European Journal of Plant Pathology (2005) 113:71–81
DOI 10.1007/s10658-005-1225-9

Host specialization not detected among isolates of the EC-1 lineage of *Phytophthora infestans* attacking wild and cultivated potatoes in Peru

Guillemette Garry[1], Alberto Salas[2], Gregory A. Forbes[2,*], Wilmer Perez[2], Magnolia Santa Cruz[2] and Rebecca J. Nelson[3]
[1]*Institut National d'Horticulture, Angers cedex 01, 49045, France;* [2]*International Potato Center (CIP), Apartado 1558, Lima 12, Peru;* [3]*Cornell University, 321/322, Plant Science Building, Ithaca, NY, 14853, USA;* **Author for correspondence (Phone: +51-1-3496017; Fax: +51-1-3175326; E-mail: g.fores@cgiar.org)*

Accepted 14 July 2005

Key words: DNA fingerprinting, host specialization, metalaxyl resistance, *Phytophthora infestans*, wild *Solanaceae*

Abstract

To determine whether populations of *Phytophthora infestans* attacking wild and cultivated potatoes in the highlands of Peru are specialized on their hosts of origin, we characterized isolates using several neutral markers, metalaxyl resistance and for aggressiveness in a detached leaf assay. One hundred and fifty-three isolates were collected from the northern and central highlands of Peru from different potato cultivars (both modern and native cultivars) and from different species of wild, tuber-bearing potatoes. All the isolates analyzed belonged to one of four clonal lineages that had been described previously in Peru: EC-1, US-1, PE-3 and PE-7. The EC-1 lineage ($n = 133$) was dominant and present in similar frequencies on wild and cultivated potatoes. PE-3 ($n = 14$) was found primarily on cultivated potatoes, with only one isolate coming from a wild host. US-1 ($n = 2$) and PE-7 ($n = 4$) were rare; all but one (PE-7) occurred on wild potatoes. Isolates from the EC-1 lineage from modern cultivars were compared in three separate detached leaf inoculation assays with EC-1 isolates from the wild potato species *S. sogarandinum, S. bill-hookerii* or *S. huancabambense*, respectively. No significant interactions between isolate type (from wild or cultivated potato) and host type (wild or cultivated) were measured for any assay. It appears that the pathogen genotypes in the EC-1 lineage indiscriminately attack both wild and cultivated tuber-bearing solanaceous hosts in Peru, and breeders should be able to select for resistance using the common EC-1 lineage.

Introduction

Late blight, caused by the oomycete pathogen *Phytophthora infestans* is the most devastating disease of cultivated potatoes worldwide. *Phytophthora infestans* causes symptoms on leaves, stems and tubers, and the disease is responsible for important economic losses. The disease is primarily controlled through frequent fungicide application; however chemical control is complemented in some locations by use of cultivars with moderate levels of resistance. Fungicide usage is not only expensive but also inconsistent with worldwide efforts to decrease pesticide dependency in agriculture. Late blight management has been complicated by frequent appearance of resistance in *P. infestans* to the phenylamide fungicide, metalaxyl (Fry et al., 1993; Perez et al., 2001b).

Disease management through host resistance has failed in some cases, because qualitative resistance conditioned by major genes from *Solanum demissum* has proven to be non-durable (Wastie, 1991; Fry and Goodwin, 1997). A recently cloned gene from the wild species

S. bulbocastanum generated significant enthusiasm because of its performance in field trials in the US and Mexico (Song et al., 2003), but its durability in large-scale production has not be evaluated. Because of the ephemeral nature of resistance based on major R genes, potato improvement efforts in both developing and industrialized countries have focused on improving the levels of quantitative resistance to late blight in breeding populations (Wastie, 1991; Ewing et al., 2000; Trognitz et al., 2002). Wild tuber-bearing potatoes from central and south America have been used as the primary sources of resistance to *P. infestans* (Glendinning, 1983; van Soest et al., 1984; Rivera-Peña, 1990; Colon et al., 1995a; Trognitz et al., 2002). Several studies have recently demonstrated the geographic (Haynes et al., 1998; Forbes et al., 2005) and temporal (Colon et al., 1995b; Grünwald et al., 2002a) stability of quantitative resistance, which can result in significant reductions in fungicide use (Grünwald et al., 2002b).

Phytophthora infestans, or close relatives of this oomycete species, attack several hosts within the plant genus *Solanum*. Several of these have been shown to have host-specific populations of *P. infestans*, including tomato (*S. lycopersicum*), pear melon (*S. muricatum*), tree tomato (*S. betaceum*) and the wild species in the *Anarrhichomenum* complex in Ecuador (Oyarzun et al., 1998; Ordoñez et al., 2000; Adler et al., 2004), and tomato in Africa (Vega-Sanchez et al., 2000). Specific adaptation on tomato has been a subject of study for many years (Berg, 1926; Oyarzun et al., 1998) but adaptation to tomato is not always clearly identifiable (Lebreton et al., 1999).

