Effect of Temperature and Photoperiod on Symptoms Associated with Resistance to *Phytophthora infestans* After Leaf Penetration in Susceptible and Resistant Potato Cultivars

O. A. Rubio-Covarrubias[1,2], D. S. Douches[3], R. Hammerschmidt[2], A. daRocha[2], and W. W. Kirk[2]*

[1]INIFAP, KM 4,5 Carretera, Toluca-Zitacuaro, Zinacantepec, Mexico
[2]Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA
[3]Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA
*Corresponding author: Tel: (517) 353-4481; Fax: (517) 353-4940; Email: kirkw@msu.edu

ABSTRACT

The effect of temperature and photoperiod on the expression of resistance against *Phytophthora infestans* in five potato cultivars with and without resistance (R) genes was investigated. Four experiments were carried out under controlled conditions in growth chambers. Two cultivars (393295.236 and 391046.22) without known R genes from the International Potato Center (CIP) in Lima, Peru, two Mexican cultivars with major R genes (Tollocan and Malinche), and a susceptible cultivar (Atlantic) were used in this study. Plants were grown for 32 days in growth chambers at two temperatures (16 and 24 C) and two photoperiods (12 and 16 h day length), then inoculated with a compatible isolate of *P. infestans* and incubated in a mist chamber at 18 C. The inoculation efficiency, the percentage of lesions that did not grow beyond the inoculation spot, the sporangia density, and the AUDPC were recorded. The percentage of arrested lesions decreased with temperature in the two most resistant cultivars (393295.236 and Malinche), and the AUDPC was lower at 16 than at 24 C in four of the five cultivars. The inoculation efficiency and the sporangia density were not affected by change in temperature. Sporangia density increased at 16 h photoperiod; however, the final infected leaf area was not affected. Our results demonstrate that the expression of horizontal and vertical resistance was affected by temperature; however, the relative resistance ranking among cultivars was the same in the four experiments with different temperatures and photoperiods. It is assumed that the resistance in the Mexican cultivars may be conferred by minor resistance genes and by the residual effect of defeated R genes. These results emphasize the difficulty in differentiating between horizontal and vertical resistance.

RESUMEN

El efecto de temperatura y fotoperíodo sobre la expresión de resistencia contra *Phytophthora infestans* fue investigada en cinco cultivares de papa con y sin genes de resistencia (R). Se hicieron cuatro experimentos en una cámara de crecimiento bajo condiciones controladas. Dos cultivares (393295.236 y 391046.22) del Centro Internacional de la Papa (CIP) Lima, Perú, dos cultivares mexicanos con genes mayores (R) (Tollocan y Malinche) y un cultivar susceptible (Atlantic) se usaron en este estudio. Las plantas se hicieron crecer por 32 días en cámaras de crecimiento a dos temperaturas (16 y 24 C) y dos fotoperíodos (12 y 16 horas de longitud del día); fueron inoculadas con un aislamiento compatible de *P. infestans* e incubadas en una cámara de crecimiento a 18 C provista de vapor, y se registró la eficiencia de inoculación, porcentaje de lesiones que no crecieron más allá del punto de inoculación, densidad de esporangios y AUDPC. El porcentaje de lesiones que no crecieron aumentó con la temperatura en dos de los cultivares más resistentes (393295.236 y Malinche) y el AUDPC fue más bajo a 16 que a 24 C en cuatro de los cinco cultivares. La eficiencia de la inoculación y la den-

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sidad de esporangios no se afectó por cambios de temperatura. La densidad de esporangios aumentó a 16 horas de fotoperíodo; sin embargo, el área final de hoja infectada no se afectó. Nuestros resultados demuestran que la temperatura afectó la expresión de las resistencias vertical y horizontal; sin embargo, la categorización de la resistencia relativa entre cultivares fue la misma en los cuatro experimentos con temperatura y fotoperíodos diferentes. Se asume que la resistencia en cultivares mexicanos puede ser conferida por genes menores y por el efecto residual de los genes R vencidos. Estos resultados enfatizan la dificultad de diferenciar la resistencia horizontal de la vertical.

