Effects of Temperature on Events in the Infection Cycle of Two Clonal Lineages of *Phytophthora infestans* Causing Late Blight on Tomato and Potato in Brazil

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ABSTRACT

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In Brazil, US-1 and BR-1 clonal lineages of *Phytophthora infestans* are widely distributed throughout the major growing areas of tomato and potato, respectively. Quantitative information regarding the effects of temperature (10, 15, 22, and 27°C) on direct and indirect sporangia germination, incubation period (IP), latent period (LP), lesion area (LA), and sporulation (SP), as well as combined temperature and leaf wetness duration periods (6, 12, 18, and 24 h) on the number of lesions (NL) was obtained under controlled conditions on either detached leaflets or whole plants. The percentage of indirect germination was higher for BR-1 isolates than for US-1. The percentage of direct germination was higher for US-1 than for BR-1. The shortest IP and LP were recorded at 22°C for both lineages: 69.3 h and 93.3 h for US-1 isolates on detached tomato leaflets, and 44.0 h and 68 h for BR-1 isolates on detached potato leaflets, respectively. US-1 isolates did not sporulate at 10°C, and BR-1 isolates did not sporulate at 27°C. Isolates of both lineages induced the largest LA at 22°C. The NL was highest at 15°C for US-1 isolates on whole tomato plants, and at 10°C for BR-1 isolates on whole potato plants. The differential effects of temperature on US-1 and BR-1 suggest that current decision support systems initially developed for controlling US-1 in Brazil may now be inaccurate for controlling BR-1, and management strategies should be properly validated before being used.

In Brazil, tomatoes (*Solanum lycopersicum* L. = *Lycopersicon esculentum* Mill.) are widely planted throughout the country, but at relatively low altitudes. In contrast, potatoes (*Solanum tuberosum* L.) are produced in areas of high altitudes (above 800 m), and confined mostly to the south and southeast regions. In each production region, several tomato and potato crops are continuously grown, with more than one planting per year. Although the same field is not cultivated with either tomato or potato successively, new tomato plantings are sometimes established near old tomato fields, and the same can occur for potatoes. The severity of late blight epidemics, caused by *Phytophthora infestans*, the most destructive disease of both host crops, has been observed to vary depending on planting date. Late blight epidemics develop with higher progress rates during the summer (rainy season) than during the winter (dry season) (24).

Late blight epidemics are favored by moderate temperatures and high humidity (12). Temperature and leaf wetness duration periods affect different stages of the life cycle of *P. infestans*. Temperature affects sporulation (1,29), infection, lesion expansion rate (15), inoculum survival (23), and sporangia germination (4). Sporangia germination is a temperature-driven process: below 15°C, zoospores are formed (indirect germination); whereas above 18°C, sporangia preferentially form germ tubes (4), a process referred to as direct germination. Once inside the plant, host colonization and pathogen sporulation are also affected by temperature, and more sporangia form optimally in the range of 18 to 22°C (12). Temperature also affects the survival of both sporangia (5,23) and oospores (6,21,33). Water, either as vapor or liquid, plays an important role on late blight development because it affects germination, sporulation, and inoculum survival. Prolonged periods with leaf wetness and/or high relative humidity are required for sporangia germination and infection establishment (12). Humidity can also affect sporulation (14) and sporangia survival (23). Abundant sporulation and extended sporangia viability also occur under high humidity conditions.

Under favorable environmental conditions, late blight outbreaks are likely to occur and fungicides are intensively applied to both tomato and potato crops in

Brazil. Depending on weather conditions, more than 15 fungicide sprays per season can be applied to each crop. Although no study has been designed to quantify fungicide usage, growers now report that more applications are being required to achieve efficient disease suppression. Similar situations have been reported elsewhere (11,17) which could be associated with changes in the pathogen population and the spread of more aggressive genotypes (7).

