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Genetic diversity and host differentiation among isolates of *Phytophthora infestans* from cultivated potato and wild solanaceous hosts in Peru

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To test the hypothesis that isolates of *Phytophthora infestans* attacking wild Solanaceae exhibit specialization for particular host species, 115 isolates of *P. infestans* were collected from cultivated potatoes, nontuber-bearing *Solanum* spp. of the *Basarthrum* section and wild tomatoes from five departments in the northern and central highlands of Peru, and characterized using several neutral markers. All isolates belonged to one of four clonal lineages described previously in Peru: EC-1, US-1, PE-3 and PE-7. There was a strong association of three lineages with host species: PE-3 was only isolated from cultivated potato, while PE-7 and US-1 were only isolated from nontuber-bearing *Solanum* spp. (*Basarthrum* section and wild tomatoes). EC-1 was isolated from all host groups sampled. A subset (n = 74) of the isolates was evaluated for metalaxyl resistance. High levels of resistance were found almost exclusively in EC-1 and PE-3, while US-1 and PE-7 isolates were generally sensitive. In a detached-leaf assay for lesion diameter using five EC-1 isolates from *S. caripense* and seven EC-1 isolates from cultivated potato, there was a significant interaction between isolate origin and inoculated host, caused by higher aggressiveness of EC-1 from cultivated potato on its host of origin. In a comparison of EC-1 (seven isolates from cultivated potato) and US-1 (three isolates from *S. caripense*), each pathogen lineage was more aggressive on its original host species, causing a highly significant interaction between isolate origin and inoculated host. Wild tomatoes and nontuber-bearing *Solanum* spp. harbour several pathogen lineages in Peru and could serve as reservoirs of inoculum that might contribute to epidemics on potato or tomato. Potential risks associated with the use of wild *Solanum* hosts as sources of resistance to *P. infestans* are discussed.

Keywords: host specificity, late blight, metalaxyl resistance, wild Solanaceae

Introduction

Late blight, caused by the oomycete pathogen *Phytophthora infestans*, is the most devastating disease of cultivated potatoes and tomatoes worldwide. Until the 1980s, only the clonal lineage US-1 was reported outside North America (Goodwin *et al*., 1994b). Since the 1980s, new clonal lineages and sexual populations with both A1 and A2 mating types have been found in different potato- and tomato-growing regions of the world (Fry *et al*., 1993). Dramatic genetic changes in *P. infestans* populations, both in recent decades and in earlier periods, were associated with migration events for which different theories have been proposed.

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Central Mexico is generally considered the centre of origin of *P. infestans* because of the apparent long-standing presence of both the A1 and A2 mating types (Goodwin *et al*., 1992; Grünwald *et al*., 2001) and a very high degree of genetic diversity in central Mexico's Toluca Valley (Niederhauser *et al*., 1954; Tooley *et al*., 1985; Goodwin & Drenth, 1997; Grünwald *et al*., 2001). *Phytophthora infestans* was apparently originally dispersed from Mexico either through a two-step migration [Mexico to USA to Europe (Niederhauser *et al*., 1954; Fry *et al*., 1993)] or a three-step migration (Goodwin *et al*., 1994b). According to the latter hypothesis, *P. infestans* migrated from Mexico to the South American Andes some centuries ago, then migrated from the Andes to North America in 1841–42, and finally moved to Europe in the 1840s from either South America or the USA, or both, leading to the late-blight epidemic that played a role in the Irish famine.

It was thought that the original introduction into Europe consisted of the clonal lineage US-1, which was subsequently spread worldwide (Goodwin *et al*., 1994a). Recent analyses,

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however, based on mitochondrial DNA (mtDNA) of *P. infestans* in herbarium material, indicate that the story may have been more complex than was originally thought, since the mtDNA sequence obtained from pathogen material dating back to the time of the Irish famine is not consistent with that of the contemporary US-1 lineage (Ristaino *et al*., 2001). Abad & Abad (1997) proposed that the Andes could be the centre of origin of *P. infestans*. This hypothesis is based on the following points: the Andes is the centre of origin for the cultivated potato; more mtDNA genotypes of *P. infestans* have been found in Peru than in central Mexico; and there are suggestive historical reports of potato disease in the Andes.

Current theories about global migrations of *P. infestans* are based primarily on what is known about isolates attacking cultivated potato and, to a lesser extent, tomato and wild tuber-bearing potatoes (Ochoa, 1954; Tooley *et al*., 1985; Goodwin *et al*., 1992, 1994a; Grünwald *et al*., 2001; Flier *et al*., 2003a). Host adaptation of *P. infestans* to tomato or potato has been examined in a number of studies, some dating back more than 80 years (for a review of the older literature, see Berg, 1926). In some studies, quantitative differentiation was detected: isolates were pathogenic on both hosts, but were differentially aggressive (Oyarzun *et al*., 1998; Vega-Sanchez *et al*., 2000; Daayf & Platt, 2003). In contrast, other authors found that isolates were classified as either aggressive on both potato and tomato, or only on tomato (Legard *et al*., 1995; Lebreton & Andrivon, 1998). Host adaptation of *P. infestans* on potato and tomato was reviewed by Oyarzun *et al*. (1998).