INTRODUCTION

The effect of environmental factors on the development of *Phytophthora infestans* (Mont.) de Bary on potato plants as well as the interaction between potato cultivar, environment, and pathogen have been studied extensively (Flores-Gutiérrez and Cadena-Hinojosa 1996; Grünwald et al. 2002). However, the systematic effect of individual environmental factors on the resistance of potato plants to late blight has received limited attention. Parker et al. (1992) demonstrated that the cultivar Alpha, which is regarded as moderately susceptible to late blight, exhibited higher resistance in northern (New York, USA, Lat ca. 44-45) than in southern latitudes (Toluca, Mexico, Lat 19). This work was done in the field and differences between sites may have been due to the presence of local races of *P. infestans* in Toluca, which could have influenced disease severity. Different climatic conditions in both sites could have also influenced the development and growth of the pathogen. However, observations made during field evaluations (Rubio et al. 1998-2001, unpublished data) of Mexican cultivars in different sites in Mexico have indicated that resistance may be influenced mainly by temperature. This hypothesis required testing under controlled conditions to avoid interference of the influence of climatic factors on *P. infestans* growth and also to avoid interference due to the presence of different races of the pathogen.

The influence of environmental factors on the expression of resistance against pathogens has been studied in other crops. Jenns and Leonard (1985) found that resistance to *Bipolaris maydis* (Nisikado and Miyake) Shoemaker in different lines of maize diminished with increase in temperature or decrease in luminance. In wheat, increasing temperatures inhibited the expression of resistance genes against *Puccinia graminis* f.sp. *tritici* Eriks. and E. Henn (Harder et al. 1979). These studies suggested a possible influence of temperature and light on the expression of resistance against pathogens.

The relationship between host resistance and environmental factors has relevance for the prediction of the behavior of potato cultivars in sites with different climatic conditions and also the effect of world climatic changes, mainly temperature, on the resistance of cultivars to *P. infestans*. This issue is also related to the stability of the different kind of resistances to late blight. Since the initial concepts of Van der Plank (1963) concerning horizontal and vertical resistance, there has been consistent interest on the differentiation of both types of resistance. However, the distinction between the two types of resistance is still not clear. Recent evidence has shown that the hypersensitive reaction, which has been regarded as exclusive to plants with R genes, may occur in host plants with horizontal as well as vertical resistance (Vleeshowers et al. 2000). Based on links between R genes and QTLs for quantitative resistance, it has been stated that there is no difference between genes controlling quantitative or qualitative resistance to potato late blight (Gebhardt and Valkonen 2001). Their studies focused on comparing the expression of both types of resistance under different temperatures, but interactions with photoperiod were not included.

The report addresses the influence of temperature and photoperiod on the resistance to penetration and growth of *P. infestans* in leaves of potato cultivars with different types and levels of resistance. The objectives of this study were to elucidate the effects of temperature and photoperiod on disease expression after inoculation of *P. infestans* onto foliar tissue of potato cultivars with and without R genes and differing in susceptibility to *P. infestans* that could influence the subsequent epidemic. The factors measured included expression of development and growth of late blight lesions on leaves, the percentage of arrested lesions on leaves and sporulation potential.