In Brazil, there are two clonal populations of *P. infestans*: a tomato adapted clonal lineage, US-1, and a potato adapted lineage, BR-1 (27). The US-1 clonal lineage, which comprises isolates of the A1 mating type, is considered to be an old population of *P. infestans* that is distributed worldwide (9). BR-1 is a new lineage first reported in Brazil in 1986 (2) and is comprised of isolates of the A2 mating type (9). Brazilian populations of *P. infestans* are host-specific: US-1 isolates cause late blight on tomato, whereas BR-1 isolates are associated with potato. Even though isolates of these two clonal lineages can infect both hosts, they are more aggressive to the host with which they are predominantly associated (30).

Host specificity and putative differences in ecological requirements of *P. infestans* lineages necessitate differential late blight management strategies, according to host-climate interaction. As previously mentioned, in Brazil, potatoes and tomatoes are cultivated in different climatic zones. Considering the differences in the planting areas, the strong association of a clonal lineage with either potato or tomato could be, at least in part, due to differences in ecological requirements of isolates of either lineage. Furthermore, there is strong evidence that the new populations of *P. infestans* have different ecological requirements than the old US-1 lineage. For example, sporangia of isolates of the US-7 and US-8 clonal lineages germinated faster at temperatures below 15°C when compared to US-1 isolates (25). Also, US-8 isolates apparently are more low-temperature tolerant than US-1 (18).

There is no quantitative information on the effects of environmental factors on Brazilian isolates of *P. infestans*. Despite the importance of late blight epidemics on tomato and potato crops, no study has yet been conducted in Brazil to address the effects of temperature and leaf wetness duration in the infection cycle of *P. in-*

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festans. Most of the information currently used in Brazil is still based on data gathered on temperate climates and on isolates of the US-1 lineage. Thus, the objective of this study was to quantify the effects of (i) temperature on sporangia germination, incubation period, latent period, lesion area, and sporulation of isolates of the two clonal lineages of *P. infestans*, and (ii) the combination of temperature–leaf wetness duration on the number of lesions incited by isolates of these lineages.

MATERIALS AND METHODS

Temperature effects on sporangia germination. *P. infestans* isolates used in the experiments were collected in different geographical regions of Brazil (Table 1). The genetic structure of the Brazilian population of *P. infestans* has previously been shown to be strictly clonal with negligible differences at the molecular level among isolates of a lineage (27). The isolates were grown on rye B (3) on petri dishes kept at 18°C with 16 h of light and 8 h of dark. After a 14-day growing period, 10 ml of sterile distilled water were added to each plate and sporangia were dislodged with a soft paintbrush. To keep isolates virulent, they were periodically inoculated on detached leaflets of susceptible (no R genes) tomato cv. Kada and potato cv. Bintje. The tomato isolates were inoculated to tomato leaflets and the potato isolates to potato leaflets. A drop of 20 µl of a suspension of 10⁴ sporangia/ml of each isolate was deposited on the abaxial side of a leaflet of the respective host. The inoculated leaflets were kept in moist chambers at 18°C, 24-h light regime (233.4 µmol/s/m²). After 10 days, the lesions that developed were gently touched with a piece of moistened sterile filter paper (1 × 1 cm) to capture sporangia, and the filter paper with sporangia was imprinted onto the surface of water agar (1.5%) in petri dishes. Three equally distant prints in a triangle shape were made per dish. The dishes were kept at 10, 15, 22, or 27°C. After 12 h, a portion of approximately 4 cm² of the agar medium containing sporangia was cut from each dish and transferred, the surface containing sporangia facing up, to a glass slide. A drop of lactophenol was added and a coverslip covered

the preparation. Direct (germ tube formation) and indirect (zoospore release) sporangial germinations were assessed observing at least 300 sporangia per slide under a microscope. All empty sporangia with clearly defined openings at the apex were considered as indirectly germinated. Empty sporangia with shrunken appearance and no opening at the apex were not counted.

