

Environment and Slow Epidemics Favor Oosporulation of *Phytophthora infestans* Mont. De Bary, on Potato Leaves in the Toluca Valley, México

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Abstract The Toluca Valley, located in the central highlands of México, has optimal climatic conditions for the development of *Phytophthora infestans* as well as for sexual reproduction due to the presence of two mating types (1:1, ratio). Therefore, it is a suitable place to study late blight epidemics on cultivated potatoes. In order to quantify oospore formation on foliage during the progress of late blight epidemics and to establish the implications of (a) genetic resistance of the host, (b) disease management (fungicide application), and (c) environmental conditions, a study was conducted in the summer of 2002 and 2003 on two potato cultivars during the progress of the epidemic

under rainfed and natural infection conditions in the Toluca Valley. The cultivars were exposed to 0.0, 0.5, and 1.0× doses of the protectant fungicide chlorothalonil (1.0×=1.15 kg a. i. ha⁻¹). Oosporulation started 56 and 46 days after planting (2002 and 2003, respectively) with a maximum peak at 72 days in the two growing cycles. The total number of oospores in both years on cultivar Zafiro, resistant to *P. infestans*, was higher than on susceptible cv. Alpha (101 vs. 67). However, there were no statistically significant differences ($P=0.40$), which suggests that the resistance level of the host did not have a direct influence on oospore formation. The epidemics obtained from each of the treatments were characterized through multivariate analysis. Initial severity (Y_0), as a percentage of damage from total foliage, time of total epidemic duration (T_t), and average of apparent infection rate (b^{-1}) were the variables that best explained the epidemics. These epidemics were organized into four groups. The group with an average rate of apparent infection of 0.010–0.015 units day⁻¹ and a duration of more than 50 days allowed higher oospore formation, regardless of host genotype. The nine days accumulated rainfall prior to the formation of oospores had a significant positive correlation ($r \geq 0.7$) with the absolute number of oospores per leaflet. It is concluded that oosporulation depended more on environmental factors (rain) and on the induction of slow epidemics (disease management), than on the genetic makeup of the host.

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Resumen El valle de Toluca, ubicado en los valles altos centrales de México, tiene las condiciones climáticas óptimas para el desarrollo de *Phytophthora infestans*, así como para su reproducción sexual debido a la presencia de los dos tipos de compatibilidad (proporción 1:1). De aquí que es un lugar ideal para estudiar las epidemias del tizón

tardío en la papa cultivada. En el verano de 2002 y 2003 se condujo un estudio en dos variedades de papa durante el progreso de la epidemia bajo condiciones de secano y de infección natural en el valle de Toluca, a fin de cuantificar la formación de oosporas en el follaje durante el progreso de las epidemias de tizón tardío, y para establecer las implicaciones de a) resistencia genética del hospedante, b) el manejo de la enfermedad (aplicación de fungicidas) y c) las condiciones ambientales. Se expusieron las variedades a dosis de 0.0x, 0.5x, y 1.0x del fungicida preventivo clorotalonil ($1.0x=1.15 \text{ kg i. a. ha}^{-1}$). La oosporulación empezó 56 y 46 días después de la plantación (2002 y 2003, respectivamente) con un pico máximo a los 72 días en los dos ciclos de cultivo. El número total de oosporas en los dos años en la variedad Zafiro, resistente a *P. infestans*, fue mayor que en la variedad susceptible Alpha (101 vs 67). No obstante, no hubo diferencias estadísticas significativas ($P=0.40$), lo cual sugiere que el nivel de resistencia del hospedante no tuvo una influencia directa en la formación de oosporas. Las epidemias obtenidas de cada uno de los tratamientos se caracterizaron con análisis multivariado. Las variables que mejor explicaron a las epidemias fueron la severidad inicial (Y_0), como porcentaje del daño del follaje total, el tiempo de la duración total de la epidemia (T_i), y el promedio del nivel aparente de infección (b^{-1}). Estas epidemias se organizaron en dos grupos. El grupo con un nivel promedio de infección aparente de 0.010–0.015 unidades día^{-1} y una duración de más de 50 días permitió la mayor formación de oosporas, independientemente del genotipo del hospedante. Los nueve días de acumulación de lluvia previos a la formación de oosporas tuvo una correlación positiva significativa ($r>0.7$) con el número absoluto de oosporas por folíolo. Se concluye que la oosporulación dependió más de factores ambientales (lluvia) y en la inducción de una epidemia lenta (manejo de la enfermedad), que de la constitución genética del hospedante.