MATERIALS AND METHODS

Two potato cultivars (Malinche and Tollocan) with major R genes, two cultivars (393295.236 and 391046.22) without known R genes and one susceptible cultivar (Atlantic; R1) were selected for this study. Malinche and Tollocan are Mexi-

can cultivars bred by INIFAP (National Institute for Forestry, Agriculture and Livestock Research). These cultivars possess moderate and high resistance against potato late blight, respectively. Their resistance is based on a combination of unknown major and minor genes derived from *Solanum demissum.* The cultivars 391046.22 (CIP1) and 393295.236 (CIP2) were bred in the CIP (International Potato Center) and form part of a group of cultivars identified by CIP as population B3 whose sources of horizontal resistance are genetic materials without R genes derived from *Solanum demissum* (Landeo 1997). The cultivars CIP1 and CIP2 have been tested in Toluca, Mexico, where they have shown medium and high resistance against late blight respectively (C. Díaz and O. Rubio 2000, unpublished data). Both cultivars are part of the group of materials known as population B derived from parents that did not show the hypersensitivity reaction after inoculation with race 0 of *P. infestans*, indicating absence of R genes (J.A. Landeo, pers comm).

Plant Growth Conditions

In vitro plantlets of each cultivar were grown for 3 wk in sterile plastic magentas, then transferred into pots of peat moss and placed in temperature-controlled environment chambers, 1.8 m^3 volume (Environmental Growth Chambers, Chagrin Falls Ohio, USA). Ten plants (one per pot) of each cultivar were grown during 32 days in independent growth chambers under the four combinations of two constant temperatures (16 and 24 C) and two photoperiods (PPD, 12 and 16 h day length). The relative humidity was programmed in the climatic chambers at 80%, and the illumination was provided by 500 W Philips-HPIT lamps. The fourth fully expanded compound leaves, counted from the apex of the main stem from each plant, were detached 32 days after plants were incubated at the temperature and photoperiod treatments.

Phytophthora infestans *Isolate and Inoculation*

The *P. infestans* isolate CO-42 (mating type A2; mefenoxam resistant; mt haplotype 1A; GPI 100/100; PEP 100/100) collected in Mexico and kindly provided by K. Deahl, USDA, was used in this study. The compatibility of this isolate with all five potato cultivars was verified by inoculating detached leaflets of each cultivar prior to imposition of the incubation treatments. In order to preserve the virulence of the isolate, it was continuously propagated on potato leaves of the cultivar CIP2. For each experiment, fresh sporangia were harvested from two leaves (8 days after inoculation) and suspended in a glass beaker with 50 mL of sterile distilled water and incubated at 4 C. After 2 h incubation, the concentration of zoospores was adjusted to $3x10^4$ mL^{-1} for inoculation.

The primary and first pair of leaflets of the fourth leaf from each plant were detached. The three leaflets were placed in a Petri dish with water-saturated filter paper and inoculated by pipetting a 10 µL droplet of inoculum suspension ($3x10^4$ of zoospores mL^{-1}) on the abaxial side of each leaflet.

The Petri dishes with the inoculated leaflets were incubated at 18 C in a dew chamber with a photoperiod of 12 h light (RH > 95%). Forty-eight hours after inoculation, leaflets were used to assess their susceptibility to potato late blight by measuring the expansion of the lesions daily for 7 to 8 days after inoculation. The largest length (L) and width (W) of each lesion were measured and used to calculate the lesion area (A); ($A = 0.25\pi LW$) and the lesion growth rate (LGR). The area under disease progress curve (AUDPC [Madden and Hughes 1995]) was calculated with the expansion of the area of the lesions over time (7-8 days after inoculation). The inoculations that did not result in lesions and the lesions that did not expand beyond the inoculum droplet area were recorded as arrested lesions and were not included in the estimation of the LGR and AUDPC but were recorded as useful components of the late blight resistance response.

Sporangial density was quantified 8 to 9 days after inoculation. Six leaf tissue discs, 12.6 mm in diameter, were removed with a cork borer from the infected tissue of two inoculated leaflets from each Petri dish. The sporangia were washed from both sides of the discs by stirring them in 5 mL of chilled (4 C) water, and the sporangial density was determined with a hemacytometer. The average measurements of the lesions on the two inoculated leaflets in each Petri dish were used for statistical analysis as a single observation. The 10 combinations of five cultivars and two temperatures (16 and 24 C) constituted a single experiment with a factorial design, which was repeated twice at a photoperiod of 12 h and another two times at a photoperiod of 16 h. The effect of photoperiod could not be statistically tested due to possible differences in the experimental conditions among the four experiments. Four variables (percent of positive inoculations, percent of arrested lesions, AUDPC, and sporangia density)

were analyzed in each of the four experiments independently as a factorial design with cultivar and temperature as the main effects (Proc GLM - SAS/Stat, SAS Institute, Cary, NC, USA).

