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Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*

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The population of *Phytophthora infestans* in Brazil consists of two clonal lineages, US-1 associated with tomatoes and BR-1 associated with potatoes. To assess whether host specificity in these lineages resulted from differences in aggressiveness to potato and tomato, six aggressiveness-related epidemiological components – infection frequency (IF), incubation period (IP), latent period (LP), lesion area (LA), lesion expansion rate (LER) and sporulation at several lesion ages (SSLA) – were measured on detached leaflets of late blight-susceptible potato and tomato plants. Infection frequency of US-1 was similar on potato and tomato leaflets, but IF of BR-1 was somewhat reduced on tomato. Incubation period was longer on both hosts with US-1, although this apparent lineage affect was not significant. Overall there was no host effect on IP. On potato, BR-1 had a shorter LP (110·3 h) and a larger LA (6·5 cm^2) than US-1 (LP = 162·0 h; LA = 2·8 cm^2). The highest LER resulted when isolates of BR-1 (0·121 $cm^2\ h^{-1}$) and US-1 (0·053 $cm^2\ h^{-1}$) were inoculated on potato and tomato leaflets, respectively. The highest values of the area under the sporulation capacity curve (AUSC) were obtained for isolates of US-1 inoculated on tomato leaflets (6146) and for isolates of BR-1 on potato leaflets (3775). In general, higher values of LA, LER, SSLA and AUSC, and shorter values of LP were measured when isolates of a clonal lineage were inoculated on their original host than with the opposite combinations. There is evidence that there are quantitative differences in aggressiveness components between isolates of US-1 and BR-1 clonal lineages that probably contribute to host specificity of *P. infestans* populations in Brazil.

Keywords: epidemiology, late blight, potato, *Solanum lycopersicum*, *Solanum tuberosum*, tomato

Introduction

In Brazil, yields of tomato (*Solanum lycopersicum* = *Lycopersicon esculentum*) and potato (*Solanum tuberosum*) are often reduced by late-blight epidemics caused by *Phytophthora infestans*. Potatoes are cultivated in areas with altitudes usually >800 m, whereas tomatoes are more widespread throughout the country. Severe late-blight epidemics occur mainly from autumn to spring, and disease control relies heavily on fungicide applications for late-blight control, the latter accounting for 20% of the production costs of both crops (Mizubuti 2001). For the past decade, growers have experienced more severe late-blight epidemics, probably because of changes in *P. infestans* populations, and have increased fungicide usage.

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The first evidence of change in the Brazilian *P. infestans* population was the detection in 1986 of isolates of the A2 mating type (Brommonschenkel, 1988). More detailed analyses revealed the occurrence of isolates of a clonal lineage of *P. infestans*, not previously reported in Brazil, that had A2 mating type, a distinct RFLP banding pattern and an isozyme genotype of 100/100 for glucose-6-phosphate isomerase (*Gpi*) and 100/100 for peptidase (*Pep*) (Goodwin *et al*., 1994). Isolates of this lineage, denominated BR-1, were always associated with potatoes, whereas those of the US-1 lineage were more commonly associated with tomatoes (Brommonschenkel, 1988; Goodwin *et al*., 1994).

The coexistence of isolates of both mating types could lead to a change in *P. infestans* population structure. Sexual reproduction is possible because tomato and potato are contiguously cultivated in many areas. Furthermore, multiple susceptible crops during the year favour late-blight epidemics and increase the chance of mating. Nonetheless, a study conducted recently to test the hypothesis of population shift from strictly clonal to randomly mating concluded that 15 years after the first report of A2 in Brazil, the population of *P. infestans* remains clonal and host-specific (Reis *et al*., 2003). The epidemiological consequences of

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host specificity in *P. infestans* are: (i) isolation of pathogen populations by prevention of mating (Goodwin *et al*., 1999) and consequently oospore formation; (ii) reduced inoculum flow between host species (Vega-Sánchez *et al*., 2000); and (iii) enhanced differences in attributes of clonal populations that affect disease management, such as fungicide resistance – as in the USA, for example, where isolates of metalaxyl-sensitive US-1 remained associated with tomatoes while isolates of metalaxyl-resistant US-8 were associated with potatoes (Legard *et al*., 1995).

Host specificity could result from differential fitness of individuals when associated with a particular host species, where fitness is defined as the contribution of an individual to the gene pool of the next generation (Antonovics & Alexander, 1989). Despite its importance to the population biology of plant pathogens, measuring fitness is not a trivial task. Nonetheless, variables used to assess pathogen aggressiveness, such as incubation period, latent period, lesion area, lesion expansion rate, sporulation capacity and infection frequency, are also considered to be related to the fitness of *P. infestans* and have been quantified in several experiments (Day & Shattock, 1997; Kato *et al*., 1997; Miller *et al*., 1998; Flier & Turkensteen, 1999; Lebreton *et al*., 1999; Carlisle *et al*., 2002).