In a few studies, *P. infestans* isolates from hosts other than potato and tomato were analysed to determine their relationship to the pathogen population attacking potato. Cooke *et al*. (2002) and Deahl *et al*. (2004) characterized isolates from *S. dulcamara* in Northern Ireland and *S. nigrum* in Wales, respectively, with biochemical and molecular markers and obtained the same genotypes as those from nearby blighted tomato and/or potato hosts. Similarly, identical pathogen genotypes were found on potato and other wild hosts in the genus *Solanum* in Canada (Punja *et al*., 1998) and the USA (Deahl *et al*., 1995; Derie & Inglis, 2001). In another study in Europe, Flier *et al*. (2003c) detected host specificity for the potato and *S. nigrum* pathosystem, which was attributed to R genes with large effects.

Research in the highlands of Ecuador has provided intriguing evidence for *Phytophthora* diversity and host differentiation. There and elsewhere in the northern Andes, potato and tuber-bearing wild *Solanum* spp. are attacked almost exclusively by the EC-1 clonal lineage of *P. infestans* (Forbes *et al*., 1997). In contrast, tomato is attacked almost exclusively by the US-1 lineage (Oyarzun *et al*., 1998), which is also found on cultivated pear melon (*S. muricatum*) and the wild nontuber-bearing *S. caripense* (Adler *et al*., 2004). Pathogen lineage EC-3 was characterized as the lineage attacking cultivated tree tomato, *S. betaceum* (Adler *et al*., 2004). Ordoñez *et al*. (2000) described a novel group of *Phytophthora* isolates similar to *P. infestans* on two nontuber-bearing *Solanum* spp., *S. brevifolium* and *S. tetrapetalum*. This pathogen group, designated the EC-2 clonal lineage, was not aggressive on tomato or potato. A more recent study demonstrated that the putative EC-2 lineage is complex and comprises at least two novel pathogen groups that attack several *Solanum* hosts, most of which are poorly defined at the species level, but belong to the series *Anarrhichomenum*. Poor host resolution has made it difficult to draw conclusions regarding host differentiation in these novel *Phytophthora* groups, which may belong to an unidentified species of the pathogen (Adler *et al*., 2004).

In addition to providing information relevant to the evolution and epidemiology of *P. infestans*, knowledge of host adaptation could also have implications for potato breeding programmes. Although wild tuber-bearing potatoes have been used as the primary sources of resistance to *P. infestans* (Glendinning, 1983; van Soest *et al*., 1984; Colon *et al*., 1995; Trognitz *et al*., 2002), an increasing palette of transgenic technologies will facilitate the introduction of novel resistance genes from more genetically distant host genotypes (Song *et al*., 2003). In this context, wild nontuber-bearing Solanaceae could also serve as sources of resistance to *P. infestans* in the future.

Peru is a centre of diversity for wild tomato species (Peralta & Spooner, 2001) and other nontuber-bearing species in the section *Basarthrum*, such as *S. caripense* (Correll, 1962). The present work was initiated in order to give new insight into the populations of *P. infestans* (or potentially related species) attacking several of these *Solanum* spp. in Peru. To determine which populations of *P. infestans* attack different *Solanum* spp., isolates from cultivated and wild Solanaceae (wild tomatoes and *Solanum* spp. of the *Basarthrum* section) were characterized for banding patterns of the allozymes glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*), mitochondrial haplotypes and RFLP DNA fingerprints. Aggressiveness of isolates from cultivated potatoes and *S. caripense* was analysed by measuring lesion diameter on inoculated leaflets.

Materials and methods

Isolates

Isolates (n = 115) were collected on a series of trips conducted between 1997 and 2000 in the departments of Cajamarca, Huancavelica, La Libertad, Lima and Piura in northern and central Peru (Table 1). Isolates were obtained from nontuber-bearing *Solanum* spp. of the *Basarthrum* group (*S. caripense*, n = 25 and *S. montanum*, n = 1); cultivated potato (n = 64); and two species of wild tomato, *S. hirsutum* and *S. peruvianum* (n = 25) (Table 2). In each case, *P. infestans* was isolated from leaflets containing a single lesion, which in the past has produced isolates of a single isozyme or RFLP genotype (unpublished data).