Keywords *Solanum tuberosum* · Late blight · Epidemics

Introduction

Recently the aggressiveness of the oomycete *Phytophthora infestans*, the causal agent of the potato late blight, has increased due to changes in the population structure generated by sexual reproduction (Drenth 1994; Fry et al. 1993; Spielman et al. 1991). Oospores are sexual reproductive structures produced by oomycetes. *P. infestans* is a heterothallic species (Gallegly and Galindo 1958; Smoot et al. 1958) thus requiring two isolates of opposite mating types, referred to as A1 and A2, to interact for formation of oospores to occur (Gallegly and Galindo 1958; Niederhauser

1991; Smoot et al. 1958). The impact of a sexual reproductive cycle on the biology and epidemiology of the disease is not completely understood despite diverse studies on oospore formation, production and survival (Cohen et al. 2000; Drenth et al. 1995; Fernández-Pavía et al. 2002; Flier et al. 2001; Grünwald and Flier 2005; Hanson and Shattock 1998a, b; Levin et al. 2001; Medina and Platt 1999; Pittis and Shattock 1994; Strömberg et al. 2001; Turkensteen et al. 2000; Zarzycka and Sobkowiak 1997).

Under natural conditions, oospores can be formed on stems, foliage (Flier et al. 2001), and tubers (Fernández-Pavía et al. 2004; Levin et al. 2001). The number of oospores produced will depend on the number of lesions per leaflet (Flier et al. 2001), on the degree of resistance of the cultivar (Fernández Elguezabal 1993; Turkensteen et al. 2000), and on the temperature (Drenth et al. 1995). The Toluca Valley, México, provides optimal climatic conditions for the natural development of late blight as well as a pathogen population with great genetic diversity that is sexually recombining (Grünwald et al. 2001; Grünwald and Flier 2005). The objectives of this effort were (1) to quantify the formation of oospores on foliage during epidemic development, and (2) to determine the effect of genetic resistance of the host, fungicide application, and environmental conditions on oospore formation.

Materials and Methods

Treatments

Tubers of the cultivars Alpha (susceptible to late blight) and Zafiro (resistant) were planted during the second week of June of 2002 and 2003 in the Toluca Valley, Mexico. Cultural practices followed standard grower practices typical for the area and included pre-plant fungicide treatments, fertilization, and insecticides. Experimental plots were $9 \times 8.1 \text{ m}$ (9 rows/plot; 36 plants/row). Three dosages (0.0, 0.5 and 1.0x) of the protectant fungicide chlorothalonil ($1.0x=1.15 \text{ kg a.i. ha}^{-1}$) were applied once a week according to the treatments. Experiments followed a factorial design with two factors including fungicide treatment (three doses) and cultivar (Alpha and Zafiro) and consisted of three replications.

Climatological Data

Two data loggers were used to record temperature and relative humidity (HOBO® Pro Series Temp, RH (C) 1998, Onset Computer Corp., Pocasset, MA, USA). These loggers were placed 50 cm above ground within the canopy of the crop. Data were logged every minute and downloaded weekly. In addition, temperature, relative humidity,

and precipitation were recorded every 5 min using a permanent meteorological station located nearby (Model 6152, Davis Vantage Pro 2, Campbell Scientific, UT, USA).

Estimation of the Disease

Disease severity (%) was estimated visually in each of the experimental units every 7 days, according to the scale of The British Mycological Society as modified by Fry (1977). Scouting for disease initiated after 50% of the plants had emerged.