The experiment was conducted twice, each in a completely randomized design with five replicates. Each petri dish was considered as an experimental unit.

Temperature and leaf wetness duration effects on the number of lesions. Two isolates of each clonal lineage (Pib214 and Pib256 of the US-1 and Pib4 and Pib198 of the BR-1) were used. Inoculum was produced as described above. Leaflets of tomato ‘Kada’ or potato ‘Bintje’ plants, 7 to 8 weeks old, were inoculated with isolates of US-1 or BR-1, respectively. Plants were sprayed with a 3 × 10⁴ sporangia/ml suspension with a hand sprayer to runoff. After inoculation, each plant was covered with a plastic bag previously wetted inside to form a moist chamber. Based on previous tests, the relative humidity provided was enough to assure a continuous leaf wetness period. The inoculated plants were transferred to growth chambers at 10, 15, 22, or 27°C with a 14-h photoperiod. After 6, 12, 18, or 24 h, the plastic bags were removed from the corresponding group of plants (treatments); the plants were dried by cool air jet from a fan and were kept inside the respective growth chamber.

The lesions that formed on 40 leaflets of each plant (an experimental unit) in each treatment (combination of isolate–temperature–leaf wetness duration) were counted 4 days after inoculation. For plants at 10°C, the lesion number was assessed 8 days after inoculation.

The experiment was conducted twice, each in a completely randomized design with three replicates.

Temperature effects on incubation period, latent period, lesion area, and sporulation. Three isolates of each lineage were used for the experiments. Detached tomato ‘Kada’ or potato ‘Bintje’ leaflets from 6- to 8-week-old plants grown in a

greenhouse were placed in 11 × 11 × 4 cm plastic boxes. Tomato leaflets were inoculated with isolates from tomato plants and potato leaflets with isolates from potato plants. Each box was lined with sterile paper towels wetted with sterile distilled water and contained three leaflets of either tomato or potato. Each leaflet was inoculated by placing a 20-µl drop of a 3 × 10⁴ sporangia/ml suspension in the central part on the abaxial side. After inoculation, the boxes were covered and transferred to incubators at 10, 15, 22, or 27°C.

The following variables were assessed:

Incubation period (IP), defined as the time (h) between inoculation and symptom appearance, was determined by visually observing the inoculated leaflets at 36, 44, 52, 60, 68, 76, 84, 92, 100, 108, and 116 h after inoculation (hai). The IP was considered completed when symptoms (tiny necrotic spots) were observed on at least two of the three inoculated leaflets in each box.

Latent period (LP), defined as the time (h) between inoculation and formation of sporangiophores with or without sporangia, was determined by inspecting each lesion under a stereoscope at the same time intervals described for IP. The LP was considered completed when sporangiophores were observed on at least two of the three inoculated leaflets in each box. This operational definition of LP was chosen to speed the assessment time and allow evaluation of all experimental units within a shorter time interval.

Lesion area (LA) was measured daily from 36 hai until 180 hai, depending on the temperature in which leaflets were maintained. Lesions were photographed with a digital camera and the images were processed with the ImageTool software (<http://ddsdx.uthscsa.edu/dig/itdesc.html>). Each image was visually inspected and the border of each lesion was manually delimited. The software measures lesion area (cm²) based on the number of pixels inside the delimited polygon.

Sporulation (SP). Two experiments using whole plants were established to quantify the effect of temperature on *P. infestans* sporulation. Fifteen leaflets of tomato ‘Kada’ or 15 of potato ‘Bintje’, 7 to 8 weeks old, were inoculated with a 50-µl drop of 3 × 10⁴ sporangia/ml suspension of each isolate of US-1 or BR-1, respectively. Immediately after inoculation, the plants were covered with plastic bags, as described above, and transferred to growth chambers at 10, 15, 22, or 27°C, with a 14-h photoperiod. After 24 hai, the plastic bags were removed. To keep the lesions wet, plants were sprayed every 8 h with distilled water for 7 days. For the plants kept at 10°C, the wetting procedure extended up to 10 days after inoculation. On the seventh or tenth day (for plants kept at 10°C), the plants were sprayed with distilled water and covered with plastic bags to provide wet conditions and stimulate sporulation.