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 transport and in an incubator

Table 1 Locations, years, habitats and numbers of isolates of *Phytophthora infestans* collected from different *Solanum* hosts in Peru

Department	Year	Location	Habitat[a]	Hosts (isolates)[b]
La Libertad	2000	Agallapampa, roads to Contumasa, and Cascas[c]	2, 4	*tub*(11), *bas*(1), *hir*(1)
Lima	1997	Lomas Lachay, Lomas Lima	1	*bas*(1), *per*(6)
	2000	Canta	2	*tub*(2), *hir*(2)
Cajamarca	1998	Between Contumasa and Cascas	2	*bas*(7), *hir* (2), *per*(2)
	1999	Salas, road to Llana	2, 4	*tub*(21), *bas*(1), *hir*(5), *per*(4)
	2000	San Juan	2	*bas*(3)
Huancavalica	2000	Colcabamba	4	*tub*(24)
Piura	1999	Huancabamba, Cancheque	3	*bas*(11), *hir*(1) *per*(2)
	2000	Huancabamba	3, 4	*tub*(6), *bas*(2)
Total				115

[a]Habitats: 1, coastal dunes where fog and mist promote disease in winter; 2, 'bosque seco tropical'; 3, 'bosque húmedo tropical'; 4, inter-Andean valley potato production. See 'Results' section for more information on habitats.
[b]Host codes: *tub*, S. *tuberosum*; *bas*, *Basarthrum* group; *hir*, *S. hirsutum*; *per*, *S. peruvianum.*
[c]Contumasa and Cascas are actually in Cajamarca, but are the closest towns to the collection area.

Table 2 Numbers of isolates belonging to one of four clonal lineages of *Phytophthora infestans* collected on different *Solanum* hosts in five departments of Peru between 1997 and 2000

Clonal	Host, department and number of isolates[a]				
lineage	Cultivated potatoes[b]	*Basarthrum*[c]	*S. hirsutum*	*S. peruvianum*	Total
EC-1	Caj99(13) Lib00(11) Lim00(2) Hua00(24) Piu00(5)	Lib00(1) Caj98(6) Caj00(3) Piu99(1)	Lib00(1) Lim00(1) Caj98(2) Caj99(2) Piu99(1)	Lim97(2) Caj99(1) Piu99(1)	77
PE-3	Caj99(8) Piu00(1)				9
PE-7		Lim97(1)	Lim00(1) Caj99(2)	Lim97(4) Caj98(2) Caj99(1)	11
US-1		Caj98(1) Caj99(1) Piu99(10) Piu00(2)	Caj99(1)	Caj99(2) Piu99(1)	18
Total	64	26	11	14	115

[a]Department code: Caj, Cajamarca; Hua, Huancavelica; Lib, La Libertad; Lim, Lima; Piu, Piura. Number of isolates in brackets.
[b]Primarily tetraploid *S. tuberosum.*
[c]All isolates were from *S. caripense* except the one isolate of PE-7 (Lim97), which was from *S. montanum.*

in the laboratory. Using this method, infected tissue could be maintained for 7–10 days between collection and isolation.

Pathogen isolation, culture and storage

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. chaucha* 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 agar and V-8 agar. After 1–2 weeks, growing colonies were transferred to rye A agar and maintained at 15°C.

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, and then examined for the presence of oospores. In previous comparisons

(Perez *et al.*, 2001), the results of similar attempted matings were confirmed using a PCR technique involving analysis of the S1 gene, which is linked to the A1-determining allele of the mating type locus (Judelson, 1996).

Metalaxyl resistance

Resistance to metalaxyl was measured with a procedure used previously for isolates collected from potato in Peru (Perez *et al.*, 2001). A randomly selected subset of isolates ($n = 74$) was plated on 10% agar containing metalaxyl at concentrations of 0, 5 and 100 p.p.m. A plug of mycelium was placed in the centre of each Petri dish and incubated in darkness at 18°C for 15 days, after which radial growth was measured. Isolates' resistance to metalaxyl was considered as follows: (i) sensitive to metalaxyl if radial growth on 5 p.p.m. metalaxyl reached 40% of the 0-p.p.m. control; (ii) intermediate if radial growth was greater than 40% of that of the control on 5 p.p.m., but not on 100 p.p.m. metalaxyl; and (iii) resistant if radial growth on 100 p.p.m. metalaxyl was greater than 40% of that of the control.

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) fingerprints were obtained using the moderately repetitive probe RG57 (Goodwin *et al.*, 1992). DNA from each isolate (3 μg) was digested with *Eco*RI. Hybridization and detection were performed using the nonradioactive kit ECL™ (Amersham Pharmacia Biotech, USA), according to the manufacturer's instructions. RFLP fingerprints were scored visually for the presence (1) or absence (0) of each polymorphic DNA fragment and compared with known published RFLP fingerprints (Forbes *et al.*, 1998).

Mitochondrial haplotype

All the isolates were analysed for mitochondrial haplotype as described by Griffith & Shaw (1998) with the following modifications: each DNA sample was amplified by the primer pairs P1f + P1r (amplicon size = 1118 bp) and P2f + P2r (amplicon size = 1070 bp) and regions of the mitochondrial genome and polymerase chain reaction products were digested with *Cfo*I (for P1) and *Msp*I (for P2). Restriction fragment patterns were classified into four different mtDNA haplotypes: Ia, Ib, IIa and IIb.

Allozyme pattern

A subset of isolates was analysed for their glucose-6-phosphate isomerase (*Gpi*, $n = 44$) and peptidase (*Pep*, $n = 25$) genotypes using one of two techniques: (i) cellulose-acetate electrophoresis (CAE), according to Goodwin *et al.* (1995), and (ii) starch-gel electrophoresis (Spielman *et al.*, 1990). 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) was used when greater resolution was needed.