RESULTS

Infection Efficiency

This parameter was measured as the percentage of the inoculated sites that developed lesions on the leaves. Testing the effect of temperature by cultivar interaction resulted in only two significant comparisons, from a total of 20, in experiments 2 and 3 (Table 1). The average effect of temperature was not significant in any of the four experiments. In experiments 1, 2, and 3 the comparison between cultivars indicated that the cultivar Malinche had the lowest percentage of positive inoculations.

Arrested Lesions

In the inoculated sites on the leaves, some lesions did not expand beyond the inoculum droplet area during the 7 to 8 days of incubation (Table 2). These arrested lesions were only in the cultivars CIP2 and Malinche, and it was evident that these arrested lesions were present in higher percentage in plants grown at 16 than at 24 C. The comparison between cultivars shows a higher percentage in Malinche than in CIP2 in three of the four experiments.

TABLE 1—*Percentage of inoculations of* Phytophthora infestans *resulting in lesions on leaves of five potato cultivars grown under two photoperiods (12 and 16 h) and at two temperatures (16 and 24 C) inoculated with* P. infestans *in four independent experiments.*

	Percentage of inoculations with *P. infestans* resulting in lesions on the leaves											
	12 h photoperiod						16 h photoperiod					
	Experiment 1 Temperature (C)			Experiment 2 Temperature (C)			Experiment 3 Temperature (C)			Experiment 4 Temperature (C)		
Cultivar	16	24	Mean	16	24	Mean	16	24	Mean	16	24	Mean
CIP1	95	100	98a[a]	75	100*[b]	88a	100	96	98a	100	96	98a
CIP2	85	100	93a	75	95	85a	90	100	95ab	96	100	98a
Malinché	70	65	68b	60	65	63b	100*	76	88b	96	96	97a
Tollocan	95	100	98a	95	100	98a	93	96	95ab	100	100	100a
Atlantic	100	100	100a	100	85	93a	96	100	98a	100	100	100a
Mean T[c]	89	93		81	89		96	94		98	98	

[a]Means of cultivar in each experiment: values followed by the same letter are not statistically different at $P < 0.05\%$, t test.
[b]The comparison of the means of each cultivar at the two temperatures is by t test ($P < 0.05\%$) and the * indicates the highest value.
[c]The main effect of temperature in each experiment was tested by ANOVA ($P < 0.05\%$) and the * indicates the highest value.

TABLE 2—*Percentage of arrested lesions on leaves of five potato cultivars grown under two photoperiods (12 and 16 h) and at two temperatures (16 and 24 C) inoculated with* Phytophthora infestans *in four independent experiments.*

	Percentage of arrested lesions on leaves after inoculation with *P. infestans*											
	12 h photoperiod						16 h photoperiod					
	Experiment 1 Temperature (C)			Experiment 2 Temperature (C)			Experiment 3 Temperature (C)			Experiment 4 Temperature (C)		
Cultivar	16	24	Mean	16	24	Mean	16	24	Mean	16	24	Mean
CIP1	0	0	0b[a]	0	0	0b	0	0	0b	0	0	0b
CIP2	0	0	0b	5	0	3b	13*	0	7a	13*	0	7b
Malinché	25*[b]	10	18a	20*	0	10a	7	0	3ab	17	17	17[a]
Tollocan	0	0	0b	0	0	0b	0	0	0b	0	0	0b
Atlantic	0	0	0b	0	0	0b	0	0	0b	0	0	0b
Mean T[c]	5	2		5*	0		4*	0		6	3	

[a]Means of cultivar in each experiment: Values followed by the same letter are not statistically different at $P < 0.05\%$, t test.
[b]The comparison of the means of each cultivar at the two temperatures is by t test ($P < 0.05\%$) and the * indicates the highest value.
[c]The main effect of temperature in each experiment was tested by ANOVA ($P < 0.05\%$) and the * indicates the highest value.