Differences in aggressiveness affect late-blight epidemics and, consequently, disease management. It has been shown that epidemics induced by more aggressive isolates are more severe, cause greater crop loss and require a higher number of fungicide sprays (Kato *et al*., 1997). In two independent studies, isolates of an introduced clonal lineage of *P. infestans* were found to be more aggressive than isolates of the original lineage (Flier & Turkensteen, 1999; Miller & Johnson, 2000). There are no studies on epidemiological attributes of *P. infestans* in Brazil, therefore it is not known if there is any difference in aggressiveness between US-1 and BR-1 isolates under Brazilian conditions. This study was conducted to test the hypothesis that aggressiveness differences between isolates of the two populations of *P. infestans* determine or contribute to their high host specificity. Based on previous work on the assessment of aggressiveness (Day & Shattock, 1997; Mizubuti & Fry, 1998; Flier & Turkensteen, 1999; Lebreton *et al*., 1999; Vega-Sánchez *et al*., 2000; Carlisle *et al*., 2002), the following aggressiveness-related epidemiological components were quantified: incubation period (IP), latent period (LP), lesion area (LA), infection frequency (IF), lesion expansion rate (LER) and sporulation at several lesion ages (SSLA) of isolates inoculated on tomato and potato leaflets. Inferences were made regarding pathogen fitness.

Materials and methods

Isolates of *P. infestans*, plant material and inoculum production

Five isolates of US-1 and five of BR-1 *P. infestans* clonal lineages from major and distinct tomato- or potato-growing areas in Brazil were selected from the collection of the Epidemiology Laboratory at the Universidade Federal de Viçosa (UFV) (Table 1). All isolates were collected in spring 1998, isolated and maintained on Rye B medium (Caten & Jinks, 1968). Slant agar cultures covered with mineral oil were stored at 10°C. Isolates were recovered by plating small culture fragments (0·25 cm^2) onto Rye B medium in Petri dishes kept at 18°C with a 16 h day length. After initial growth, isolates were again transferred to Rye B medium under similar conditions. Mycelium plugs (0·8 cm diameter) cut from cultures with a cork borer were transferred to slices of potato tuber (cv. Bintje) ≈0·5 cm thick (potato isolates) or to slits (1 cm deep × 2 cm long) in green fruits of tomato cv. Kada (tomato isolates). Inoculated plant parts were kept either in 9 cm diameter Petri dishes (potato slices) or plastic bags (tomato fruits) maintained at 18°C with a 16 h day length. After sporulation, mycelium and sporangia were scraped from tuber slices or fruit surfaces and transferred to 50 mL sterile distilled water. Suspensions were filtered through two layers of cheesecloth to remove mycelium fragments and subsequently adjusted to a concentration of 10^3 sporangia mL^{-1}. Detached lateral leaflets from potato cv. Bintje or tomato cv. Kada leaves, from the middle third of 6- to 8-week-old plants grown in the glasshouse, were placed abaxial side up inside square plastic boxes (11 cm square × 3·5 cm deep) containing a cotton swab soaked with distilled water to produce a moist chamber. One 20 μL droplet of inoculum suspension was deposited on each leaflet. Boxes were kept at 18°C with a 16 h day length. After 10 days lesions were cut and washed in distilled water to obtain sporangial suspensions for use in the experiments.

For IF experiments, 10^3 sporangia mL^{-1} was previously established as a suitable concentration of inoculum to obtain individualized lesions. For IP, LP, LA, LER and SSLA, an inoculum suspension of 2×10^4 sporangia mL^{-1} was kept for 1 h at 4°C to enhance zoospore formation. Sporangial suspensions were prepared so that the timespan between inoculum preparation and inoculation never exceeded 2 h.