Oospore Incidence and Density

During the first experiment conducted in 2002, 1373 leaflets with multiple coalescent lesions were collected from the middle part of the plant between July 19 and September 14. In 2003, disease incidence was lower, and thus only 459 samples were obtained between July 18 and September 12. A 10 mm diameter sample was taken from each infected area of a given leaflet. Each circular sample was then placed in ethanol (95%) overnight to remove chlorophyll. The tissue samples were then bleached with NaOCl (5.5% available Chlorine). Each sample was placed on a glass slide with glycerol and examined under the microscope at 100 and 400 \times magnification. The numbers of leaflets with oospores as well as the average number of oospores formed per leaflet were recorded for statistical analysis. Given that foliage was collected weekly in the same experimental units, there was a dependence of the variable measured between one sampling and another. This autocorrelation was taken into account by conducting repeated measurement analyses. Repeated measurement analysis was conducted using the statement REPEATED in PROC GLM (SAS Institute, Cary, NC, USA) to determine statistical significance of differences among treatments over time. Independent variables included cultivar and fungicide treatments. A *t* test was used to characterize differences among both years by cultivar and to determine the effect of host resistance across cultivars.

Effect of Epidemic on Oospore Formation

Disease progress curves were obtained for a total of 36 epidemics (18 for each cultivar at three dosages, with three replications in two crop cycles). Epidemics were compared according to a set of descriptive variables as suggested by Mora-Aguilera et al. (1996). Each epidemic was characterized by eight variables: (1) total epidemic duration in days (T_i), (2) the time, in days, from planting until appearance of first symptoms (X_0), (3) percentage of initial disease severity (Y_0), (4) and final disease severity (Y_{max}), (5) area under

disease progress curve (AUDPC), (6) AUDPC standardized by the duration of the epidemic T_i in days ($AUDPC_s = AUDPC/T_i$), (7) the shape of the disease progress curve (c), and (8) the apparent infection rate (b^{-1}). The last two variables were calculated using the Weibull distribution function modified as a two-parameter model, which is a flexible model suitable for fitting to disease progress data ($y = \exp [-(t/b)^c]$, $t > 0$, Pennypacker et al. 1980).

In which y = disease severity, t = time of disease assessment (days) after planting, b = scale parameter, and c = shape parameter.

The initial values of c and b were obtained empirically at the start of the interactions in a non-linear regression using the Does Not Use Derivatives method in PROC NLIN (SAS Institute). The values obtained for b were inversely transformed to reflect that, this resulting value corresponds to the apparent infection rate (b^{-1}). Principal components analyses (PCA, PROC PRINCOMP, SAS Institute, Cary, NC, USA) was used to reduce the original set of descriptive variables, allowing the elimination of the variables whose explanatory capacity was lower and whose correlation was relatively high. The reduction of the original set of variables was made in three steps. In the first step, the eight variables of the 36 epidemics were subjected to PCA, integrating factor analysis using PROC FACTOR (SAS Institute, Cary, NC, USA) and the biplot technique. Biplot displays were obtained by varimax rotation of the highest principal components. Pearson correlation matrices and the biplot displays were examined to detect variables with a high degree of correlation ($r \geq 0.6$). In a second step, the variables were arranged in two groups with lower among-variable correlation. Each group of variables was subjected to PCA. PCA separated the variables with highest and lowest variance, grouping the latter within the lower principal components ($\lambda < 0.7$). The selected variables were those that had a higher weight only in the higher principal components and a lower among-variable correlation. The varimax rotation in the factor analysis also helped in the elimination of variables. In a third step, the remaining variables of both groups were again subjected to a PCA until there were as few variables as significant principal components. The variables that had the best explanatory capacity were used to form groups of epidemics. The AVERAGE method was used to classify epidemics into various groups using cluster analysis (CA, PROC CLUSTER, SAS Institute, Cary, NC, USA). The groups formed by CA were separated via a discriminant analysis using PROC CANDISC (SAS Institute, Cary, NC, USA) by determining the Mahalanobis squared distance among them. To determine whether the squared distances among groups were significant, discriminating functions derived from the same procedure were used. The discriminating functions are analogous to a means test in univariate analyses.

Climatic Variables in Oospore Formation

The maximum, minimum and average temperature, relative humidity, and total precipitation were recorded daily. The number of hours with relative humidity greater than or equal to 90% and cumulative precipitation were calculated from this data. In previous studies it had been reported that the formation of oospores on leaflets with multiple lesions occurs after nine days of incubation in vitro (Drenth et al. 1995); based on this observation time-lag climatic variables up to 9 days prior to the day of oospore observation were included in the analysis. Given that oospores observations were obtained in weekly intervals rather than continuously, the present study intervals of 9 days were dephased by the LAG function (SAS Institute, Cary, NC, USA). Simple correlations were calculated among the climatic variables and the average number of oospores observed per leaflet.