AUDPC

Three days after inoculation, the lesions in all five cultivars were apparent and expanded very slowly during the following two days. Then after the fifth day, the lesions abruptly increased their expansion rate (Figure 1). The same trend was observed in each of the four experiments and only the disease progress curves from experiment 1 are shown. The average AUDPC of each experiment is presented in Table 3. The interaction between cultivar and temperature was significant in all experiments (data not shown) and AUDPC in CIP2, Malinche, Tollocan, and Atlantic increased when grown at 24 C (Table 3). However, AUDPC in CIP1 decreased when grown at 24 C (Table 3). CIP1 did not adapt well at 24 C, and the leaves generally looked wilted even though well watered. The interaction between cultivar and temperature, in three of the four experiments, indicated that the effect of temperature was significant and the AUDPC was higher at 24 C than at 16 C.

The comparison between cultivars indicated that Malinche and CIP2 had lower AUDPC and Atlantic had the highest AUDPC (Table 3). Even though the effect of photoperiod could not be tested statistically, there seemed to be no effect of the photoperiod on the AUDPC of the different cultivars (Table 3).

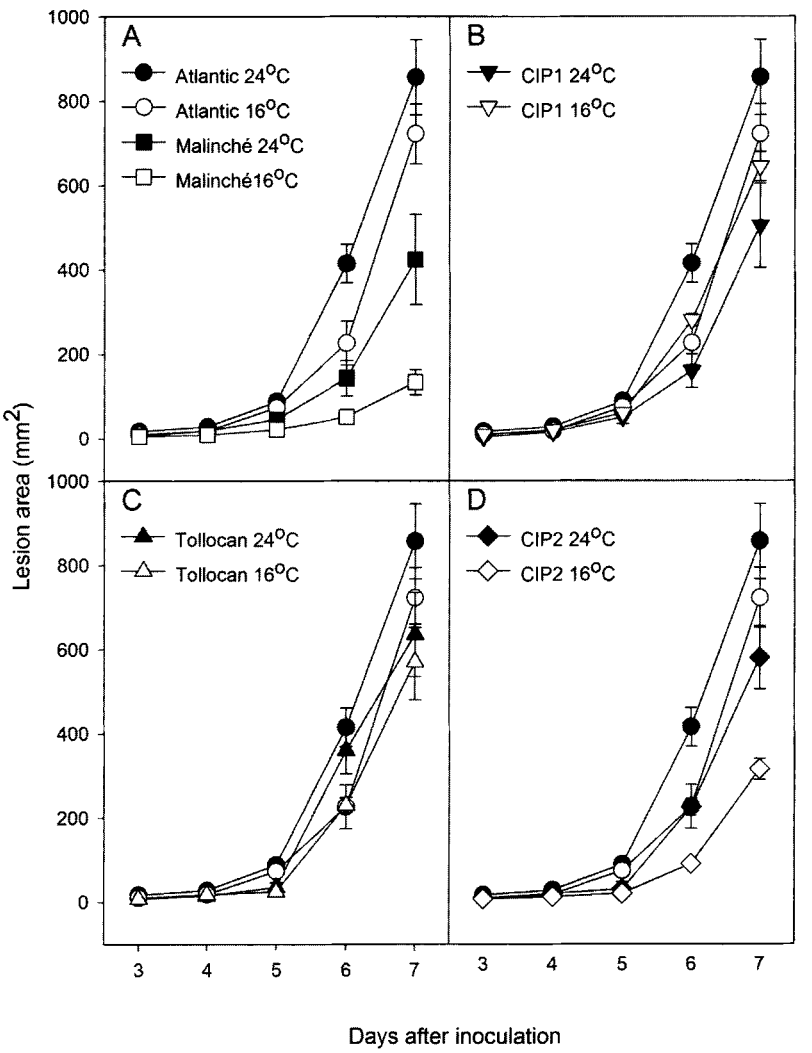

FIGURE 1 A-D.
Effect of the pre-innoculatoin temperature (15 or 24 C) on the expansion of leaf lesions of *Phytophthora infestans*. The graphs are from experiment 1 under a photoperiod of 12 h. The vertical bars indicate the standard error of the mean. The response of the cultivar Atlantic is included on each of the four graphs as a reference. A: Malinché; B, CIP1; C, Tollocan; D, CIP2.

Sporangia Density

There was no consistency in the effect of the temperature on the production of sporangia (Table 4). Testing the effect of the temperature by cultivar interaction resulted in only three significant comparisons, from a total of 20, favoring the sporangia density under 16 C and three significant comparisons with higher production of sporangia in plants that were grown at 24 C. There was no significant effect in the comparison of the average effect of the