Table 1 Isolates of *Phytophthora infestans* from Brazil assessed for aggressiveness components on detached tomato and potato leaflets (all isolates collected in spring 1998)

Isolate	Clonal lineage	Mating type	Host	Place of origin
Pib-4	BR-1	A2	Potato	Urubici, SC
Pib–18	BR-1	A2	Potato	Carandaí, MG
Pib–121	BR-1	A2	Potato	Passo Fundo, RS
Pib–193	BR-1	A2	Potato	Paraguaçu, MG
Pib-272	BR-1	A2	Potato	Venda Nova, ES
Pib 51	US-1	A1	Tomato	Barbacena, MG
Pib 105	US-1	A1	Tomato	Viçosa, MG
Pib 214	US-1	A1	Tomato	Santo Amaro, SC
Pib 239	US-1	A1	Tomato	Alfenas, MG
Pib 256	US-1	A1	Tomato	Botucatu, SP

Inoculation

To quantify IF, potato and tomato leaflets obtained as described above were placed, abaxial surface up, completely

covering the internal surface of two transparent plastic boxes previously disinfested with ethanol (70%) and internally lined with wet paper towel. The total area covered by leaflets (242 cm^2) was sprayed as evenly as possible with 1 mL of a suspension of 10^3 sporangia mL^{-1}. The same suspension was also sprayed on the internal surface of a 9 cm diameter Petri dish containing 63·62 cm^2 water agar (15 g L^{-1}). The number of sporangia deposited on each Petri dish was counted microscopically (×100 magnification) 12 h later. The total number of sporangia deposited on 242 cm^2 of leaflets was estimated based on the density of spores per cm^2 in the Petri dishes. After inoculation the boxes were initially kept at 16°C in the dark for 12 h, and thereafter at 19 ± 2°C with a 16 h day length.

To quantify IP, LP, LA, LER and SSLA, three potato or tomato leaflets were placed in separate boxes (three per box) lined with wet paper towel. To provide enough moisture and prevent early wilting and senescence, the basal part of each leaflet petiole was wrapped with a cotton swab soaked with distilled water. Each leaflet was inoculated on the abaxial side with one 50 μL drop of a suspension of 2×10^4 sporangia mL^{-1}. Isolates from either tomatoes or potatoes were inoculated on both tomatoes and potatoes. After inoculation, the boxes were kept for 7 days at 18°C with a 16 h day length.

To assess IP, LP, LA, LER and SSLA, each experiment was conducted twice. Each experiment was set in a nested-crossed design (isolates nested in clonal lineages and crossed with hosts), with four blocks, each block being a different inoculation date. Each box with three leaflets was considered an experimental unit.

Assessments

Infection frequency
IF was calculated as the number of individualized lesions divided by the total number of sporangia deposited on 242 cm^2, determined by counting the lesions under a stereoscope 96 h after inoculation (HAI). However, for one US-1 isolate (Pib-51) inoculated on tomato leaflets, sporulation was noticed before tissue necrosis. Thus a second, more detailed evaluation was performed, counting the number of sporulating points at 108 HAI.

Incubation period
For the first experiment run, leaflets were visually inspected at 6 h intervals from 36–120 HAI. After 120 HAI leaflets were examined at 24 h intervals until 210 HAI. For the second run, leaflets were examined at the same intervals but the assessments ended at 216 HAI. IP was considered completed when typical blight symptoms were observed on at least two of the three leaflets of a replication.

Latent period
Inoculated leaflets were observed under a stereoscope at the same time intervals used for assessing IP. LP was considered completed when sporangiophores were visible in at least two of the three lesions of a replication.

Lesion area
Six days after inoculation all lesions were photographed with a digital camera. Lesion images were analysed and lesion areas determined using the IMAGETOOL software (UTHSCSA, University of Texas Health Science Center, San Antonio).

Lesion expansion rate
In each assay, lesion area was determined at 24 h intervals, as described previously. Lesion area was evaluated from 66–210 and from 96–216 HAI for the first and second runs, respectively. Only lesions that did not reach the leaflet edge were photographed. When water-soaked areas associated with saprotrophic bacteria were present, lesion area was measured only if a typical late-blight lesion could be clearly identified and the water-soaked area occupied less than 50% of the affected area and did not extend beyond the lesion border.

Sporulation at several lesion ages
To measure SSLA accurately, assessments were conducted daily from the third to the sixth day after inoculation (DAI) and from the third to the ninth DAI for the first and second experiments, respectively. The three leaflets in each box were photographed, and lesion areas determined as described above. Lesions were cut from the leaflets and transferred to test tubes containing 2 mL of a preservation solution (1 mL distilled water and 1 mL of 0·04 M $CuSO_4$/0·2 M sodium acetate/acetic acid pH 5·4) (Mizubuti & Fry, 1998). Sporangia were released from lesions by vortexing the tubes for 10 s. Plant tissue was removed and sporangia were counted within 36 h using a haemocytometer. Each sporangial suspension was counted at least twice, counts were averaged and sporangia production per unit lesion area (sporulation capacity) calculated by multiplying the average sporangial concentration (spores mL^{-1}) by the volume of preservation solution and dividing by the total lesion area (cm^2) (Kadish *et al*., 1990). Sporulation capacity of lesions at different ages was determined daily. Sporulation capacity data were plotted against lesion age, and the area under the sporulation capacity curve (AUSC) was calculated for each replicate using the average value of three leaflets in each replicate. For both experiments AUSC values were standardized to allow data comparisons (Fry, 1977).