Table 1. Characteristics of the Brazilian US-1 and BR-1 isolates of *Phytophthora infestans* used in the experiments

Isolate	Clonal lineage	Host of origin	Mating type	State	Region	Metalaxyl sensitivity
Pib-4	BR-1 ^a	Potato	A2	SC ^b	S ^c	Intermediate ^a
Pib-193	BR-1	Potato	A2	MG	SE	Resistant
Pib-198	BR-1	Potato	A2	MG	SE	Resistant
Pib-214	US-1	Tomato	A1	SC	S	Sensitive
Pib-239	US-1	Tomato	A1	MG	SE	Sensitive
Pib-256	US-1	Tomato	A1	SP	SE	Sensitive

^a Clonal lineage and metalaxyl sensitivity of each isolate was determined as described elsewhere (27).

^b SC, MG, and SP = Santa Catarina, Minas Gerais, and São Paulo states, respectively.

^c S and SE = south and southeast Brazilian regions, respectively.

Based on data collected on different sets of leaflets of tomato and potato plants, equations were generated to estimate lesion area by measuring length and width of 100 lesions and calculating the area of an ellipsis. To assess the “real” area of a lesion, an image was obtained with a digital camera and the lesion area was measured using the ImageTool software. Through regression analysis of the estimated area (ellipsis) versus real area (digital images), two equations were generated: Tomato lesion area = $-2.090 + 1.213 \text{ length} + 1.634 \text{ width}$ ($R^2 = 0.85$); and Potato lesion area = $-2.331 + 1.703 \text{ length} + 1.117 \text{ width}$ ($R^2 = 0.88$).

In the experiment, length and width of 12 lesions per plant per treatment were measured and the areas were estimated using the proper equation. Lesions were then cut and transferred to test tubes with 5 ml of preservation solution (2.5 ml of distilled water and 2.5 ml of 0.04 M $\text{CuSO}_4/0.2$ M sodium acetate/acetic acid, pH 5.4). Tubes were vortexed for 20 s to dislodge sporangia. The plant tissue was removed, and the suspension was kept at 4°C. Sporangia were counted using a hemacytometer. The number of sporangia per unit of lesion area was determined as previously described (25).

All experiments were conducted twice, each in a completely randomized design. Three replicates were used in the experiments to assess temperature effects on IP, LP, and LA. A plastic box with three leaflets comprised an experimental unit. For the SP experiment, there were 15 replicates; a pot with one plant comprised an experimental unit.

Statistical analyses. All data were analyzed with the SAS System version 8.0 (SAS Institute Inc., Cary, NC). The homogeneity of variances between experiments was tested using PROC MIXED, based on the difference of $-2\text{REML Log Likelihood}$ values and the significance level calculated based on the chi-square distribution (20). Data of two experiments were pooled when the hypothesis of equal variances was not rejected.

To analyze the effect of temperature on sporangia germination, IP, LP, and SP, the treatment design was a nested-crossed factorial, and the mixed effects linear model used for the analyses of variance was: $Y_{ijkl} = \mu + \alpha_i + b_{j(i)} + \gamma_k + \alpha\gamma_{ik} + bc_{jk(i)} + e_{ijkl}$ (μ = overall mean, α_i = fixed effect of clonal lineage, $b_{j(i)}$ = random effect of isolates nested within clonal lineages, γ_k = fixed effect of temperature, $\alpha\gamma_{ik}$ = interaction term of clonal lineage and temperature [fixed], $bc_{jk(i)}$ = interaction term of isolates nested within clonal lineages and temperature [random], and e_{ijkl} = random experimental error) (19). Similar models were fit to analyze the effect of temperature and leaf wetness on the number of lesions as well as the effect of temperature and time on LA.