While evidence is growing for host specialization in pathogen populations attacking genetically distant species in the genus *Solanum*, the situation for wild tuber-bearing species is less clear. Part of the problem arises from lack of clarity about species boundaries between the excessive number of tuber-bearing species that have been named to date (Spooner et al., 2004). Furthermore, although extensive research has been done on resistance to *P. infestans* in this group of hosts, little has been done to determine whether host specificity occurs in the pathogen genotypes that attack tuber-bearing *Solanum* spp.; we are aware only of work done in central Mexico. Until recently, researchers in central Mexico generally agreed that inoculum from cultivated potato growing in the valley bottoms was the source annually of epidemics on wild hosts growing at higher altitudes. The epidemics on wild hosts always occurred significantly later in the season and no host-related differentiation was found in the pathogen population using several neutral markers (Grünwald et al., 2001) or metalaxyl (Matuszak et al., 1991; Grünwald et al., 2001). However, more recent AFLP analysis of pathogen populations in this region indicated that the situation is probably more complex (Flier et al., 2003a). The host populations from cultivated and wild hosts could be differentiated genetically, with the latter having several unique AFLP markers. The authors hypothesized that the genetic make-up of the population attacking wild hosts was probably influenced by the R-gene structure of the wild host population and genetic drift.

In spite of the historical and contemporary interest in identifying new sources of resistance in wild tuber-bearing potatoes, in Peru and in south America in general, *P. infestans* populations attacking these hosts have not been studied for potential host specialization. If host specialization exists, it should be considered in the use of wild potatoes in plant breeding, or at least in the selection of pathogen genotypes used in screening for resistance (Flier et al., 2003b). It is therefore important to establish the potential effects of using isolates from wild potatoes *versus* isolates from cultivated potato for resistance screening.

The present work was conducted to test whether isolates of *P. infestans* attacking wild and cultivated tuber-bearing *Solanum* spp. in the highlands of Peru are host-specialized. We sampled and characterized isolates from wild and cultivated tuber-bearing *Solanum* species with phenotypic (mating type, metalaxyl resistance) and molecular markers (RFLP, allozyme, mitochondrial haplotype). To determine whether isolates were more fit on their host of origin, we analyzed aggressiveness by measuring lesion diameter on inoculated leaflets.

Materials and methods

Pathogen isolation, culture, and storage

Isolates (n = 153) were collected in a series of trips conducted between 1998 and 2000 in the departments of Ancash, Cajamarca, Huancavelica, La Libertad, Lima and Piura in northern and

central Peru (Table 1, Figure 1). Isolates (n = 55) were obtained from 22 wild tuber-bearing *Solanum* species, five modern cultivars (n = 71, note: four came from unidentified plants) and diverse native cultivars (n = 27) (Table 2). In each case, *P. infestans* was isolated from leaflets containing a single lesion. After collection, each infected leaflet was maintained in the lid of an inverted, sealed Petri dish containing a layer of 1.5% water agar in the base. Petri dishes were maintained in an insulated cooler during travel and in an incubator in the laboratory. Using this method, we could maintain infected tissue for 7–10 days between collection and isolation. Prior to isolation, Petri dishes containing infected tissue were exposed to 12 h of light per day to promote sporulation. For each isolation, sporangia from an individual lesion were washed into a container, collected on a 10-μm filter, and rinsed with sterile water. The filter system allowed efficient recovery of sporangia, even when the infected tissue was several days old and contaminated with bacteria and saprophytes. The sporangial suspension was refrigerated at 5–8 °C to promote the liberation of zoospores. Potato tuber slices (*S. choucha* cv. Huayro) were inoculated with 20 μl of the zoospore suspension per slice, and incubated at 18 °C for 5–7 days in a moist chamber. Mycelial fragments were transferred aseptically to Rye B and V-8 agar plates. After 1–2 weeks, growing colonies were transferred to Rye A agar plates and maintained at 15°C.

Table 1. Isolates of *Phytophthora infestans* collected between 1998 and 2000 in Peru from modern and native potato cultivars and wild tuber-bearing species of *Solanum*

Host	1998	1999	2000	Total
Modern cultivars				
Amarilis-INIA		5	6	11
Canchan		6	9	15
Mix			4	4
Molinera			1	1
Perricholi		5	11	16
Yungay		6	18	24
Native cultivars				
S. tuberosum ssp. *andigena*		1	6	7
S. goniocalyx		4	11	15
S. chaucha cv. *Huayro*			1	1
S. phureja			3	3
S. stenotonum			1	1
Wild potatoes				
S. acaule	1			1
S. ancophilum		1		1
S. bill-hookerii		5	1	6
S. cajamarquense	2			2
S. cantense		1		1
S. chiquidenum	1			1
S. chomatophilum	1		1	2
S. gracilifrons		2	2	4
S. hastiforme			1	1
S. huancabambense		4	3	7
S. hypacarthrum		1	1	2
S. jalcae	1			1
S. medians		1	4	5
S. mochiquense			2	2
S. multiinterruptum	1			1
S. orophylum		1		1
S. paucissectum		2	1	3
S. piurae		1	2	3
S. raquialatum		2		2
S. simplicissimum		1		1
S. sogarandinum		3		3
S. wittmackii		1	4	5
Total	7	53	93	153

Mating type determination

Mating type was determined by pairing each isolate in the collection with two isolates of known A1 mating type (Peruvian isolates 228 and 1696) on 10% clarified V-8 agar. It was not possible to use A2 test isolates for quarantine reasons; the A2 mating type has never been reported in Peru and could not be introduced. Petri dishes were incubated in darkness at 15 °C for 4 weeks, then examined for presence of oospores. This method was previously compared to a PCR technique by Judelson (1996) using S1A and SB primers to amplify the S1 locus. The latter is linked to the A1-determining allele of the mating type locus.