temperature in any of the four experiments. The variation in sporangia density depended mainly on the cultivar. Malinche, Tollocan, and CIP2 had the lowest density of sporangia per unit area of lesion. These cultivars also had the lowest AUDPC values and the correlation between sporangia density and AUDPC was significant ($P < 0.01$) in the four experiments (data not shown). Even though the effect of photoperiod can not be tested statistically, it was evident that all cultivars had higher sporangia density at 24 h than at 12 h of photoperiod (Table 4).

DISCUSSION

This study dealt with changes in resistance to *Phytophthora infestans* in potato cultivars with and without R genes, induced by changes in temperature and photoperiod dur-

TABLE 3—*Average AUDPC of lesions on leaves inoculated with* Phytophthora infestans *of five potato cultivars grown under two photoperiods (12 and 16 h) and at two temperatures (16 and 24 C) in four independent experiments.*

	AUDPC of leaves inoculated with *Phytophthora infestans*											
	12 h photoperiod						16 h photoperiod					
	Experiment 1[a] Temperature (C)			Experiment 2 Temperature (C)			Experiment 3 Temperature (C)			Experiment 4 Temperature (C)		
Cultivar	16	24	Mean	16	24	Mean	16	24	Mean	16	24	Mean
CIP1	660	517	589bc[b]	1041	819	930a	582	739	660b	1117*	723	920b
CIP2	300	644*[c]	472cd	570	808	689b	321	872*	597b	199	673*	436d
Malinché	169	550*	359d	359	858*	608b	258	244	251d	296	375	336d
Tollocan	611	732	672b	1001	1195	1098a	425	485	455c	567	876*	721c
Atlantic	698	993*	845a	1341*	763	1052a	965	1130	1047a	1184	1272	1228a
Mean T[d]	488	687*		862	889		510	694*		673	784*	

[a]The AUDPC in the experiments 1, 3, and 4 were calculated over a period of 7 days after inoculation and in experiment 2 over 8 days.
[b]Means of cultivar in each experiment: values followed by the same letter are not statistically different at $P < 0.05\%$, t test.
[c]The comparison of the means of each cultivar at the two temperatures is by t test ($P < 0.05\%$) and the * indicates the highest value.
[d]The main effect of temperature in each experiment was tested by ANOVA ($P < 0.05\%$) and the * indicates the highest value.

