*	*100/100*	IIa
1999	Atlantic	San José (2)	R	A2	*100/100*	*100/100*	IIa
1999	Atlantic	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Atlantic	San José (1)	I	A2	*100/100*	*100/100*	IIa
1999	Breeder's clone	Canelones (1)	R	A2	*100/100*	*100/100*	IIa
1999	Breeder's clone	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Chieftain	San José (4)	R	A2	*100/100*	*100/100*	IIa
1999	Chieftain	Canelones (1)	R	A2	*100/100*	*100/100*	IIa
1999	Chieftain	Maldonado (1)	R	A2	*100/100*	*100/100*	IIa
1999	Chieftain	Río Negro (1)	R	A2	*100/100*	*100/100*	IIa
1999	Chieftain	San José (1)	I	A2	*100/100*	*100/100*	IIa
1999	Chipeta	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Ipora	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Kennebec	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Liseta	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Mondial	Río Negro (1)	R	A2	*100/100*	*100/100*	IIa
1999	Mondial	San José (1)	R	A2	*100/100*	*100/100*	IIa
1999	Red Pontiac	San José (1)	R	A2	*100/100*	*100/100*	IIa

[a]Uruguay is sub-divided into 18 departments; the four sampled represent the main potato-growing areas.
[b]S = metalaxyl sensitive, I = metalaxyl intermediate, or R = metalaxyl resistant response.

All of the 25 isolates proved to be of the A2 mating type (Table 1). *In vitro* metalaxyl sensitivity assays indicated that 23 isolates were resistant (mycelial growth on agar containing 10 mg metalaxyl l^{-1} ranged from 62% to 158% of the growth on the control), two isolates were intermediate in response (growth on 10 mg metalaxyl l^{-1} was 40–50% of control) and none was sensitive. Several of the metalaxyl-resistant isolates came from sites that had not been treated with metalaxyl. By cellulose-acetate electrophoresis (CAE), all 25 of the isolates were *Gpi 100/100* and *Pep 100/100*, regardless of the site sampled, cultivar or year of isolation. MtDNA haplotyping of the 25 isolates revealed that all were the IIa haplotype. RG57 fingerprinting of the 1999 isolates showed that all shared a common fingerprint viz. 101 110 100 000 110 000 111 101 1 (Figure 1).

Pathotype determination revealed greater diversity within the Uruguayan *P. infestans* isolates than was shown by other markers. Nine pathotypes were identified (Table 2). None of the isolates was virulent to R9, whereas all were virulent to R1 and R4. The mean number of virulence factors per isolate was 7.8 for 1998 and 6.7 for 1999 with complexity ranging from 4 to 10 of the 11 virulence factors tested. Excluding virulence to R9, the rarest virulence genes, those to resistance genes R5 and R6, were found predominantly in the most complex pathotypes.

All of the characters analysed indicate that the isolates studied, in common with those elsewhere in South America, belong to the new *P. infestans* population. This is shown most clearly by the fact that all belonged to the A2 mating type, which has not previously been reported from Uruguay, whereas the old population consists exclusively of the A1 mating type. The allozyme genotype analysis further supports the attribution of the Uruguayan *P. infestans* isolates to the new population, since none of the old dilocus genotypes, *Gpi 86/100 Pep 92/100, Gpi 86/100 Pep 100/100* or *Gpi 100/100 Pep 92/100* (Spielman et al., 1991) was found. All the Uruguayan isolates were monomorphic at both the *Gpi* and *Pep* loci under the conditions employed, but since CAE using tris-glycine buffer (Goodwin et al., 1995) is unable to resolve the cryptic *Gpi 90* allele found in some South American isolates of the *Gpi 90/100* genotype (Erselius et al., 2000), this genotype might be present within the Uruguayan populations.