Metalaxyl resistance

A randomly selected subset of 137 isolates was plated onto 10% V-8 agar containing metalaxyl at concentrations of 0, 5 and 100 ppm. A plug of mycelium was placed in the centre of each Petri dish, incubated in darkness at 18 °C for 15 days, after which radial growth was measured. Isolates were considered sensitive to metalaxyl if radial growth on 5 ppm metalaxyl reached 40% of the 0 ppm control; moderately resistant if radial growth was greater than 40% of the control on 5 ppm but not on 100 ppm; and resistant if radial

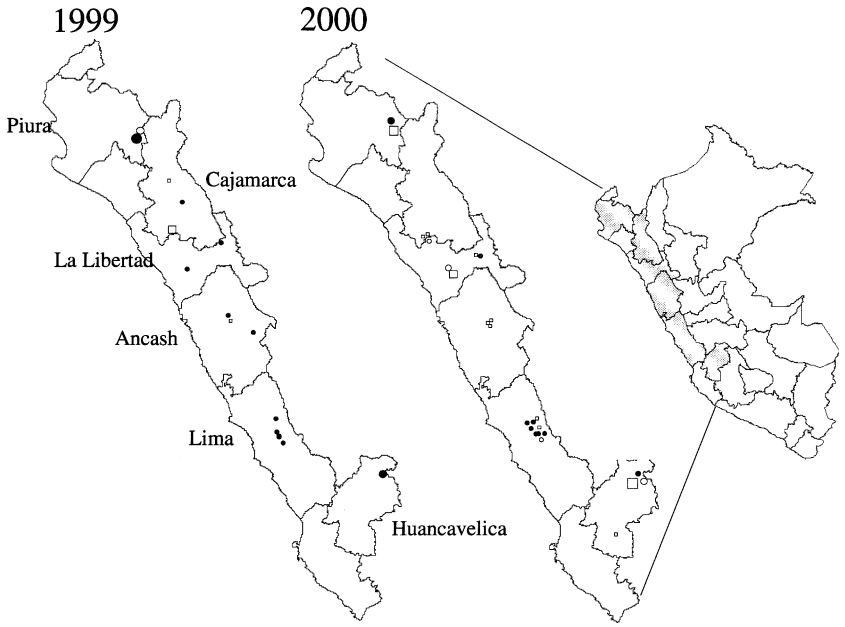

Figure 1. Location of isolates of *Phytophthora infestans* collected in Peru from wild tuber-bearing species of *Solanum* and cultivated (modern and native) potatoes in 1999 and 2000. Code: open square = modern cultivars; open circles = native cultivars; solid dots = wild species. Seven isolates collected in 1998 (Table 1) are not shown here. Symbols have been moved slightly to facilitate viewing. Larger symbols roughly represent a large number of overlapping collection locations.

Table 2. Number, description and location of clonal lineages of *Phytophthora infestans* found in Peru between 1998 and 2000 on cultivated and wild potatoes

Clonal lineage	RG57 fingerprint[a]	mtDNA	*GPI*[b]	*PEP*[b]	No. of isolates on[c]			Total	Location[d]
					Modern	Native	Wild		
EC-1	1110101001001101000111011	IIa	90/100	96/100	61	23	49	133	An, Ca, Hca, Lib, Li, Pi
PE-3	1100100001001100100111011	Ia	100/100	100/100	10	3	1	14	Ca, Li, Pi
US-1	1011101011001101000110011	Ib	86/100	92/100	0	0	2	2	Pi
PE-7	1110101001001100101111011	Ia	100/100	96/98	0	1	3	4	Hca, Li
Total					71	27	55	153	

[a]RFLP banding pattern as described previously (Forbes et al., 1998).
[b]Glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*).
[c]Modern = potato cultivars modern national institutions an a product of hybridization; Native = native cultivars of unknown origin; wild = wild tuber-forming species in the genus *Solanum*.
[d]Listed by department code: An = Ancash, Ca = Cajamarca, Hca = Huancavelica, Jun = Junin, Li = Lima, Lib = La Libertad, Pi = Piura.

growth on 100 ppm was greater than 40% of the control (Perez et al., 2001b).

Molecular analyses

RFLP fingerprints were obtained for 186 isolates using the moderately repetitive probe RG57 (Goodwin et al., 1992). Three micro grams of DNA from each isolate were digested with *Eco*RI. Hybridization and detection were conducted using the non-radioactive kit ECL^{TM} (Amersham, Inc., Piscataway, NJ, USA), according to the manufacturer's instructions. RFLP fingerprints were scored visually for the presence (1) or absence (0) of each polymorphic DNA fragment. Diversity estimates were calculated using Nei's formula (Nei, 1973): $H = [n/(n-1)] \times [1-\Sigma X_i^2]$; where X_i is the frequency of the ith genotype in the population, and n is the

number of isolates examined. Cluster analysis of the binary data was conducted using the unweighted pair group method with arithmetic mean (UPGMA) algorithm in the software programme NTSYS-pc (Rohlf, 1997). A similarity matrix was constructed using the Dice coefficient. All the isolates were analysed for mitochondrial haplotypes as described by Griffith and Shaw (1998) with the following modifications: each DNA sample was amplified by the primer pairs P1f + P1r (1118 bp) and P2f + P2r (1070 bp) and polymerase chain reaction products were digested with restriction enzymes *Cfo*I (for P1), and *Msp*I (for P2). Restriction fragment patterns were classified into four mtDNA haplotypes: Ia, Ib, IIa, IIb.