RESULTS

Temperature effects on sporangia germination. Data were pooled for analysis because variances of both experiments did not differ ($P = 0.053$). The average clonal lineage effect was estimated. There was significant clonal lineage $\times$ temperature interaction when analyzing direct ($F = 23.9$; $P < 0.0001$) and indirect germination ($F = 27.3$; $P < 0.0001$). Temperature had a differential effect on sporangia germination depending on the clonal lineage. For isolates of both lineages, the indirect germination was highest at 10°C (Fig. 1A). For both US-1 and BR-1, indirect germination decreased as temperature increased,

but the indirect germination of US-1 was lower than that of BR-1 isolates. At 22°C, sporangia of US-1 did not germinate indirectly, while the germination of BR-1 was 0.57%. At 27°C, indirect germination of isolates of both lineages was minimal (Fig. 1A). For both lineages, highest direct germination was at 22°C (Fig. 1B). There was no direct germination at 10°C for either clonal lineage. Direct germination levels of US-1 isolates were higher than those of the BR-1 isolates at 15, 22, and 27°C (Fig. 1B). Direct germination of BR-1 isolates was low at 15 and 27°C (Fig. 1B).

Number of lesions caused by isolates of US-1 and BR-1 clonal lineages. Tem-

Fig. 1. Indirect (A) and direct (B) germination of sporangia of *Phytophthora infestans* isolates of BR-1 and US-1 clonal lineages at different temperatures. Data from two experiments were pooled. Error bars represent the standard deviation. All regression models were significant ($P = 0.05$).

perature and leaf wetness duration differentially affected the isolates of the two lineages. The US-1 isolates produced almost no lesions at 10°C, whereas for BR-1 the maximum number of lesions occurred at 10°C (Fig. 2). For US-1 isolates, the maximum number of lesions occurred at 15°C, with slightly fewer lesions at 22°C, and still some lesions at 27°C. BR-1 isolates produced no lesions at 27°C. In general (except at 27°C), US-1 isolates produced fewer lesions than BR-1 isolates. For isolates of both lineages, more lesions were produced when the duration of leaf wetness lasted 18 or 24 h.

Temperature effects on incubation period, latent period, lesion area, and sporulation. For all variables assessed, data from the two experiments were pooled for analysis after testing for homocedasticity.

Incubation period. The interaction between clonal lineage and temperature was significant ($P < 0.001$) (Table 2). Shortest IP for both lineages occurred at 22°C, and

was 69.3 h for US-1 in both experiments (Fig. 3A), and 44.0 and 49.3 h for BR-1 in experiments 1 and 2, respectively. The longest IP times were at 10 and 27°C, for US-1 and BR-1, respectively (Fig. 3A).

The variance component for isolates within clonal lineages was low (0.89) compared to the variance component of the residual (44.3) (Table 2). The latter estimates the amount of variance that is left over after the variance due to isolates within clonal lineages and the interaction temperature $\times$ isolates nested in clonal lineages have been explained. Thus, the variation between isolates was not high and the differences between clonal lineages were more evident ($F = 214.4$; $P < 0.001$) (Table 2).

Latent period. The interaction between clonal lineages and temperature was significant ($P < 0.001$) (Table 2). Thus, temperature differentially affected LP of the clonal lineages, but for all temperatures, US-1 isolates took longer to develop than did BR-1 isolates. The general trend for

isolates of both lineages was a reduction of LP with increasing temperatures up to 22°C. Between 22 and 27°C, LP tended to increase (Fig. 3B). No sporangiophores were produced by US-1 isolates at 10°C or by BR-1 isolates at 27°C (Fig. 3B). Thus, in both cases the LP was not recorded. Shortest LP values were recorded at 22°C for isolates of both lineages in both experiments. At 22°C, LP values for US-1 in experiments 1 and 2 were 93.3 and 106.0 h, respectively, and for BR-1 in experiments 1 and 2 were 68.0 and 76.7 h, respectively (Fig. 3B). Most variability was due to the residual term (66.6); variation among isolates within clonal lineages was low, 2.7 (Table 2).