Statistical analyses

For IF, IP, LP, LA and AUSC, the treatment design was a nested-crossed factorial and the linear model used for ANOVA was:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_{k(i)} + \beta\gamma_{jk(i)} + \delta_l + e_{ijklm}$$

where μ = overall mean, α_i = fixed effect of clonal lineage, β_j = fixed effect of host, $\alpha\beta_{ij}$ = interaction term of clonal lineage and host, $\gamma_{k(i)}$ = fixed effect of isolates nested within clonal lineages, $\beta\gamma_{jk(i)}$ = interaction term of host and isolates within clonal lineages, δ_l = effect of blocks and e_{ijklm} = random experimental error (Kuehl, 1994). This model allowed testing for differences between clonal lineages as well as among isolates within a clonal lineage.

Lesion expansion rates (LER) were estimated by regressing LA averages from three leaflets of each replicate on time (LA = α + β HAI). The slope of the regression line (β) was interpreted as LER (cm^2 h^{-1}). LERs for combinations of interest between host and clonal lineage were compared using 95% confidence intervals (95% CI; Draper & Smith, 1998). The general effect of clonal lineages was also evaluated using the mean value for each isolate, calculated for both experiments.

The relationship between aggressiveness components was estimated through Pearson's correlation coefficients.

All data were analysed with the SAS System ver. 8·0 (SAS Institute Inc., Cary, NC, USA). Using PROC MIXED a test of equal variances was obtained based on the difference of –2REML log likelihood values and the significance level calculated based on a χ^2 distribution (Littell *et al*., 1996). Data were pooled only when the hypothesis of equal variances was not rejected.

Results

Infection frequency

Data for IF were not pooled for the ANOVAs and each experiment was analysed independently. For both experiments there was no difference between IF values for clonal lineages, and the clonal lineage × host interaction was not significant (results not shown).

Incubation and latent periods

Data for IP and LP were pooled after testing for equality of variances. For IP there were no significant effects of clonal lineage, host or clonal lineage × host interaction (results not shown).

The LP varied according to the combination of host and clonal lineage (P = 0·017) (Table 3). The LP of BR-1 (110·3 h) was significantly shorter than that of US-1 (162·0 h) on potato (P = 0·007), but LP values of the lineages did not differ on tomato (Tables 2 and 3). Isolates also varied within clonal lineages (Table 3).

Lesion area

Analyses were carried out with pooled data, and a significant interaction between clonal lineages and host species was detected (P = 0·003) (Table 3). BR-1 caused larger lesions on potato (6·5 cm^2), its original host, than did US-1 (1·3 cm^2). No significant difference was found between LA values caused by BR-1 and US-1 isolates on tomato.

Table 2 Epidemiological components of BR-1 and US-1 clonal lineages of Brazilian isolates of *Phytophthora infestans* inoculated on potato and tomato leaflets

Epidemiological component	Clonal lineage			
	US-1		BR-1	
	Potato	Tomato	Potato	Tomato
Infection frequency[a]	0·022 (0·0026)[b]	0·024 (0·0031)	0·020 (0·0023)	0·012 (0·0015)
Incubation period (h)	75·5 (12·4)	83·3 (31·9)	66·7 (10·4)	62·4 (11·0)
Latent period (h)	162·0 (33·1)	114·9 (34·1)	110·3 (22·5)	104·6 (23·2)
Lesion area (cm^2)	1·3 (1·5)	2·8 (2·7)	6·5 (3·8)	0·8 (0·8)
LER[c] (cm^2 day^{-1})	0·030 (0·027)	0·053 (0·018)	0·121 (0·046)	0·020 (0·006)
AUSC[d]	740·52 (62·79)	6145·86 (299·55)	3775·43 (281·02)	1012·40 (267·03)

[a]Number of developed lesions per sporangium deposited.
[b]Mean values of two experiments (SE).
[c]Lesion expansion rate.
[d]Area under sporulation capacity curve.