Allozyme analysis

A subset of 55 isolates (all the isolates obtained in 1999) were analyzed for their glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) genotypes using three techniques: (i) cellulose-acetate electrophoresis (CAE) according to Goodwin et al. (1994), (ii) starch-gel electrophoresis (Spielman et al., 1990), and (iii) polyacrylamide gel electrophoresis (PAGE) (Vega-Sanchez et al., 2000). Allozyme genotypes were scored as described by Spielman et al. (1990), by representing the mobilities of the enzyme alleles relative to an allele arbitrarily designated as 100. CAE was the simplest technique for determining the 86/100 *Gpi* and 92/100 *Pep* genotypes. Starch-gel electrophoresis (for the 86/100, 90/100 and 100/100 *Gpi* genotypes) and PAGE (for the 92/100, 96/100 and 100/100 *Pep* genotypes) were used when greater resolution was needed.

Aggressiveness

Plants for cross-inoculation assays were grown in ll. pots filled with a 1:1:1 (v/v/v) soil—sand–peat mixture in a glasshouse at CIP headquarters in Lima (400 m above sea level) with about 12 h of natural light per day. Relative humidity was 70–95% and temperature was 15 ± 5 °C in the glasshouse. Three modern potato cultivars were used in each of the three detached leaf assays: Amarilis-INIA (CIP 384866.5), moderately resistant to late blight; Canchan (CIP 380389.1), susceptible; and Yungay (CIP 720064), susceptible, four genotypes of the wild potato species, *S. huancabambense* (from accession number CIP 761238), one genotype of *S. sogarandinum* (from accession number CIP 761046) and one genotype of *S. bill-hookerii* (from accession number CIP 762926). In each assay, we compared the pathogenic aggressiveness of nine isolates of *P. infestans* from cultivated potato (three isolated from each cultivar) with a set of isolates from wild potatoes: three each in the cases of *S. sogarandinum* and *S. bill-hookerii*, and two in the case of *S. huancabambense*. Each isolate was inoculated on its host of origin and all other hosts in the assay. All isolate by plant genotype combinations were tested in a preliminary inoculation trial to confirm that there were no incompatible reactions as a result of major resistance genes (R genes). In each case there was a clearly visible lesion with sporulation and it was assumed that all isolates were virulent (i.e., compatible) on all plant genotypes.

Inoculum for tests was obtained from previously inoculated tuber slices of *S. choucha* cv. Huayro incubated for 6–7 days at 18 °C. Each host–pathogen combination was represented by two leaflets in a Petri dish, each leaflet with one lesion. The Petri dish was considered the experimental unit and the mean of the two lesions was used as the basic unit for analysis. There were two Petri dishes for each host-pathogen combination, which served as replications in the analysis of variance. For all assays, fully expanded leaflets from plants between 6-weeks-old and flowering initiation were placed in the lids of inverted Petri dishes with 1.5% water agar in the base. Leaflets were inoculated by placing one 20 μl drop of inoculum containing 5×10^3 sporangia ml^{-1} on the midrib, thus producing one lesion per leaflet. Inoculated leaflets were incubated at 18 °C with a 14 h light/day cycle for 5 days (Lamps LUCALOX 400 W GE, light intensity: 0.5 μE m^{-2} s^{-1}) after which lesion diameter was measured along the leaflet midrib.

The host–pathogen interaction, between isolate origin (i.e., coming from a cultivated or wild host) and inoculated host species, was tested using a general linear model (Proc GLM, SAS, Cary NC, USA) because the lesion diameter data were distributed normally. The model used for the analysis of variance was

$$\mathrm{LD} = u + a + b + a \times b + c(a) + d(b) + c \times d(a \times b) + e$$

in which LD = lesion diameter, u = the overall mean, a = origin of isolate (cultivated or wild potatoes), b = type of inoculated host species (wild or cultivated potatoes), c = specific isolate nested in origin, d = plant genotype nested in host species, e = error. The interaction effect c × d(a × b) was used as the denominator in the F test of the interaction $a \times b$, which was the primary interest of this test.

Results

Overall, 153 isolates were collected over a 3-year period from three classifications of tuber-bearing hosts (Table 1): modern cultivars, native cultivars and wild species. Modern potato cultivars are those produced by hybridization in a potato breeding programme and named by Peruvian governmental authorities. These are tetrapolid potatoes, derived primarily from *S. tuberosum*, although other species may also be involved in the pedigrees. Native cultivars are potato varieties or land races of unknown origin. Those we collected from comprised five different *Solanum* species of variable ploidy. Most isolates were collected in 1999 and 2000; only seven were collected in 1998 and all of these from wild species. All three host classifications were sampled in 1999 and 2000 (Table 1).