Lesion area. In general, at higher temperatures more lesions were incited by the US-1 isolates than by the BR-1 isolates ($P = 0.0006$). At 10°C, the US-1 isolates induced no lesions, whereas the BR-1 isolates did (Fig. 4). Conversely, at 27°C, US-1 induced lesions, whereas no BR-1 isolates did. For US-1, lesion growth rates

Fig. 2. Number of lesions caused by isolates of US-1 and BR-1 clonal lineages of *Phytophthora infestans* in plants that were kept in different combinations of temperature and leaf wetness duration after inoculation. Values are averages from the two experiments. Isolates of US-1 or BR-1 were inoculated in tomato or potato plants, respectively.

increased with temperature from 15 through 27°C. For BR-1 isolates, lesion growth rates increased with temperature from 10 through 22°C. Variation among isolates within clonal lineages was low, 0.17 (Table 2).

Sporulation. Temperature differentially affected sporulation of the clonal lineages ($P = 0.0260$), and the maximum sporulation of the US-1 isolates occurred at slightly higher temperatures than that of the BR-1 isolates. Maximum sporangia production of US-1 isolates was at 22°C, and they did not sporulate at 10°C (Fig. 3C). BR-1 isolates sporulated most at either 15 or 22°C, and they did not sporulate at 27°C (Fig. 3C).

DISCUSSION

Important events during the infection cycle of the Brazilian clonal lineages of *P. infestans* were differentially affected by temperature. In general, the optimum temperature for BR-1 isolates to develop faster, to cause more lesions, and to produce more sporangia were lower than the optimum for the US-1 isolates. Indirect germination, which is favored by low temperatures (4,22), was higher for BR-1 isolates. Direct germination, which is favored by temperatures above 20°C, was higher for US-1 isolates. Effects of temperature on sporangia germination are well known (4). Even though detailed information on how temperature affects genes involved in zoospore formation has been gathered (31,32), isolates of the new and old lineages of *P. infestans* have not yet been compared regarding differential response to temperature at the gene level. Differences in temperature responses between US-1 and BR-1 lineages could be related to potentially different alleles/genes present in isolates of these lineages and/or to the variable amount of expression of simi-

lar genes. At this point, there are no data to support this claim, and further research to assess differences in gene expression of new versus old lineages of *P. infestans* is needed.

The low temperature requirement for high germination levels of BR-1 isolates could restrict epidemic development in subtropical/tropical areas. However, this seems not to be the case for late blight epidemics on potatoes in Brazil that are caused by BR-1, as production areas are located at higher altitudes where night temperatures are suitable for sporangia germination. At nighttime, the combination of mild temperatures and high humidity creates favorable microclimatic conditions for sporangia germination. Historical monthly average temperatures from April to October, the major potato growing season, in the producing areas where BR-1 isolates were collected, ranged from 9.5 to 21°C. Therefore, periods with favorable temperatures do occur and epidemic development follows.

The combined effects of temperature and leaf wetness duration periods on the number of lesions were largely influenced by temperature. Extending the leaf wetness period to 24 h did not compensate for the effects of limiting temperatures. At 27°C and 24 h of leaf wetness duration, there were no lesions on potato leaflets inoculated with BR-1 isolates, probably because no sporangia germinated at 27°C. US-1 isolates caused no lesions at 10°C, in both hosts, even when the leaflets remained wet for 24 h, despite the fact that sporangia germinated at this temperature. Most likely, the low temperature was the major factor that limited lesion formation. It is known that optimum temperatures for late blight epidemics are between 16 and 23°C (12), and there is practically no epidemic at 7°C (16) or above 28°C (28). Develop-

ment of lesions by BR-1 and US-1 at 10 and 27°C, respectively, reinforces the idea that BR-1 isolates are favored by low temperatures and US-1 isolates by higher temperatures.