Table 3 ANOVA from pooled data of two experiments that tested the effect of host (potato or tomato), clonal lineages of *Phytophthora infestans* and Brazilian isolates of the pathogen nested in clonal lineages, on epidemiological components evaluated in detached leaflet inoculation experiments

Source	Latent period				Lesion area				Area under sporulation capacity curve			
	DF	MS	F	$P > F$	DF	MS	F	$P > F$	DF	MS	F	$P > F$
Host	1	12656·4	9·69	0·0135	1	339·7	14·38	0·0053	1	41891152·5	8·88	0·0247
Clonal lineage	1	27247·8	7·13	0·0284	1	249·8	6·78	0·0314	1	26423352·7	4·34	0·0823
Host × CL	1	11408·7	8·98	0·0171	1	415·2	17·57	0·0030	1	400334054·2	84·83	<0·0001
Isolate(CL)[a]	8	3821·8	9·14	<0·0001	8	36·8	20·97	<0·0001	6	6087576·4	6·98	<0·0001
Host × isolate(CL)[b]	8	1270·1	3·04	0·0047	8	23·6	13·45	<0·0001	6	4719279·5	5·41	<0·0001

[a]Isolate nested within clonal lineage.
[b]Interaction between host and isolate nested within clonal lineage.

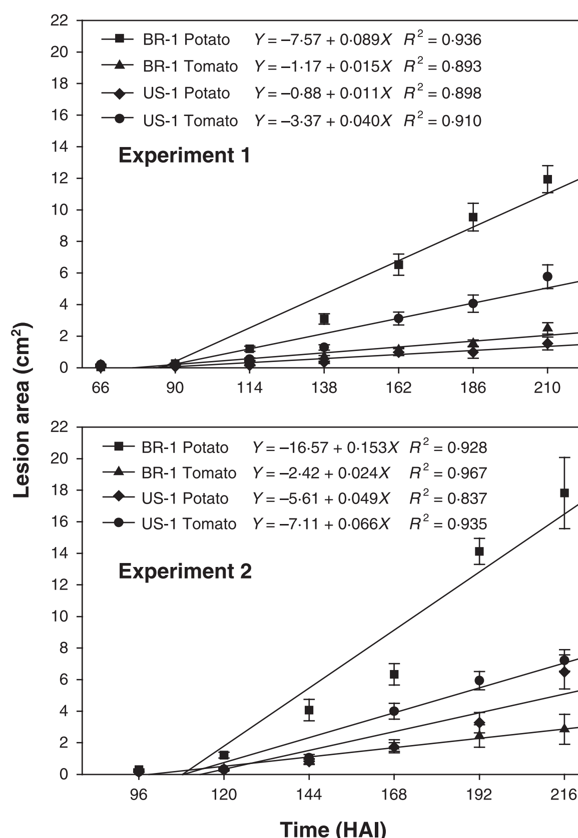

Figure 1 Lesion area (Y) on potato and tomato leaflets inoculated with Brazilian isolates of the US-1 or BR-1 clonal lineages of *Phytophthora infestans* plotted against time (X) (HAI). Bar = SEM.

Lesion expansion rate

In both experiments, higher LER values were recorded for BR-1 isolates inoculated on potato and for US-1 isolates on tomato than for the opposite combinations (Fig. 1). The lower and upper limits of 95% CIs for the difference of LER for BR-1 on potato vs tomato were 0·050–0·096 and 0·080–0·178 in experiments 1 and 2, respectively. Zero was not included in the 95% CIs, so differences were significant. LER for US-1 on tomato was significantly higher than on potato in experiment 1 (95% CI = 0·016–0·042), but there was no difference in experiment 2 (95% CI = –0·015–0·049).

The LER averages of both experiments with US-1 isolates on tomato leaflets (0·053 $cm^2\ h^{-1}$) were higher than with BR-1 isolates (0·020 $cm^2\ h^{-1}$) on the same host. There was also a difference between clonal lineages on potato leaflets: BR-1 isolates had a higher LER value (0·121 $cm^2\ h^{-1}$) than US-1 isolates (0·030 $cm^2\ h^{-1}$) (Table 2).

Sporulation at several lesion ages

Sporulation capacity values varied according to the combination of host and clonal lineage, and higher values were estimated when isolates were inoculated on their original host (Fig. 2). The highest values of sporulation capacity were recorded for US-1 isolates on tomato. Similarly, there was a significant interaction between clonal lineage and host ($P < 0·0001$). Higher AUSC values were recorded when tomato leaflets were inoculated with US-1 isolates and potato leaflets were inoculated with BR-1 isolates, whereas lower AUSC values were estimated for crossed inoculation combinations (Tables 2 and 3).

Relationship between aggressiveness components of *P. infestans*

In general, values of Pearson's correlation coefficient between aggressiveness components were small and nonsignificant (Table 4). Latent period was the only component that correlated with others: LA ($r = –0·497$) and AUSC ($r = –0·472$) (Table 4).