Aggressiveness

A cross-inoculation experiment was conducted to test for differential aggressiveness between isolates from cultivated hosts and *S. caripense*. Cuttings of plant genotypes were grown in 1 L pots filled with a 1:1:1 (v/v/v) soil-sand-peatmoss mixture in a glasshouse in Lima [(400 m above sea level (asl)] with about 12 h of natural light per day. Relative humidity was 70–95% and the temperature in the glasshouse was 15 ± 5°C. Three potato cultivars were used in each detached-leaf experiment: Amarilis-INIA (CIP 384866·5), with general resistance to late blight; Canchan (CIP 380389·1), moderately resistant; and Yungay (CIP 720064), susceptible. Four genotypes of the wild *S. caripense* (CIP 762640) were also used in the experiment. All cultivated and wild plants used in these assays were first tested to confirm that they were free of major resistance genes interacting with the pathogen isolates tested. Lack of major genes was determined by the presence of a lesion with sporulation.

Fully expanded leaflets from plants between 6 weeks old and initiation of flowering were placed in inverted Petri dishes lined with 1·5% water agar in the base (leaflets lay in the lids below the agar layer). Each host–pathogen combination was represented by two Petri dishes, each containing two leaflets with one lesion per leaflet. The Petri dish was considered the experimental unit, and the mean of two lesions was used as the basic unit for analysis. The two Petri dishes were used as replicates and all Petri dishes were randomly placed in the incubation chamber.

Inoculum for the experiment was obtained from previously inoculated tuber slices of *S. choucha* cv. Huayro incubated for 6–7 days at 18°C. Leaflets were inoculated by placing a 20 μL drop of inoculum containing 5×10^3 sporangia mL^{-1} on the midrib; thus each droplet produced a single lesion. Inoculated leaflets were incubated at 18°C with 14 h light per day. Five days after inoculation, the lesion diameter was measured along the leaflet midrib.

Statistical analyses

Two different combinations of data were analysed separately in order to explore two-way interactions with simple biological interpretation. The first analysis compared EC-1 lineage isolates from both cultivated potato and *S. caripense*. Five EC-1 lineage isolates of *P. infestans* were from *S. caripense* growing along a roadway in the province of Cajamarca in 1998. Collections were separated by a distance of 0·5–5 km along the roadway in the tropical dryland forest. These were compared with seven EC-1 lineage isolates also collected in Cajamarca, but in 1999 from cultivated potato cvs Amarilis ($n = 2$), Yungay ($n = 2$) and Canchan ($n = 3$), growing in the areas of Cajeron and Cruz Blanca.

Table 3 Number, description and location of clonal lineages of *Phytophthora infestans* found in different departments in Peru between 1997 and 2000 on wild nontuber-bearing *Solanum* species and cultivated potatoes

Clonal lineage	mtDNA haplotype	*Gpi*[a]	*Pep*[b]	RG57 fingerprint[c]	Cultivated potatoes	*Basarthrum* section	Wild tomatoes
EC-1	IIa	90/100 (19)	96/100 (4)	1110101001001101000111011	55	11	11
PE-3	Ia	100/100 (8)	100/100 (6)	1100100001001100100111011	9	0	0
US-1	Ib	86/100 (14)	92/100 (12)	1011101011001101000110011	0	14	4
PE-7	Ia	100/100 (3)	96/98 (3)	1110101001001100101111011	0	1	10
Total					64	26	25

[a]Glucose-6-phosphate isomerase genotype (see 'Materials and methods'). Number of isolates in brackets.
[b]Peptidase genotype (see 'Materials and methods'). Number of isolates in brackets.
[c]Restriction fragment length polymorphism (RFLP) banding pattern as described previously (Forbes *et al.*, 1998).

In the second analysis, the seven EC-1 isolates from cultivated potato described above were compared with three US-1 isolates collected from *S. caripense* growing in the humid tropical forest area near Huancabama, Piura.

In each analysis, the interaction between isolate origin (cultivated potato or *S. caripense*) and inoculated host species (cultivated potato or *S. caripense*) was tested by ANOVA. The experimental unit for this analysis was the average diameter of the two lesions in each Petri dish. The model used for the analysis of variance was:

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

where LD is the lesion diameter, u is the overall mean, a is the origin of isolate (cultivated potato or *S. caripense*), b is the type of host species (cultivated potato or *S. caripense*), c is the isolate nested in origin, and d is the plant genotype nested in host species. Factors c and d and the interaction of c and d were treated as random. F-tests for fixed factors were constructed by PROC MIXED of SAS.