TABLE 4—*Average sporangia density (number of sporangia mm^{-2}) from lesions on leaves inoculated with* Phytophthora infestans *of five potato cultivars grown under two photoperiods (12 and 16 h) and at two temperatures (16 and 24 C) in four independent experiments.*

	Average density of sporangia (Number of sporangia mm^{-2}) in leaves inoculated with *P. infestans*											
	12 h photoperiod						16 h photoperiod					
	Experiment 1 Temperature (C)			Experiment 2 Temperature (C)			Experiment 3 Temperature (C)			Experiment 4 Temperature (C)		
Cultivar	16	24	Mean	16	24	Mean	16	24	Mean	16	24	Mean
CIP1	128	113	121bb	99	142	120b	215	210	212a	293*	82	188b
CIP2	83	83	83c	72	151*	112b	180	170	175b	83	95	89c
Malinché	39	68*[c]	54d	38	74	56c	106	114	110c	80	29	54c
Tollocan	36	46	42d	118	121	120b	77	77	77d	68	42	55c
Atlantic	141	152	147a	224*	135	179a	259*	175	217a	227	461*	344a
Mean T[d]	86	93		110	125		167	149		150	142	

[a]Average sporangia densities in experiments 1, 3, and 4 were measured 8 days after inoculation and in experiment 2 after 9 days.
[b]Means of cultivar in each experiment: values followed by the same letter are not statistically different at $P < 0.05\%$, t test.
[c]The comparison of the means of each cultivar at the two temperatures is by t test ($P < 0.05\%$) and the * indicates the highest value.
[d]The main effect of temperature in each experiment was tested by ANOVA ($P < 0.05\%$) and the * indicates the highest value.

ing the pre-inoculation growing conditions of the plants. The results indicated that temperature had no effect on the inoculation efficiency or sporangia density; however, the percentage of arrested lesions increased with temperature in the two most resistant cultivars and the AUDPC was lower at 16 than at 24 C in four of the five cultivars. These results suggest that the different components of resistance may be differently affected by climatic conditions during the pre-inoculation period. Our observations are similar to Jenns and Leonard (1985) in that lesion length and sporulation increased with increasing temperature, and, as in corn plants inoculated with *B. maydis*, the infection efficiency was not affected by the pre-inoculation temperature. There is no available information in the literature about the pre-inoculation effect of temperature on each one of the resistance components of the potato late blight pathosystem, and generally, the AUDPC has been taken as the final result of the expression of resistance. The influence of climatic conditions on resistance against *P. infestans* in potato has been previously reported by Parker et al. (1992), who observed that the cultivar Alpha had higher AUDPC in Freeville, NY, USA, than in Toluca, Mexico. In that study, the average temperature during the epidemic was lower in Toluca (18-20 C) than in Freeville (20-22 C). This may be interpreted as a negative effect of the temperature on the resistance to *P. infestans*,

which is opposite to what was observed in our study. However, it is important to point out the study of Parker et al. (1992) was carried out in the field and the influence of different races of *P. infestans* and of different climatic conditions on the growth of the pathogen could not be eliminated. The present study was conducted under controlled conditions and results clearly indicated a negative effect of higher temperature (24 C) on the total area of infected leaf tissue. Our results are in agreement with the results of Jenns and Leonard (1985), who observed that different maize lines diminished their resistance to *B. polaris maydis* with increasing temperature, and also with the findings of Harder et al. (1979), who demonstrated that increasing temperatures inhibited the expression of resistance genes against *P. graminis* f.sp. *tritici* in wheat.

The effect of photoperiod during plant growth on the percentage of successful inoculations, the percentage of arrested lesions and the AUDPC did not show a clear trend. The photoperiod only had an observable effect on the increment of sporangia density under 16 h of day length, which did not affect the final infected leaf area. This is not in agreement with the postulations of Parker et al. (1992), who suggested that photoperiod may be the most important environmental factor that influenced the susceptibility to *P. infestans* of the potato cultivar Alpha in Toluca, Mexico, and in Freeville, NY. The discrepancy between results of these two studies may be explained by the different experimental conditions mentioned above. The effect of photoperiod could not be statistically tested in this study and further experiments are needed to clarify this issue.