Discussion

This study provides evidence that differential aggressiveness between isolates of US-1 and BR-1 clonal lineages contributes to host specificity of *P. infestans* populations in Brazil. Isolates from a particular clonal lineage were more aggressive on their original host, but establishment of a host–parasite relationship and host cell damage occurred on both hosts regardless of pathogen genotype. As reported in Uganda and Kenya (Vega-Sánchez *et al*., 2000) no qualitative differences could be detected regarding IF. Nevertheless, under field conditions BR-1 isolates have never been found on tomatoes in Brazil, whereas US-1 isolates have been associated with a late-blight epidemic in potato in a tomato-producing area (Reis *et al*., 2003).

Symptoms and sporulating lesions developed in potato and tomato as a result of infection by either US-1 or BR-1 isolates. For pathogens with short generation periods, such as *P. infestans*, symptom development should occur quickly and little difference in IP can be expected. The shorter LP for BR-1 than for US-1 isolates on potato appears to indicate higher specific aggressiveness to this host. The reasons for the lack of statistical difference in LP on tomatoes are not known, but the high susceptibility of tomato cv. Kada to both potato and tomato isolates could have made differences more difficult to detect. Shorter latent periods of other lineages of *P. infestans* on the original host were reported in previous studies (Kato *et al*., 1997; Flier & Turkensteen, 1999; Lebreton *et al*., 1999). Interestingly, for one US-1 isolate (Pib-51) the LP was shorter than the IP. This was observed in both experiments, and suggests that there are variations not only in LP duration, but also in the way some isolates colonize the host. Isolates that sporulate without causing concomitant necrosis have a more biotrophic colonization mode, with almost no visible cell damage. *Phytophthora infestans* is a hemibiotrophic pathogen of aerial plant parts (Smart *et al*., 2003) and a biotrophic colonization phase has been reported for tomato-adapted isolates on tomato (Vega-Sánchez *et al*., 2000; Smart *et al*., 2003). Delay in the hypersensitive response in the compatible interaction of *P. infestans* and

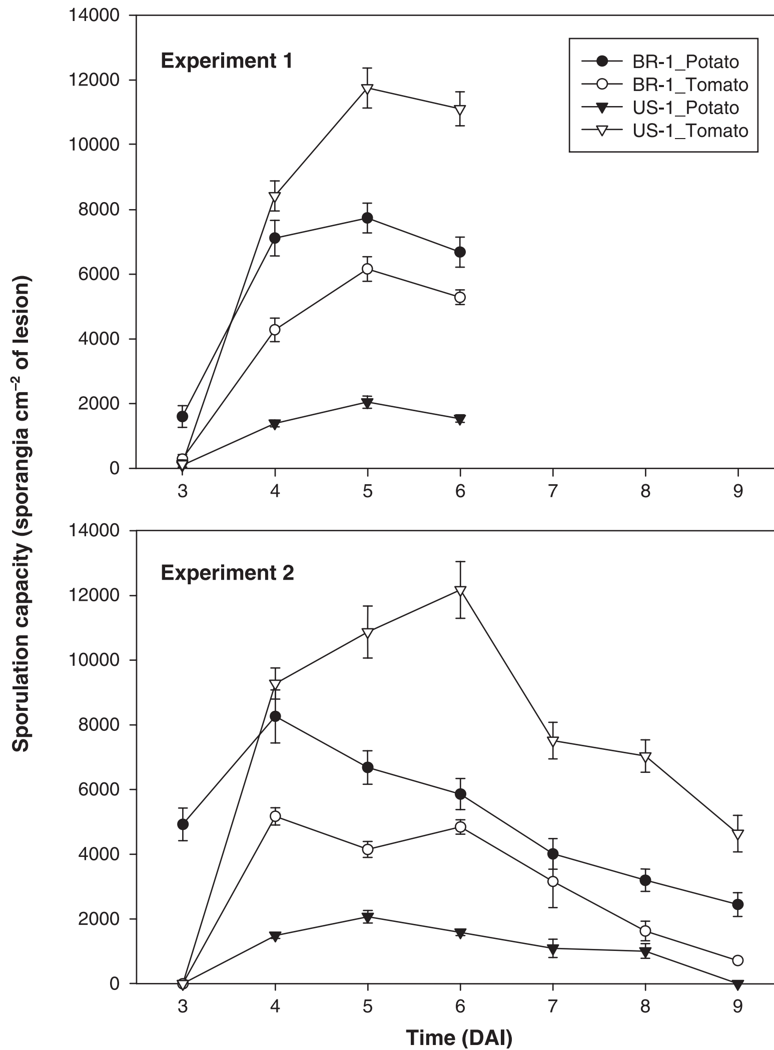

Figure 2 Sporulation capacity (sporangia cm^{-2} of lesion) of Brazilian *Phytophthora infestans* clonal lineages on potato and tomato leaflets plotted against time (DAI) for two experiments. Bar = SEM.