Results

Isolates were collected in areas ranging from about 700 to over 3000 m asl. Four habitats were identified, although they overlapped and there were many intermediate environments. These habitats were as follows: (1) coastal dunes < 300 m asl, characterized by high humidity in the winter, with mist and fog frequently producing appropriate conditions for disease; (2) 'bosque tropical seco' from 600 to 2500 m asl, characterized by dry cool winters and rainfall only in the summer months (November to March); (3) 'bosque tropical humido' from 700 to 2500 m asl, characterized by seasonal rainfall patterns, but with rainfall possible throughout the year; and (4) Andean valleys between 1500 and 3500 m asl, where potatoes are commonly produced on valley bottoms or slopes. Collections from cultivated potato were generally from habitat 4, *Basarthrum* from habitats 2 or 3 and wild tomatoes from habitats 2 or 4 (Table 1).

Mating-type determination

After 4 weeks of incubation with A1 testers, none of the isolates produced oospores, indicating that all were of the A1 mating type. These results are consistent with published descriptions from other locations of both the EC-1 (Forbes *et al.*, 1997) and US-1 (Goodwin *et al.*, 1994a) lineages.

Characterization of the isolates by molecular markers and sensitivity to metalaxyl

Isozyme patterns, mtDNA haplotypes and RFLP fingerprints (Table 3) were also consistent with those published for the different lineages: EC-1 (Forbes *et al.*, 1997); US-1 (Goodwin *et al.*, 1994a); PE-3 (Perez *et al.*, 2001); and PE-7 (Garry *et al.*, 2001). PE-3 and PE-7 have only been described in Peru.

The EC-1 lineage was the most common and was found in all departments sampled and on all hosts (Table 2). PE-3 was only found on cultivated potatoes, in strong contrast to lineages PE-7 and US-1, which were only found on nontuber-bearing species. Collections were made in different locations and at different times, so results may have been influenced not only by host/pathogen genetics, but also by spatial and temporal substructuring of the pathogen population. Nonetheless, in some cases different pathogen genotypes associated with different host types were isolated from the same department (e.g. Cajamarca in 1999, see Table 2). In contrast, for other collections, there was no association between host and pathogen lineage. In Cajamarca in 2000, only EC-1 was isolated from cultivated potato and *S. caripense*. Nonetheless, very small sample sizes make any temporal or spatial associations of hosts and lineages tenuous.

Metalaxyl resistance

An association between metalaxyl resistance and pathogen lineage was evident. Fully resistant isolates were only found in the EC-1 lineage, although some intermediate isolates were found in PE-3 and US-1 (Table 4). Most of the US-1 and all of the (few) PE-7 genotypes were sensitive to metalaxyl. Within EC-1, resistance was more common on cultivated potatoes, but the sample size for nontuber-bearing wild *Solanum* spp. was too small to draw conclusions.

Aggressiveness of *P. infestans*

In each of the two analyses of the data from the detached-leaf assay, the interaction of primary interest was significant

Table 4 Number of isolates of *Phytophthora infestans* collected from cultivated potatoes and wild nontuber-bearing *Solanum* spp. between 1997 and 2000 in Peru found to be sensitive, moderately resistant or resistant to metalaxyl

Clonal lineage	Sensitivity to metalaxyl	Cultivated potatoes	*S. caripense*	Wild tomatoes	Total
EC-1	Resistant	33	1	4	38
	Intermediate	9	0	0	9
	Sensitive	1	1	2	4
PE-3	Resistant	0	0	0	0
	Intermediate	0	0	0	0
	Sensitive	7	0	0	7
US-1	Resistant	0	1	0	1
	Intermediate	0	2	0	2
	Sensitive	0	5	4	9
PE-7	Sensitive	0	0	4	4
Total		50	10	14	74

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

Table 5 Analysis of variance for diameter (cm) of lesions caused by isolates of the EC-1 lineage of *Phytophthora infestans* on leaflets of *Solanum caripense* and cultivated potatoes

Source	N-df[a]	D-df[a]	*F*-value[b]	*P* > *F*
Isolate origin: O[c]	1	10	6·52	0·0287
Inoculated host type: H[d]	1	5	1·91	0·2251
O × H	1	54	6·84	0·0115

[a]N-df, numerator degrees of freedom; D-df, denominator degrees of freedom.
[b]*F*-tests were constructed using PROC RANDOM in SAS. Random factors were: (i) specific isolate embedded within isolate origin (e.g. isolate × from *S. caripense*); (ii) specific host embedded within host type (e.g. cv. Yungay within cultivated host); and (iii) the interaction of these factors.
[c]Host species from which isolates were collected. This trial compared isolates of the EC-1 lineage coming from both *S. caripense* (*n* = 5) and cultivated potatoes (*n* = 7).
[d]Host type (*S. caripense* or cultivated potato) on which the isolates were inoculated in the trial.