The effects of temperature on IP, LP, and LA cannot be properly explained at this time due to the lack of detailed data on how pathogen mycelium inside host tissues is affected. Lesion area can be influenced by the intensity of pathogen colonization in the host. The amount of pathogen hyphae of an isolate putatively of the US-1 lineage in potato leaflets was lower at 13°C than at 17°C (13). To date, there is no report on mycelial growth of isolates of the new lineages inside host tissues. The absence of pathogen structures on lesions of BR-1 isolates at 27°C could be attributed to unfavorable temperature for growth and/or sporulation. After 15 days at 10°C or 8 days at 27°C, US-1 or BR-1 isolates, respectively, did not complete the LP in the inoculated leaflets. However, when the leaflets were transferred to 20°C, the lesions started to produce sporangia after 12 h (data not shown). This suggests that the pathogen can remain viable inside the host under unfavorable conditions; as soon as the conditions become favorable, it may quickly return to an active state.

The continuous moist conditions in which the inoculated leaflets were kept allowed sporulation over a wide range of temperatures. High numbers of sporangia/unit area were recorded at 15 and 22°C. This agrees with the optimum temperature range reported previously in which plenty of sporangia formed from 18 to 22°C under high relative humidity (1,25). Observing that there was no sporulation of US-1 isolates at 10°C or of BR-1 isolates at 27°C reinforces the findings of the experiments with detached leaflets in which no lesion developed at 10 or 27°C

Table 2. Analysis of variance after fitting a mixed model to incubation period (IP), latent period (LP), lesion area (LA), and sporulation per cm² of lesion, with data from two experiments pooled

Component	Test of fixed effects	df num ^a	df den ^a	F value	Probability	Covariance parameter ^b	Estimate
IP	Clonal lineage (CL)	1	4	214.4	0.0001	Isolate(CL) ^c	0.89
	Temperature (Temp)	3	12	354.7	<0.0001	Temp*Isol(CL) ^c	3.73
	CL*Temp ^c	3	12	147.6	<0.0001	Residual	44.3
LP	CL	1	5.6	370.9	<0.0001	Isolate(CL)	2.69
	Temp	3	10.6	180.8	<0.0001	Temp*Isol(CL)	28.70
	CL*Temp	2	10.7	134.6	<0.0001	Residual	66.56
LA	CL	1	2	15.5	0.059	Isolate(CL)	0.17
	Temp	3	6	72.8	<0.0001	Temp*Isol(CL)	0.47
	CL*Temp	3	6	28.8	0.0006	Residual	2.81
Spor/area ^d	CL	1	5.99	9.9	0.020	Isolate(CL)	0.00
	Temp	3	6.05	65.4	<0.0001	Temp*Isol(CL)	0.007
	CL*Temp	1	5.99	8.7	0.026	Residual	0.096

^a df num = degrees of freedom of the numerator; df den = degrees of freedom of the denominator, which were estimated through the Satterthwaite approximation for unbalanced data sets.

^b Covariance parameter of random effects.

^c CL*Temp = clonal lineage × temperature interaction; Isolate(CL) = isolates nested within clonal lineages; and Temp*Isol(CL) = interaction between temperature and isolates nested within clonal lineages.

^d Spor/area = log₁₀ (number of sporangia per cm² of lesion).