Mating type

After 4 weeks of incubation with A1 testers, none of the isolates produced oospores, indicating that all the isolates were of the A1 mating type which is consistent with previous reports of the four pathogen lineages detected (Goodwin et al., 1994; Forbes et al., 1997; Garry et al., 2001; Perez et al., 2001b).

Characterization of the isolates by molecular markers and sensitivity to metalaxyl

All the isolates analyzed belonged to one of four lineages (Table 2), which have all been described before. The lineage EC-1 was first described in Ecuador by Forbes et al. (1997), but was also previously found to be dominant on potato in Peru (Perez et al., 2001b). PE-3 and PE-7 have also been described previously in Peru (Garry et al., 2001; Perez et al., 2001b) and US-1 is a lineage of global distribution (Goodwin et al., 1994). *Gpi* and *Pep* allozyme patterns and the mtDNA haplotype markers for the lineages EC-1, PE-7 and US-1 (Table 2) were as reported previously; however, this is the first time the unusual *Pep* pattern has been shown (Figure 2). The *Pep* pattern of PE-7 is apparently unique; however, isozyme technology has developed over the years and comparisons with historical data must be interpreted with caution. PE-3 was previously reported to have an electrophoretic banding pattern of 100/100 for *Pep*, but our analysis demonstrated that the apparent homodimer migrates slightly faster than the 100 band that is characteristic of the US-1 lineage (Figure 2). The EC-1 lineage was dominant among the isolates collected from wild and cultivated potatoes, accounting for 133 of the 153 isolates characterized (Table 2) EC-1 was found in relatively similar proportions on all three host types and was also found in all departments. The PE-3 lineage was the second most common with 14 isolates, of which all but one occurred on cultivated potatoes. PE-3 was found in the northern (Cajamarca and Piura) and central parts (Lima) of the country. PE-7 and US-1 were rare with four and two isolates, respectively. Isolates of the PE-7 lineage were collected from two species of wild potatoes in the department of Lima (*S. medians* and *S. wittmackii*) and two native potato cultivars in Huancavelica. The US-1 lineage was only found on the wild potato species *S. raquialatum* and *S. piurae*.

Although the small sample size precluded rigorous testing, there appeared to be differences among lineages for resistance to metalaxyl (Table 3). Most isolates from the EC-1 lineage were resistant to metalaxyl, while all isolates from the other lineages were moderately resistant or sensitive. Within EC-1, resistant isolates were found on modern, native and wild potatoes.

Aggressiveness of P. infestans

In the detached leaf assay, the effects of isolate origin (factor O, Table 4) or host (factor H, Table 4) sometimes resulted in significant differences in lesion diameter at $P < 0.05$. However, the effect on lesion diameter of the interaction between host species and isolate origin was not significant for any of the comparisons made (Table 4). There were no cases in which isolates were clearly more

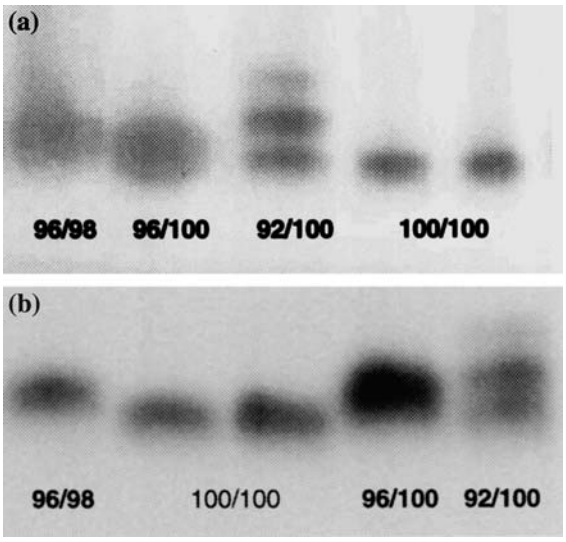

Figure 2. Peptidase banding pattern of *Phytophthora infestans* produced on polyacrylamide (a) and cellulose acetate (b). The direction of migration is from the top to bottom. Lanes identified as 96/100 are characteristic of the EC-1 clonal lineage; those identified as 96/98 are from PE-7; 92/100 is from US-1 and the lanes identified as 100/100 are from EC-3. Note that the 100/100 homodimer appears to have migrated farther than the 100 mark of the US-1 pattern indicating that 100/100 may not be correct for EC-3.

aggressive on the host type (cultivated or wild) from which they were isolated.

Discussion

We found that the pathogen population attacking wild and cultivated potato in northern and central Peru is primarily EC-1. The dominance of EC-1 on cultivated potato was reported earlier for southern Peru (Perez et al., 2001b) and Ecuador (Forbes et al., 1997). Limited information from Colombia and Venezuela indicates that EC-1 is, or until recently was, dominant on potato in those countries also (Forbes et al., 1998). EC-1 has similarities with European populations of the pathogen, from where it was probably introduced (Forbes et al., 1997). There is less certainty about the possible origin of PE-3, which Perez et al. (2001b) noted has some characteristics of EC-1 and US-1 and could result from recombination events (parasexual) between those lineages. PE-7 appears to be rare in Peru but is primarily associated with wild species (Garry et al., 2001). Given the unusual *Pep* banding pattern and its apparent association of PE-7 with wild species, its origin is also unclear.