Table 6 Analysis of variance for diameter (cm) of lesions caused by isolates of EC-1 and US-1 lineages of *Phytophthora infestans* on leaflets of *Solanum caripense* and cultivated potato

Source	N-df[a]	D-df[a]	*F*-value[b]	*P* > *F*
Isolate origin: O[c]	1	8	9·90	0·0137
Inoculated host type: H[d]	1	5	0·01	0·9079
O × H	1	44	26·80	< 0·0001

[a]N-df, numerator degrees of freedom; D-df, denominator degrees of freedom.
[b]*F*-tests were constructed using PROC RANDOM in SAS. Random factors were: (i) specific isolate embedded within isolate origin (e.g. isolate × from *S. caripense*); (ii) specific host embedded within host type (e.g. cv. Yungay within cultivated host); and (iii) the interaction of these factors.
[c]Host species from which isolates were collected. This trial compared isolates of the EC-1 lineage coming from both *S. caripense* (*n* = 5) and cultivated potatoes (*n* = 7).
[d]Host type (*S. caripense* or cultivated potato) on which the isolates were inoculated in the trial.

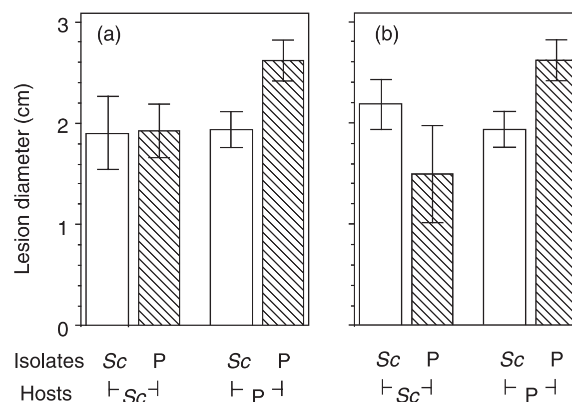

Figure 1 Average lesion diameter of isolates of *Phytophthora infestans* on four genotypes of *Solanum caripense* (*Sc*) or cultivated potatoes (P, cvs Yungay, Amarilis and Canchan). In the first analysis (a), isolates from *Sc* and from P belonged to the EC-1 lineage; in the second analysis (b), isolates from *Sc* were from the US-1 lineage, while isolates from P were from the EC-1 lineage [as in (a)]. In both graphs, open bars refer to lesion diameter when isolates came from *Sc* and shaded bars refer to lesion diameter when isolates were from P. Error bars indicate ± SEM.

at 0·05% (Tables 5 and 6). This interaction indicated that isolates from *S. caripense* and isolates from cultivated potato were generally more aggressive on their host of origin. Nonetheless, in the first analysis, which only involved isolates from the EC-1 lineage, those coming from *S. caripense* were of similar aggressiveness on both *S. caripense* and potato. The interaction effect was therefore caused by the isolates from cultivated potato, which were more aggressive on their hosts of origin (Fig. 1a). In the second experiment, involving isolates of US-1 lineage from *S. caripense* and isolates of EC-1 lineage from cultivated potato, there was a stronger interaction (Table 5), which was also more evident graphically (Fig. 1b). In this experiment, the isolates were more aggressive on their hosts of origin.

Discussion

Evidence presented in this study supports the hypothesis that some Peruvian clonal lineages of *P. infestans* are differentially adapted to their wild hosts. The results suggest that lineages PE-7 and US-1 are better adapted to nontuber-bearing species than to cultivated potato. This observation is based on isolation frequencies and, in the case of US-1, on a detached-leaf assay. The detached-leaf study indicated that US-1 is more aggressive than EC-1 on the wild species *S. caripense*. It is not known if this is also the case for US-1 on wild tomatoes, but it is a plausible hypothesis. In Ecuador, US-1 is better adapted than EC-1 to cultivated tomato (Oyarzun *et al*., 1998). PE-7 should also be tested for differential aggressiveness on wild tomatoes and cultivated potato.

In contrast to the lineages PE-7 and US-1, EC-1 was found on all host groups. Inoculation trials with only EC-1 gave unclear results, as isolates from both cultivated potato and *S. caripense* were equally aggressive on the wild host, but those from cultivated potato were more aggressive on their original host.

In the absence of clear results from cross-inoculation studies designed to measure pathogenic aggressiveness, the relationship between isolation frequency and host adaptation is tenuous. Pathogen genotypes may be isolated from alternative hosts if they comprise the majority (or all) of the available inoculum. All isolates collected from *S. ochrantum* in northern Ecuador belonged to the EC-1 lineage, but these were subsequently determined to be less well adapted pathogens coming from neighbouring potato fields (Erselius *et al*., 1999). Similarly, potato-adapted genotypes of low aggressiveness on tomato were isolated from tomato plants growing near severely infected potato fields (Adler *et al*., 2004). The fact that EC-1 was found on nontuber-bearing *Solanum* spp. in the present study may be explained by the absence of inoculum of US-1 or PE-7.

Detection of specialized pathogen isolates on nontuber-bearing *Solanum* spp. had two potential limitations in the present study: limited sample size and the possibility that the isolation technique of trapping *P. infestans* in potato tuber slices may have biased the sample by eliminating some host-specific isolates that were not well adapted to tuber tissue. While this potential source of bias cannot be ruled out, it is unlikely to be an issue in practice. Even isolates strongly adapted to tomato can grow on potato tuber slices, although they do not grow as well as isolates adapted to potato (data not shown). Therefore, while this baiting technique may be ideal for potato-adapted isolates, it also works for other isolates. With a mixture of isolates, the technique may lead to systematic selection of the one most adapted to growth on tuber slices. In future studies, several different isolation techniques should be used, including selective media (Adler *et al*., 2004). Isolation frequencies should be analysed to measure potential bias.