Table 4 Pearson's correlation coefficient between average values of aggressiveness components: incubation period, latent period, infection frequency, lesion area and area under the sporulation capacity curve (AUSC) of *Phytophthora infestans*

Components	Incubation period	Latent period	Infection frequency	Lesion area	AUSC
Incubation period	1·0	0·233 (0·223)[a]	0·132 (0·580)	−0·248 (0·202)	0·231 (0·278)
Latent period		1·0	0·339 (0·144)	−0·497 (0·007)	−0·472 (0·023)
Infection frequency			1·0	−0·294 (0·207)	−0·249 (0·201)
Lesion area				1·0	0·186 (0·395)
AUSC					1·0

[a]*P* value in parentheses.

tomato was postulated to be of epidemiological importance by favouring disease development with less cell damage by tomato-specialized isolates (Smart *et al*., 2003).

In both experiments higher LER values for the combinations of BR-1 with potato and US-1 with tomato were recorded. Previous reports showed that lesion growth rates for isolates of newly introduced clonal lineages were higher than those for US-1 isolates on potato leaflets (Kato *et al*., 1997; Mizubuti & Fry, 1998). In the present study, LER values of US-1 isolates on potato leaflets were higher than the values for BR-1 isolates on tomato. US-1 isolates possess some aggressiveness to potato, but seem less adapted to it than to tomato. Previous association of the US-1 lineage with potato in Brazil might explain why isolates of this lineage retain a certain degree of aggressiveness on potato.

When assessing pathogen aggressiveness, lesion expansion rate can be a useful component because it encompasses the temporal dynamics of pathogen development in the host. Under similar environmental conditions and the same level of host resistance, this component reflects the colonization ability of an individual and summarizes several pathogenesis processes (Ordoñez *et al*., 1998). The ability of the pathogen to colonize host tissues more quickly can give it an advantage when environmental conditions are marginally favourable. In tropical and subtropical areas, favourable environmental conditions (high relative humidity and mild temperatures) for late-blight epidemic development can occur throughout the year. In Brazil, favourable periods are more frequent and last longer in autumn or spring, but are limited to short intervals, at dawn, during dry winters and warm summers. Fast colonization during the short timespan of favourable periods can assure lesion formation and continuous inoculum supply. Thus isolates on their host of origin will have a better chance of successful infection under marginal conditions than will nonadapted isolates.

In a pathogen population, individuals that sporulate more abundantly have better chances of increasing in number and of contributing to the gene pool of the next generations. As with most other components, higher sporulation was observed when isolates were inoculated on their host of origin. In polycyclic diseases such as late blight, secondary cycles and new lesion formation are determined to a large extent by the amount of spores produced. Isolates adapted to a host will produce more sporangia, increasing the number of lesions of the same genotype, which contributes to the maintenance of separate populations of *P. infestans* in Brazil. Although no competitive fitness experiments were conducted in this study, other studies have demonstrated the importance of sporulation capacity in the development of late-blight epidemics in the field (Spielman *et al*., 1992; Lebreton *et al*., 1999).

Pathogen sporulation is an important aggressiveness component, but its quantification is subject to errors that could prevent detection of differences among isolates. In crossed inoculations AUSC for BR-1 on tomato was higher than for US-1 on potato in both experiments. Although lesion area was measured accurately, estimating numbers of sporangia per lesion area was difficult because of variability at the beginning of the infectious period when sporulation was scarce. Additionally, for the BR-1 isolates Pib-121 and Pib-18 inoculated on tomato no sporangia were observed in spore-suspension preparations, although a few sporangia were visualized under a stereoscope in the lesions caused by both isolates. Temporal analysis of sporulation capacity through the AUSC allowed integration of sporulation values over time and provided a reliable measurement of this component.

Despite the clonal structure of *P. infestans* populations in Brazil, there were differences between isolates within a lineage for all components measured. Variation among isolates within a clonal lineage is known (Anderson & Kohn, 1995). In the present study variability among isolates within a lineage was high, but in most trials variability between lineages was significant.