Most of the evidence presented in this study supports the hypothesis that cultivated and wild potatoes in Peru are attacked by the same population of *P. infestans*. Thus, host specificity does not appear to play an important role in the structure of the pathogen population attacking tuber-bearing *Solanum* species in the Peruvian highlands. None of the neutral markers employed indicated pathogen population sub-structuring based on host. Furthermore, the detached leaf aggressiveness test demonstrated no interaction between isolate origin and inoculated host. This result is somewhat different from those of an apparently more complex situation in central Mexico (Grünwald et al., 2001; Flier et al., 2003a). Earlier studies on frequency of metalaxyl resistance (Matuszak et al., 1994) and also on allozyme and RFLP data (Grünwald et al., 2001) provided no evidence for host-related sub-structuing in

Table 3. Number of isolates of *Phytophthora infestans* collected between 1998 and 2000 in Peru found to be sensitive, moderately resistant or resistant to metalaxyl

Lineage	Sensitivity[a]	Number of isolates per host origin			Total
		Modern cv.	Native cv.	Wild spp.	
EC-1	Resistant	40	9	28	77
	Moderately resistant	17	11	6	34
	Sensitive	1	2	5	8
PE-3	Moderately resistant	1	1	0	2
	Sensitive	7	2	1	10
US-1	Sensitive	0	0	2	2
PE-7	Moderately resistant	0	1	0	1
	Sensitive	0	0	3	3
Total		66	26	45	137

[a]Sensitivity to metalaxyl determined as described in Materials and methods.

Table 4. Analysis of variance for diameter (cm) of lesions caused by *Phytophthora infestans* on leaflets of wild and cultivated potatoes in three separate detachedleaf assays

Source	df[a]	Mean square	*F* value[b]	*P* > *F*
Assay 1: *Solanum huancabambense* and cultivated potato				
Isolate origin: O[c]	1	3.94	27.16	< 0.0001
Host species: H[d]	1	1.26	8.69	0.0044
O × H	1	0.07	0.48	04917
Isolate (O)[e]	9	0.94	6.47	< 0.0001
Plant (H)[f]	5	0.88	6.07	0.0001
Isolate × plant (O × H)	58	0.36	2.49	0.0002
Residual error	69	0.14		
Assay 2: *Solanum sogarandinum* and cultivated potato				
Isolate origin: O	1	3.73	17.24	0.0002
Host species: H	1	2.12	9.8	0.0032
O × H	1	0.00	0.00	0.9792
Isolate (O)	10	0.61	2.81	0.0093
Plant (H)	2	1.89	8.72	0.0007
Isolate × plant (O × H)	31	0.45	2.07	0.0142
Residual error	42	0.21		
Assay 3: *Solanum bill-hookerii* and cultivated potato				
Isolate origin: O	1	0.09	0.38	0.0006
Host species: H	1	3.28	13.89	0.5412
O × H	1	0.55	2.33	0.1338
Isolate (O)	10	0.73	3.11	0.0045
Plant (H)	2	4.33	18.36	< 0.0001
Isolate×plant (O × H)	31	0.58	2.48	0.0031
Residual error	43	0.23		

[a]df = degrees of freedom.
[b]*F* = The *F*-test of interest in these assays was isolate origin by host species (O × H), which was tested with the error term of isolate × plant (O × H).
[c]Host classification (wild or cultivated) from which isolates were collected.
[d]Host classification (wild or cultivated) on which the isolates were inoculated in the trial.
[e]Specific isolate imbedded within isolate origin (e.g., isolate × from a wild host). This was considered a random factor.
[f]Specific host embedded within host classification (e.g., cv. Yungay within cultivated host). This was considered a random factor.

central Mexico; it appeared that the pathogen population on wild species was derived annually from the population on cultivated potato. More recent analysis of AFLP data, however, indicated that the population on wild potatoes is genetically distinct from that cultivated potatoes.

In central Mexico, the epidemics on wild and cultivated hosts initiate at different times, with disease occurring approximately 2 months earlier on the cultivated potatoes in the lower altitudes (Grünwald et al., 2001). In our field visits we found disease occurring simultaneously on wild and cultivated hosts, but we do not know when the epidemics started. In the highlands of Peru, the temporal dynamics of epidemics on wild and cultivated hosts are certainly affected (and probably complicated) by the fact that in many parts of the highlands conditions are appropriate for disease to occur year round.

Pathogen population sub-structuring in Mexico has been attributed to genetic drift and selection by R genes (Flier et al., 2003a). Based on segregation patterns of resistance in families of wild species from south America it would appear that unidentified R genes conferring immunity to pathogen genotypes in the EC-1 lineage are quite common (Perez et al., 2001a), although the R genes from south American species have received much less attention than those from *S. demissum*. One gene from the south American species *S. berthaultii* was mapped and shown not to be one of the known genes from *S. demissum* (Ewing et al., 2000). Therefore, R-genes may also contribute to pathogen population sub-structuring in Peru.

Nonetheless, in testing the isolates and host genotypes to be used in the detached leaf experiments in this study, we did not find any avirulence reactions.