Similarly to results reported earlier (Krug 1965; Steward et al. 1981), we observed that the lower temperature and the shorter photoperiod induced earliness in all the tested potato cultivars. Using molecular techniques, it has been shown that resistance to late blight in potato is correlated with maturity and vigor (Collins et al. 1999; Visker et al. 2003a). It was therefore expected that a resistant cultivar may express decreased resistance as a consequence of its reduction in maturity under the influence of a lower temperature and shorter photoperiod. This effect was not observed in our study. A partial explanation is that changes of resistance with respect to leaf position, plant age, and leaf age (Dorrance and Inglis 1997; Visker et al. 2003a), were avoided in our study by inoculating leaves from the same position on the plant and before tuber growth started. The methodology for measuring the infection severity may also influence the interpretation of the results. We calculated the AUDPC with the expansion of the area of the lesions in a period of 7 to 8 days after inoculation; however, an AUDPC based on the percentage of foliar infection during the total life of the plant may lead to different results because the length of the vegetative phase of the plants may be changed by environmental factors.

Our results obtained under controlled conditions in growth chambers, confirmed what was previously observed in field tests in Toluca, Mexico (C. Díaz and O. Rubio 2002, unpublished data) where Malinche and CIP2 had high resistance to late blight, CIP1 and Tollocan medium resistance, and Atlantic was susceptible. Comparing the five cultivars, it seems that regardless of their type and level of resistance, their relative resistance (resistance ranking among the five cultivars) was not affected by changes in temperature and photoperiod; however, the AUDPC values were affected differently by temperature. All the cultivars, except CIP1, showed a general trend of lower AUDPC at the lower temperature (16 C). The cultivar CIP1 showed no adaptation to the high temperature (24 C), and the extreme heat stress may have induced some resistance to *P. infestans* in the wilted leaves.

The comparison of the tested potato cultivars provides information for a better understanding of horizontal and vertical resistance. Horizontal or quantitative resistance to late blight has been described as stable and durable (Parlevliet 1979; Van der Planck 1968). In the present study, it was demonstrated that the resistances of cultivars with horizontal resistance (CIP1 and CIP2) and those with a combination of horizontal and vertical resistance (Malinche and Tollocan) were affected by temperature; however, their resistance ranking was the same in the four experiments with different temperatures and photoperiods. Therefore, the tested cultivars with horizontal and vertical resistance were not stable in terms of their susceptibility to infection but were stable in terms of their relative resistance expression.

It is well known that the durability of resistance based on major (R) genes can be shortened by continuous changes in the pathogen (Goodwin et al. 1995; Van der Planck 1968); however the combination of major and minor resistance genes has been a successful strategy in many Mexican potato cultivars selected under natural infection conditions in the Toluca Valley, Mexico (Flores-Gutiérrez and Cadena-Hinojosa 1996; Grünwald et al. 2002). The four resistant potato cultivars used in this study were previously tested in the field in Toluca, where at least 11 known races of *P. infestans* are present, and

it is expected that the resistance of the four cultivars may be durable. Resistance in the cultivars free of R genes (CIP1 and CIP2) is assumed to be conferred by minor genes (Landeo et al. 2000). The observed resistance in the Mexican cultivars (Malinche and Tollocan) after inoculation with a compatible isolate of *P. infestans* may be conferred by minor resistance genes and by the residual effect of defeated R genes, which was previously demonstrated in potato (Stewart et al. 2003) and other crops (Durel et al. 2000; Li et al. 1999).

In conclusion, our results indicate that resistance against *P. infestans* in potato cultivars with and without R genes can be affected by the temperature at which the plants have been growing before inoculation. The general tendency was to decrease resistance with increase in temperature from 16 to 24 C; however, there may be some cultivars that respond differently. The relative stability of the resistance among the tested cultivars did not change with changes in temperature and photoperiod. These results emphasize the difficulty in differentiating horizontal and vertical resistance and confirm that a combination of both kinds of resistance is the best strategy to develop cultivars with stable and durable resistance.

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