One of the lineages found in Peru was intriguing; PE-7, which was mostly found on wild tomatoes, had isozyme alleles not reported in a global database (Forbes *et al*., 1998), nor, apparently, elsewhere. However, isozyme identification has improved with adoption of techniques with greater resolution and this makes comparison with historical data difficult. Nonetheless, it is interesting that PE-7 has for the second time been associated with wild tomatoes in Peru (see Garry *et al*., 2001). In addition to an unusual peptidase pattern and a new RFLP genotype, PE-7 is also universally sensitive to metalaxyl, further distinguishing it from the potato population of *P. infestans*.

As in a previous study (Perez *et al*., 2001), the majority of isolates of the EC-1 lineage were resistant to metalaxyl, whereas most of the isolates from other lineages were intermediate or sensitive. While it is tempting to hypothesize that the potato population is resistant because it is exposed to this compound, while the populations on wild plants are not, this is not consistent with the fact that all seven of the PE-3 isolates from cultivated potato were highly sensitive (Table 4). Nonetheless, it does seem plausible that sensitivity in US-1 and PE-7 is to be expected, because they are rarely found on cultivated plants sprayed with this product. A high level of resistance to metalaxyl was found in isolates from unsprayed wild host species in central Mexico (Matuszak *et al*., 1994), but more recent studies demonstrated that this population is derived seasonally from epidemics on cultivated potatoes (Grünwald *et al*., 2001; Flier *et al*., 2003a).

Host specificity has implications for breeding, because of the possibility that the genetic mechanisms governing general resistance and host specificity may differ. The results of previous work indicate that even a susceptible potato cultivar can appear resistant against some tomato-adapted isolates of the pathogen (Oyarzun *et al*., 1998; Vega-Sanchez *et al*., 2000). It can be speculated that, conversely, resistance derived from wild species might be effective against pathogen strains adapted to potato, but not against strains adapted to the wild host. For this reason, knowledge of potential host adaptation in pathogen populations may improve the efficiency of selection for resistance. Recently, Flier *et al*. (2003b) reported pathogen adaptation to specific cultivated potato genotypes and suggested that selection for resistance should be carried out using a mixture of diverse and highly aggressive isolates.

From an epidemiological point of view, wild *Solanum* spp. (*Basarthrum* section), as well as wild tomatoes, could serve as reservoirs of inoculum for the epidemics in potato crops as both wild and cultivated hosts can harbour common lineages of *P. infestans*. The pathogen may remain active in humid refugia on wild plants during periods when conditions are not conducive to survival in potato-growing areas.

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References

Abad ZG, Abad JA, 1997. Another look at the origin of late blight of potatoes, tomatoes, and pear melon in the Andes of South America. *Plant Disease* **81**, 682–8.

Adler NE, Erselius LJ, Chacón MG, Flier WG, Ordoñez ME, Kroon LPNM, Forbes GA, 2004. Genetic diversity of *Phytophthora infestans sensu lato*. Ecuador provides new insight into the origin of this important plant pathogen. *Phytopathology* **94**, 154–62.

Berg A, 1926. *Tomato Late Blight and its Relation to Late Blight of Potato*. Morgantown, WV, USA: Agricultural Experiment Station, College of Agriculture, West Virginia University.

Colon LT, Budding DJ, Keizer LCP, Pieters MMJ, 1995. Components of resistance to late blight (*Phytophthora infestans*) in eight South American *Solanum* species. *European Journal of Plant Pathology* **101**, 441–56.

Cooke LR, Carlisle DJ, Wilson DG, Deahl KL, 2002. Natural occurrence of *Phytophthora infestans* on woody nightshade (*Solanum dulcamara*) in Ireland. *Plant Pathology* **51**, 392.

Correll DS, 1962. *The Potato and its Wild Relatives*. Renner, TX, USA: Texas Research Foundation.

Daayf F, Platt HW, 2003. Differential pathogenicity on potato and tomato of *Phytophthora infestans* US-8 and US-11 strains isolated from potato and tomato. *Canadian Journal of Plant Pathology* **25**, 150–4.

Deahl KL, Demuth SP, Inglis DA, 1995. Occurrence of metalaxyl resistant isolates of *Phytophthora infestans* on hosts not treated with metalaxyl. *American Potato Journal* **72**, 619.

Deahl KL, Shaw DS, Cooke LR, 2004. Natural occurrence of *Phytophthora infestans* on black nightshade (*Solanum nigrum*) in Wales. *Plant Disease* **88**, 771.

Derie ML, Inglis DA, 2001. Persistence of complex virulences in populations of *Phytophthora infestans* in western Washington. *Phytopathology* **91**, 606–12.