Fig. 3. Incubation period (A), latent period (B), and sporulation per unit of lesion area (C) of isolates of US-1 and BR-1 clonal lineages of *Phytophthora infestans* in leaflets kept at different temperatures after inoculation, averaged from data of two experiments. Isolates of US-1 or BR-1 were inoculated in tomato or potato leaflets, respectively. Incubation and latent periods were assessed in detached leaflets. Sporulation per unit of lesion area was assessed in whole plants. Error bars represent the standard deviation.

for US-1 and BR-1, respectively. Additional experiments in which lesions would be allowed to develop under fluctuating temperature conditions would provide more detailed information on how temperature affects pathogen sporulation.

An interesting question could be raised regarding the effects of temperature on isolates of a lineage when they infect the nonpreferred host. Unfortunately, this effect could not be tested in this study due to differences in aggressiveness between the two lineages when they infect the non-preferred host. Isolates of US-1 are more aggressive on tomato than on potato, whereas isolates of BR-1 are more aggressive on potato than on tomato (30). In cross-inoculation experiments, the effects of temperature would be confounded with those related to host-specificity.

Populations of *P. infestans* with distinct ecological requirements could have originated from the introduction of genotypes having different requirements and/or from the adaptation of lineages to the climatic conditions where potato and tomato are grown. Recent migrations (gene flow) of *P. infestans* have resulted in the introduction and subsequent distribution of new genotypes in many countries (8,9). These genotypes are distinct from the old US-1 lineage in many epidemiologically important traits such as lower metalaxyl sensitivity (10), higher aggressiveness (17), and differential response to temperature (18,25). Isolates of BR-1 with different ecological requirements might have been introduced to Brazil via seed tubers imported from Europe. The patterns of the *Gpi* and *Pep* (100/100) isozymes, typical of BR-1 isolates, are commonly found in European populations of *P. infestans* (27), and in the past there was intensive trade of potato seed tubers with the Netherlands.

Selective adaptation of BR-1 isolates to high altitude climatic conditions, after their introduction in Brazil, is not a plausible hypothesis to explain the differential temperature responses noted in its comparisons with US-1 in this study. Although late blight is known to have occurred in Brazil since 1898 (26), based on all evidence gathered, only A1 mating type isolates, presumably the US-1 lineage, were most likely associated with late blight epidemics until the early 1980s (8) when BR-1 first was detected (2). In contrast, the location of tomato producing areas at lower altitudes, the higher fitness of US-1 on tomato, and the longer time of co-evolution of US-1 with tomato plants may have contributed to the adaptation of US-1 isolates to higher temperatures. Curiously, sporulation of US-17, a new tomato adapted lineage, inoculated on tomato kept at 28°C was almost nil (1), whereas the sporulation of US-1 isolates at 27°C was similar to that recorded at the optimum temperature.

The marked effects of temperature in the events of the infection cycle of *P. infestans*

Fig. 4. Lesion area caused by isolates of US-1 and BR-1 clonal lineages of *Phytophthora infestans* in detached leaflets that were kept at different temperatures and measured at different time intervals, after inoculation, averaged from data of two experiments. Isolates of US-1 or BR-1 were inoculated in tomato or potato leaflets, respectively. Differences in measurement times are due to different leaflet sizes and times at which lesions reached the borders of the leaflets.

lineages in Brazil could affect late blight management, whether epidemics develop on tomato or on potato. The fact that the optimum temperature for US-1 isolates was higher than that for BR-1 isolates helps to explain the frequent outbreaks of severe late blight epidemics on tomato crops during the summer in Brazil. On the other hand, late blight epidemics are likely to be less severe on potato because BR-1, the potato-associated lineage, develops at a slower rate under high temperatures. However, potatoes are grown in areas of high altitude, and temperatures at night can be favorable for infection. Additionally, frequent rains during the summer also favor epidemics. During the winter, temperatures are suitable for late blight epidemics in both hosts, but rains are not common, relative humidity is low, and disease intensity is lower than during the rainy season. The differential responses to ecological variables must be taken into account when implementing disease management tools such as forecast systems and also when using late blight simulation models for the evaluation of control practices.

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