The detection of mtDNA haplotype IIa in all characterised isolates is also consistent with their belonging to the new population; haplotype Ib, characteristic of the old population, was not detected. The predominance of mtDNA haplotype IIa in Uruguay is in agreement with previously published studies of isolates from other South American countries

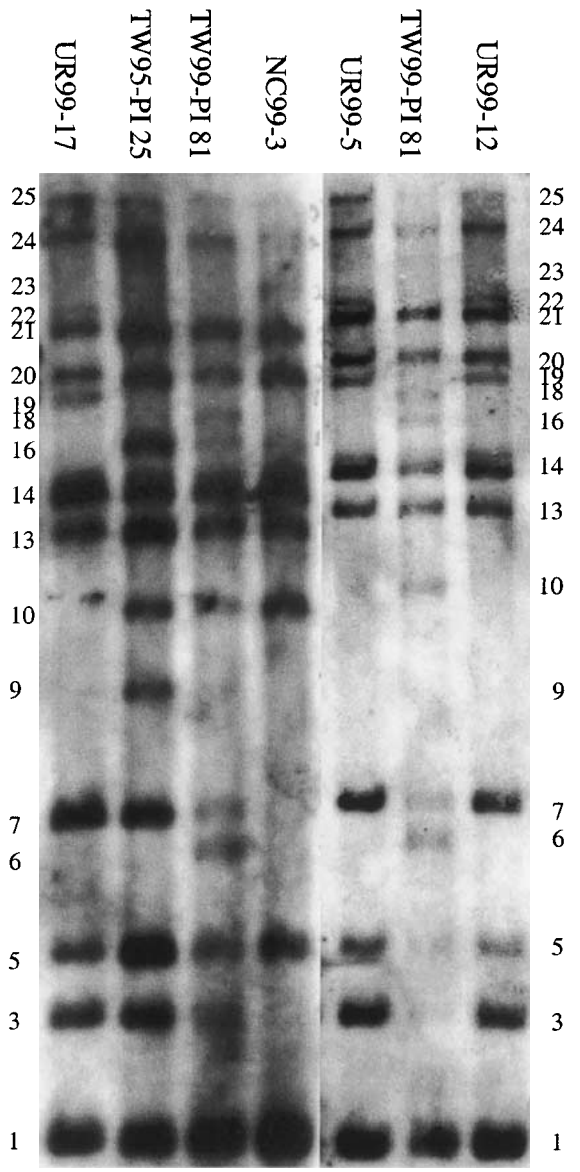

Figure 1. RG57 fingerprints of isolates of *P. infestans* from Uruguay (lanes 1,5,7; UR-99-17, UR-99-5, UR-99-12) compared with those of representative US-1 (lane 2; TW95-PI 25), US-8 (lane 4; NC99-3, lane 4) and US-11 (lanes 3 and 6; TW99-PI 81) isolates, showing that the fingerprint of the Uruguayan isolates is distinct from those of the US genotypes and corresponds to that of genotype BR-1 (101 110 100 000 110 000 111 101 1; Forbes et al., 1998). Band numbers are indicated to the right and left.

Table 2. Pathotypes and complexity characteristics of Uruguayan isolates of *P. infestans* collected in 1998 and 1999

Virulence of pathotype[a]	Number of isolates in population
0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 11	5
0, 1, 2, 3, 4, 5, 6, 7, 8, 11	3
0, 1, 2, 3, 4, 6, 7, 10, 11	2
0, 1, 2, 3, 4, 7, 8, 10, 11	6
0, 1, 2, 3, 4, 7, 11	3
0, 1, 2, 3, 4, 8, 11	1
0, 1, 4, 8, 10, 11	2
0, 1, 2, 3, 4, 7	2
0, 1, 4, 8, 11	1

[a]Based on the number of susceptible or resistant interactions on a set of differential (R gene) potato genotypes.

(Goodwin et al., 1994; Forbes et al., 1998; Gavino, 1999; Perez et al., 2001). Gavino (1999) found that IIa was the commonest haplotype in isolates from Argentina, Bolivia, Colombia, Ecuador and Peru. Although haplotype IIa is widely distributed in Europe, Asia and South America, it has not been so far reported from the United States and Canada (Gavino, 1999; K.L. Deahl, unpubl.). Haplotype Ia has been found in isolates associated with diverse genotypes of *P. infestans* in Peru, Argentina and Brazil (Gavino, 1999), but was not found in Uruguay in the present study.

The presence of metalaxyl-resistant strains is a character often, although not invariably, associated with new populations of *P. infestans*. Such strains have been found within Ecuador (Forbes et al., 1997) and several other countries of South America (Ñústez, 1999), including Argentina and Brazil.