Metalaxyl sensitivity may be one of the factors explaining the apparent association of certain rare lineages with non-cultivated potatoes, as well as the predominance of EC-1 on cultivated potato. Genotypes of US-1 and PE-7, which are predominantly sensitive to metalaxyl, were only isolated from native or wild potatoes where metalaxyl is not used or used only rarely. Metalaxyl frequency has been used as a marker for population studies in central Mexico (Matuszak et al., 1994; Grünwald et al., 2001) but this was subsequently questioned by Flier et al. (2003a). Regardless, much larger samples sizes and knowledge of fungicide application practices are needed to determine whether metalaxyl is affecting the population structure of *P. infestans* in the highlands of Peru.

The detached leaf assay we used represented a single cycle of a polycyclic disease. Epidemiologically significant factors might be hard to measure in single infection events on detached leaves. Similar studies on potato- and tomato-specific pathogen populations from Ecuador (Oyarzun et al., 1998) and sub-Saharan Africa (Vega-Sanchez et al., 2000) demonstrated that very small effects in detached leaf assays translated into near exclusivity in the field. Nonetheless, in both cases the authors were able to consistently detect host-pathogen interactions on detached leaves. These interactions were quantitative as isolates were universally pathogenic but more aggressive on their host of origin. The fact that we could not measure interactions would seem to indicate that either they do not exist or are so small as to be of negligible importance. Smaller effects that still may have significance in the field may not be detectable in a detached leaf assay.

Our results showing that the EC-1 lineage causes epidemics with equal aggressiveness on wild and cultivated hosts suggest that it is appropriate to screen for resistance in segregating host populations in areas where this pathogen lineage is dominant, which appears to be the case for the northern Andes. Screening of segregating populations in the field in Peru with naturally occurring populations of *P. infestans* should give an accurate prediction of the host–pathogen reaction, even if that host is eventually grown on a large scale. Since the most common genotypes of *P. infestans* (EC-1 lineages) were found on both wild and cultivated hosts, it appears that wild potatoes could serve as reservoirs of inoculum for the epidemics in potato crops.

Acknowledgements

This work was supported financially by the French Ministry of Foreign Affairs. We thank S. Gamboa for her technical assistance; E. de la Torre, F. Ventura, and J. Paredes for technical support; Dr. Bodo Trognitz for providing the isolates of *P. infestans* of the 1997–1998 collection; R. Hijmans for providing maps of the geographic location of the isolates of *P. infestans*; Dr. William Fry of Cornell University, USA, for providing the probe RG57; and F. de Mendiburu for statistical analyses.

References

Adler NE, Erselius LJ, Chacón MG, Flier WG, Ordoñez ME, Kroon LPNM and Forbes GA (2004) Genetic diversity of *Phytophthora infestans sensu lato* in Ecuador provides new insight into the origin of this important plant pathogen. Phytopathology 94: 154–162.

Berg A (1926) Tomato late blight and its relation to late blight of potato, Agricultural Experiment Station, College of Agriculture, West Virginia University, Morgantown, VA.

Colon LT, Budding DJ, Keizer LCP and Pieters MMJ (1995a) Components of resistance to late blight (*Phytophthora infestans*) in eight South American *Solanum* species. European Journal of Plant Pathology 101: 441–456.

Colon LT, Turkensteen LJ, Prummel W, Budding DJ and Hoogendoorn J (1995b) Durable resistance to late blight (*Phytophthora infestans*) in old potato cultivars. European Journal of Plant Pathology 101: 387–397.

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD and Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Molecular Breeding 6: 25–36.

Flier WG, Grunwald NJ, Kroon LPNM, Sturbaum AK, Bosch TBMvd, Garay-Serrano E, Lozoya-Saldana H, Fry WE and Turkensteen LJ (2003a) The population structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum* spp. Phytopathology 93: 382–390.

Flier WG, vanden Bosch GBM and Turkensteen LJ (2003b) Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. Plant Pathology 52: 326–337.

Forbes GA, Chacón G, Kirk HG, Huarte M, Damme MV, Distel S, Capezio S, Mackay G, Stewart H, Lowe R, Duncan J, Mayton H, Fry WE, Andrivon D, Ellissèche D, Pellé R, Platt H, MacKenzie G, Tarn R, Colon LT, Budding DJ, Lozoya-Saldaña H and Hernandez-Vilchis A (2005) Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathology 54: 364–372.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K and Fry WE (1997) Population genetic structure of *Phytophthora infestans* in Ecuador. Phytopathology 87: 375–380.

Forbes GA, Goodwin SB, Drenth A, Oyarzún P, Ordoñez ME and Fry WE (1998) A global marker database for *Phytophthora infestans*. Plant Disease 82: 811–818.

Fry WE and Goodwin SB (1997) Resurgence of the Irish potato famine fungus. Bioscience 47: 363–371.

Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA and Sandlan KP (1993) Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. Plant Disease 77: 653–661.

Garry G, Forbes G, Salas A, Perez W, Santa Cruz M, Pinedo HM, Gonzales E, Rivera M and Nelson RJ (2001) Characterization of *Phytophthora infestans* colonizing different Solanaceous species in Peru, with implications on the control of potato late blight. In: Ferguson P, Parrott S, Sheridan K, Smith B and Stares J (eds) Scientist and Farmer: Partners in Research for the 21st Century. Program Report 1999–2000 (pp. 39–47) International Potato Center, Lima

Glendinning DR (1983) Potato introductions and breeding up to the early 20th century. New Phytologist 94: 479–505.