Erselius LJ, Hohl HR, Ordoñez ME, Jarrin F, Velasco A, Ramon MP, Forbes GA, 1999. Genetic diversity among isolates of *Phytophthora infestans* from various hosts in Ecuador. In: Arthur C, Ferguson P, Smith B, eds. *Impact on a Changing World. Program Report, 1997–8*. Lima, Peru: International Potato Center, 39–48.

Flier WG, Grunwald NJ, Kroon LPNM, Sturbaum AK, van den Bosch TBM, Garay-Serrano E, Lozoya-Saldana H, Fry WE, 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**, 382–90.

Flier WG, van den Bosch GBM, Turkensteen LJ, 2003b. Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. *Plant Pathology* **52**, 326–37.

Flier WG, van den Bosch GBM, Turkensteen LJ, 2003c. Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*. *Plant Pathology* **52**, 595–603.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* **87**, 375–80.

Forbes GA, Goodwin SB, Drenth A, Oyarzún P, Ordoñez ME, Fry WE, 1998. A global marker database for *Phytophthora infestans*. *Plant Disease* **82**, 811–8.

Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA, Sandlan KP, 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. *Plant Disease* **77**, 653–61.

Garry G, Forbes G, Salas A, Perez W, Santa Cruz M, Pinedo HM, Gonzales E, Rivera M, 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, Stares J, eds. *Scientist and Farmer: Partners in Research for the 21st Century. Program Report, 1999–2000.* Lima, Peru: International Potato Center, 39–47.

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

Goodwin SB, Cohen BA, Deahl KL, Fry WE, 1994a. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology* **84**, 553–8.

Goodwin SB, Cohen BA, Fry WE, 1994b. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Science, USA* **91**, 11591–5.

Goodwin SB, Drenth A, 1997. Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology* **87**, 992–9.

Goodwin SB, Drenth A, Fry WE, 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* **22**, 107–15.

Goodwin SB, Schneider RE, Fry WE, 1995. Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Disease* **79**, 1181–5.

Griffith GW, 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**, 4007–14.

Grünwald NJ, Flier WG, Sturbaum AK, Garay-Serrano E, van den Bosch TBM, Smart CD, Matuszak JM, Lozoya-Saldana H, Turkensteen LJ, Fry WE, 2001. Population structure of *Phytophthora infestans* in the Toluca Valley region of Central Mexico. *Phytopathology* **91**, 882–90.

Judelson HS, 1996. Chromosomal heteromorphism linked to the mating type locus of the oomycete *Phytophthora infestans*. *Molecular and General Genetics* **252**, 155–61.

Lebreton L, Andrivon D, 1998. French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology* **104**, 583–94.

Legard DE, Lee TY, Fry WE, 1995. Pathogenic specialization in *Phytophthora infestans*: aggressiveness on tomato. *Phytopathology* **85**, 1356–61.

Matuszak JM, Villarreal-Gonzalez MJ, Gu WK, Villarreal-Gonzalez MJ, Fry WE, 1994. Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: distribution and dynamics. *Plant Disease* **78**, 911–6.

Niederhauser JS, Cervantes J, Servin L, 1954. Late blight in Mexico and its implications. *Phytopathology* **44**, 406–8.

Ochoa C, 1954. Northern Peru, a possible new source of potatoes resistance to *Phytophthora infestans*. *American Potato Journal* **31**, 371.

Ordoñez ME, Hohl HR, Velasco JA, Ramon MP, Oyarzun PJ, Smart CD, Fry WE, Forbes GA, 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, Forbes GA, 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* **88**, 265–71.

Peralta IE, Spooner DM, 2001. Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section

Lycopersicon [Mill.] Wettst. subsection *Lycopersicon*). *American Journal of Botany* **88**, 1888–902.

Perez WG, Gamboa JS, Falcon YV, Coca M, Raymundo RM, Nelson RJ, 2001. Genetic structure of Peruvian populations of *Phytophthora infestans*. *Phytopathology* **91**, 956–65.

Punja ZK, Forster H, Cunningham I, Coffey MD, 1998. Genotypes of the late blight pathogen (*Phytophthora infestans*) in British Columbia and other regions of Canada during 1993–97. *Canadian Journal of Plant Pathology* **20**, 274–82.

Ristaino JB, Groves CT, Parra GR, 2001. PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature* **411**, 695–7.

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

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP, 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**, 9128–33.

Spielman LJ, Sweigard JA, Shattock RC, Fry WE, 1990. The genetics of *Phytophthora infestans*: segregation of allozyme markers in F2 and backcross progeny and the inheritance of virulence against potato resistance genes *R2* and *R4* in F1 progeny. *Experimental Mycology* **14**, 57–69.

Tooley PW, Fry WE, Villarreal Gonzalez MJ, 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *Journal of Heredity* **76**, 431–5.

Trognitz F, Manosalva P, Gysin R, Niño-Liu D, Simon R, Herrera MR, Trognitz B, Ghislain M, 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**, 587–97.

Vega-Sanchez ME, Erselius LJ, Rodriguez AM, Bastidas O, Hohl HR, Ojiambo PS, Mukalazi J, Vermeulen T, Fry WE, 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**, 531–9.

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