Host specificity is normally thought of as a qualitative response, but in nature quantitative responses can occur. Quantitative differences in aggressiveness components, such as faster symptom development and differences in lesion formation (LA and LER) and sporulation (LP, SSLA and AUSC), contribute to the host specificity of *P. infestans* populations in Brazil. Host specificity resulting from differences in aggressiveness, as reported in the USA (Legard *et al*., 1995), Ecuador (Oyarzun *et al*., 1998) and Uganda and Kenya (Vega-Sánchez *et al*., 2000), has important epidemiological implications for late-blight management. Two components of late-blight epidemics affected by host specificity are inoculum dynamics and reproductive isolation.

Inoculum dynamics are influenced by differential host availability, which in turn favours maintenance of host-adapted isolates already present in the population. In most growing areas of Brazil, up to two tomato and three potato crops can be grown throughout the year. In regions where the cultivation of one host is more widespread than that of another, inoculum from this more abundant source can initiate epidemics in another host cultivated in more restricted areas located in the same region. Absence of BR-1 inoculum probably explains why US-1 was found in potato crops cultivated in a tomato-producing area in Brazil. It is not known, however, if these US-1 isolates were highly aggressive on potato. In Africa, potato-adapted and tomato-adapted isolates of US-1 were described, but no US-1 isolates were found that were highly aggressive on both hosts (Vega-Sánchez *et al*., 2000). In Ecuador EC-1, a potato lineage, was isolated from *Solanum ochrantum* growing near late blight-infected potatoes. These isolates were later found to be weakly pathogenic on *S. ochrantum*, which is generally attacked aggressively by the US-1 lineage (G. Forbes, CIP, Lima, Peru, personal communication). For Brazil, more information is needed to establish the epidemiological significance and management implications of US-1 passing from tomato to potato. In general, however, if both hosts are grown in the same region at similar acreages, the host-adapted population will cause the epidemic on its primary host and the management of late blight needs to consider two distinct *P. infestans* populations.

Even though reproductive isolation can result from host specificity, it is not certain that this is happening in Brazil. The hypothesis of reproductive isolation in the Brazilian population of *P. infestans* has not yet been properly tested. There is evidence of reproductive isolation because no oospores or recombinant genotypes of *P. infestans* have been found (Reis *et al*., 2003). However, other barriers in addition to host specificity, such as genetic incompatibility and oospore sterility, may also prevent the establishment of a panmictic population and contribute to reproductive isolation.

The results reported here support the idea of stability of US-1 on tomato (Erselius *et al*., 2000). In many Latin American countries, such as Ecuador (Erselius *et al*., 2000), Peru (Perez *et al*., 2001) and Colombia (Cuéllar & Domínguez, 1998), US-1 was probably the dominant lineage on potato and while it was displaced on that host it

remained on tomato. In Nepal, the RG57 fingerprinting pattern of all 12 isolates collected from tomatoes was characteristic of the US-1 lineage (Ghimire *et al*., 2003). In sub-Saharan Africa (Vega-Sánchez *et al*., 2000) and South Africa (McLeod *et al*., 2001), US-1 causes late blight on both potato and tomato. It is possible that in these areas potatoes have not yet been challenged by potato-adapted isolates. In Brazil, *P. infestans* isolates of the A1 mating type were reported associated with potato late-blight epidemics (Ciccarone *et al*., 1959) and could be of the US-1 lineage. Recently, a study revealed that all 267 isolates from tomato were US-1, and only four out of 184 isolates collected from potato were US-1 (Reis *et al*., 2003). These four isolates came from a potato experimental field located in a tomato-producing region, where BR-1 may have been absent or very low in frequency. There is evidence that US-1 is a stable lineage in tomatoes in Brazil.

The quantification of epidemiological components related to *P. infestans* aggressiveness is also important for understanding the evolution of pathogen populations. Evolution of pathogen populations is influenced by differences in fitness, a complex attribute (Antonovics & Alexander, 1989) potentially determined by epidemiological components and other factors not measured in the present study. The fact that no survival-related stage was quantified in the present study precludes a deeper discussion on differences in fitness between the two clonal lineages of *P. infestans* in Brazil. Nevertheless, this does not invalidate the inferences made here because in most growing areas of Brazil host tissue and inoculum are likely to be available year-round, and survival out of the host may be of little or no importance. However, more detailed studies on survival of *P. infestans* in tropical and subtropical conditions are necessary to determine the epidemic significance of potential inoculum sources such as plant debris, tubers or voluntary plants.

In summary, the most likely hypothesis resulting from the data presented here is that quantitative differences in aggressiveness determine host specificity and enhance the chance of maintaining a clonal population of *P. infestans* in Brazil. Host specialization contributes to reproductive isolation, although other barriers may also be involved. The absence of oospores suggests lower genetic variability and reduced survival efficacy, therefore strategies for managing late blight in Brazil should be directed towards the asexual stage of *P. infestans*.

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