Goodwin SB, Cohen BA and Fry WE (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proceedings of the National Academy of Science, USA 91: 11591–11595.

Goodwin SB, Drenth A and Fry WE (1992) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Current Genetics 22: 107–115.

Griffith GW and Shaw DS (1998) Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. Applied and Environmental Microbiology 64: 4007–4014.

Grünwald NJ, Cadena Hinojosa MA, Rubio Covarrubias OA, Rivera Peña A, Niederhauser JA and Fry WE (2002a) Potato cultivars from the Mexican national program: sources and durability of resistance against late blight. Phytopathology 92: 688–693.

Grünwald NJ, Flier WG, Sturbaum AK, Garay-Serrano E, Bosch TBMvd , Smart CD, Matuszak JM, Lozoya-Saldana H, Turkensteen LJ and Fry WE (2001) Population structure of *Phytophthora infestans* in the Toluca Valley region of Central Mexico. Phytopathology 91: 882–890.

Grünwald NJ, Romero Montes G, Lozoya Saldaña H, Rubio Covarrubias OA and Fry WE (2002b) Potato late blight management in the Toluca Valley: Field validation of SimCast modified for cultivars with high field resistance. Plant Disease 86: 1163–1168.

Haynes KG, Lambert DH, Christ BJ, Weingartner DP, Douches DS, Backlund JE, Secor G, Fry W and Stevenson W (1998) Phenotypic stability of resistance to late blight in potato clones evaluated at eight sites in the United States. American Journal of Potato Research. 75: 211–217.

Judelson HS (1996) Chromosomal heteromorphism linked to the mating type locus of the oomycete *Phytophthora infestans*. Molecular and General Genetics 252: 155–161.

Lebreton L, Lucas JM and Andrivon D (1999) Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. Phytopathology 89: 679–686.

Matuszak JM, Goodwin SB, Fry WE, Villarreal-Gonzalez MJ and Fernandez-Esqabal J (1991) The genetic structure of *Phytophthora infestans* populations in the Toluca Valley as determined by molecular markers. Phytopathology 81: 1191

Matuszak JM, Villarreal-Gonzalez MJ, Gu WK, Villarreal-Gonzalez MJ and Fry WE (1994) Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: Distribution and dynamics. Plant Disease 78: 911–916.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Science USA 70: 3321–3323.

Ordoñez ME, Hohl HR, Velasco JA, Ramon MP, Oyarzun PJ, Smart CD, Fry WE, Forbes GA and Erselius LJ (2000) A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. Phytopathology 90: 197–202.

Oyarzun PJ, Pozo A, Ordoñez ME, Doucett K and Forbes GA (1998) Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. Phytopathology 88: 265–271.

Perez W, Salas A, Raymundo R, Huaman Z, Nelson R and Bonierbale M (2001a) Evaluation of wild potato species for resistance to late blight. In: Ferguson P, Parrott S, Sheridan K, Smith B and Stares J (eds). Scientist and Farmer: Partners in Research for the 21st Century. Program. Report 1999–2000 (pp. 49–62) International Potato Center (CIP), Lima

Perez WG, Gamboa JS, Falcon YV, Coca M, Raymundo RM and Nelson RJ (2001b) Genetic structure of Peruvian populations of *Phytophthora infestans*. Phytopathology 91: 956–965.

Rivera-Peña A (1990) Wild tuber-bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the western slopes of the volcano Nevado de Toluca. 5. Type of resistance to *P. infestans*. Potato Research 33: 479–486.

Rohlf FJ (1997) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 1.70, Exeter Software, Setanket, NY, USA.

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP and Jiang J (2003) Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proceedings of the National Academy of Science, USA 100: 9128–9133.

Spielman LJ, A SJ, Shattock RC and Fry WE (1990) Segregation of allozyme markers in F_2 and backcross progeny and the inheitance of virulence against potato resistance genes R2 and R4 in F_1 progeny. Experimental Mycology 14: 57–69.

Spooner DM, vanden Berg RG, Bryan G and delRio A (2004) Species concepts and reduction of species in wild and cultivated potatoes (*Solanum* section Petota). Acta Horticulturae 619: 63–75.

Trognitz F, Manosalva P, Gysin R, Niño-Liu D, Simon R, Herrera MR, Trognitz B, Ghislain M and Nelson R (2002) Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* × Dihaploid *S. tuberosum* hybrids. Molecular Plant Microbe Interactions 15: 587–597.

vanSoest LJM, Schöber B and Tazelaar MF (1984) Resistance to *Phytophthora infestans* in tuber-bearing species of *Solanum* and its geographical distribution. Potato Research 27: 393–411.

Vega-Sanchez ME, Erselius LJ, Rodriguez AM, Bastidas O, Hohl HR, Ojiambo PS, Mukalazi J, Vermeulen T, Fry WE and Forbes GA (2000) Host adaptation to potato and tomato within the US-1 clonal lineage of *Phytophthora infestans* in Uganda and Kenya. Plant Pathology 49: 531–539.

Wastie RL (1991). Breeding for resistance. In: Ingram DS and Williams PH (eds) *Phytophthora infestans*: The Cause of Late Blight of Potato (pp 193–224) Academic Press, San Diego, CA.