The occurrence of complex pathotypes of *P. infestans* in the absence of selection pressure in their favour, is typical of new populations of *P. infestans* (e.g. Drenth et al., 1994). Although a range of different commercial potato cultivars is grown in Uruguay and eight of these were sampled in this study, few contain specific resistance to *P. infestans* other than the R1 gene in Kennebec and Atlantic. Thus selection of complex pathotypes by R genes is highly unlikely. In the present study, virulence tests revealed that 19 (76%) of the isolates collected in 1998 and 1999 showed virulence to at least six of the 11 R genes tested. In a comparison of structure and pathotype diversity among populations of *P. infestans* collected worldwide during 1970–1995, Andrivon (1994) and Andrivon et al. (1994) found that most populations contained a large proportion of unnecessary virulences, including virulence to R5, R7 and R8, which have never been employed in commercial potato cultivars. When the pathotype diversity was calculated according to the Gleason index used by Andrivon (1994), the figure for the Uruguay isolates in the present study was 2.49, which is comparable to the figures given by Andrivon (1994) for populations in the early 1990s in France (2.08–3.56), slightly less than in the US (4.33), but contrasts markedly with the diversity among isolates from the genetically heterogeneous, putative sexually reproducing populations of Central Mexico (10.82). One exception noted by Andrivon (1994) was Peru, where the population showed very low level complexity, but a recent study by Pérez et al. (2001) shows that the Peruvian *P. infestans* population has shifted dramatically since the 1980s and complex pathotypes now predominate.

The genotype of the Uruguayan isolates in the present study (A2 mating type, *Gpi 100/100*, *Pep 100/100*, mtDNA haplotype IIa) appears identical to the multilocus genotype BR-1, reported from Brazil, which is A2, *Gpi 100/100*, *Pep 100/100* and has the same RG57 fingerprint 101 110 100 000 110 000 111 101 1 (Goodwin et al., 1994; Forbes et al., 1998). Recently, 14 isolates from Brazil collected in 1998 and characterised as BR-1 by mating type, allozyme genotype, RG57, and metalaxyl resistance were shown to be haplotype IIa (E.S.G. Mizubuti, pers. comm.), providing further confirmation that they and the isolates from Uruguay belong to the same multilocus genotype. Genotype BR-1 is also found in Bolivia, but is distinct from the A2 genotypes found in Argentina, which possess different *Pep* alleles and RG57 fingerprints (Forbes et al., 1998).

The new *P. infestans* population found on potato in Uruguay was most probably introduced by migration within the last 20 years by routes which could include aerial spread from neighbouring potato-producing countries and import in seed potato tubers, the latter being the commonest method of long-distance spread (Fry et al., 1993). The clonal nature of the group of isolates used in the present study in terms of all characters apart from specific virulence suggests that they may have been derived from a common origin relatively recently. This is also supported by the very marked predominance of metalaxyl-resistant strains associated with this clonal lineage, which is more likely to have resulted from migration of metalaxyl-resistant genotypes into the region rather than from selection on an existing metalaxyl-sensitive population.

Many seed tubers are imported into Uruguay from Canada, the US and the Netherlands. However, introduction via North American seed is improbable since haplotype IIa has not yet been detected in *P. infestans* populations in North America. The characteristics of the Uruguayan isolates are consistent with an introduction from the Netherlands and some researchers have suggested that the new populations in South America may represent secondary migrations from Europe (Fry and Smart, 1999). However, since the same clonal lineage appears to occur in Brazil, Bolivia and Uruguay, any one of these countries might represent its point of arrival in South America; for example, the Netherlands is the major supplier of seed potatoes to Brazil. Characterisation of a much greater number of isolates obtained from all three countries is needed to provide a fuller picture of the current *P. infestans* population structure in this part of South America, which would contribute to development of improved strategies for control of late blight of potato in this region and/or indicate any changes which might lead to more severe problems. Among possible risks is that of a sexually reproducing population of the pathogen developing in the southern Andes if the clonal lineage BR-1 spreads north to encounter the A1 clonal lineages of Ecuador and Peru.

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