Results

Epidemic Onset and Oospore Density

First lesions were observed on July 19 in 2002 and July 12 in 2003. The epidemic was delayed in 2002 due to lower rainfall (Fig. 1e). The first leaflets with oospores were observed 21 and 16 days after the start of the epidemic in 2002 and 2003, respectively (Fig. 1a, b). In 2002, the first oospores were detected in the treatments without application and with half the fungicide dose in the cultivar Alpha (susceptible), whereas in 2003 all of the treatments with this cultivar had oospores on the first date oospores were detected (Fig. 1a). In 2002 the treatments without application and with half the fungicide dose of the cultivar Zafiro (resistant) had leaflets with oospores 35 days after the onset of the epidemic. In 2003, leaflets with oospores were only found in the treatment without fungicide 28 days after the onset of the epidemic in this cultivar. The greatest numbers of leaflets with oospores were found 35 and 42 days after the onset of the epidemic in 2002 and 2003, respectively (Fig. 1b). At the end of the growing season, the treatment without fungicide of the cultivar Zafiro had more oospore infected leaflets and more oospores per leaflet than cv. Alpha without chemical protection. There were significant differences among the treatments with respect to the number of oospores per leaflet (Wilks' Lambda, $P=0.0018$) and the number of leaflets with oospores (Wilks' Lambda, $P=0.0088$). On the other hand, there was no statistically significant difference between the two crop cycles with respect to the total number of oospores on Alpha ($t=-0.77$; $P=4524$) or on Zafiro ($t=-0.09$; $P=0.9269$); neither were there differences among the number of total oospores formed between cultivars ($t=-0.85$; $P=0.4014$). However, there were differences when comparing

the treatment without fungicide on both cultivars ($t=-3.13$; $P=0.0106$). Generally, oospores were detected when disease severity got above 15%. However, the treatment with half the fungicide dose of cv. Zafiro produced oospores at about 1% disease severity 35 days after the onset of the epidemic. The treatments with half the fungicide dose on cv. Alpha and without fungicide on cv. Zafiro showed oospores during almost the entire crop season in both years. Cultivar Zafiro showed more leaflets with oospores than cv. Alpha, although without a statistically significant difference between these two cultivars.

Type of Epidemic and Oospore Formation

In both years, the epidemics on cv. Alpha without fungicide treatment had an asymmetric, sigmoidal curve with duration of no more than 35 days (Fig. 1c). The epidemics for other treatments had a more gradual disease progress over 40 to 60 days. The eight descriptive variables associated with the 36 epidemics in both crop cycles were subjected to a multivariate analysis to determine which combination of variables best distinguished epidemic development among treatments. A high positive correlation was obtained among b^{-1} , Y_{max} , $AUDPC$ and $AUDPCs$ ($r \geq 0.8$); a negative correlation was obtained between c and T_i ($r=-0.68$), and between c and $AUDPCs$ ($r=0.74$; Table 1). The biplot displays also revealed high correlations among different variables after the maximum variance had been rotated (Fig. 2). The variables were separated into two groups; variables with lower correlations into one group and high correlations into another group. The variables X_0 , T_i and b^{-1} formed one group, and Y_{max} , Y_0 , $AUDPC$ and c formed another group. The variable $AUDPCs$ was excluded from the later analyses because of its high correlation with both groups of variables (Table 1). For the following analyses, the variables $AUDPC$, Y_{max} and c were eliminated, because of the high Eigenvalues they contributed to the minor principal components ($\lambda < 0.7$). In the PCA, the variables that had the highest explanatory capacity were Y_0 , T_i and b^{-1} . CA based on these variables clustered epidemics into four groups. The first group consisted of the treatment without fungicide on susceptible cultivar Alpha, which was characterized by a very explosive initial epidemic, with a high value of initial disease severity and a short epidemic duration (Table 2). The second group contained epidemics with very low initial severities but with an early onset, and a very prolonged duration with moderate intensity. The half and full fungicide dose treatments on susceptible cv. Alpha, as well as the treatments without application, half and full fungicide doses on cv. Zafiro were included in this group (Table 2). A third group included epidemics on Zafiro with half the fungicide dose in 2002; these epidemics had a shorter duration than the previous group and a low apparent

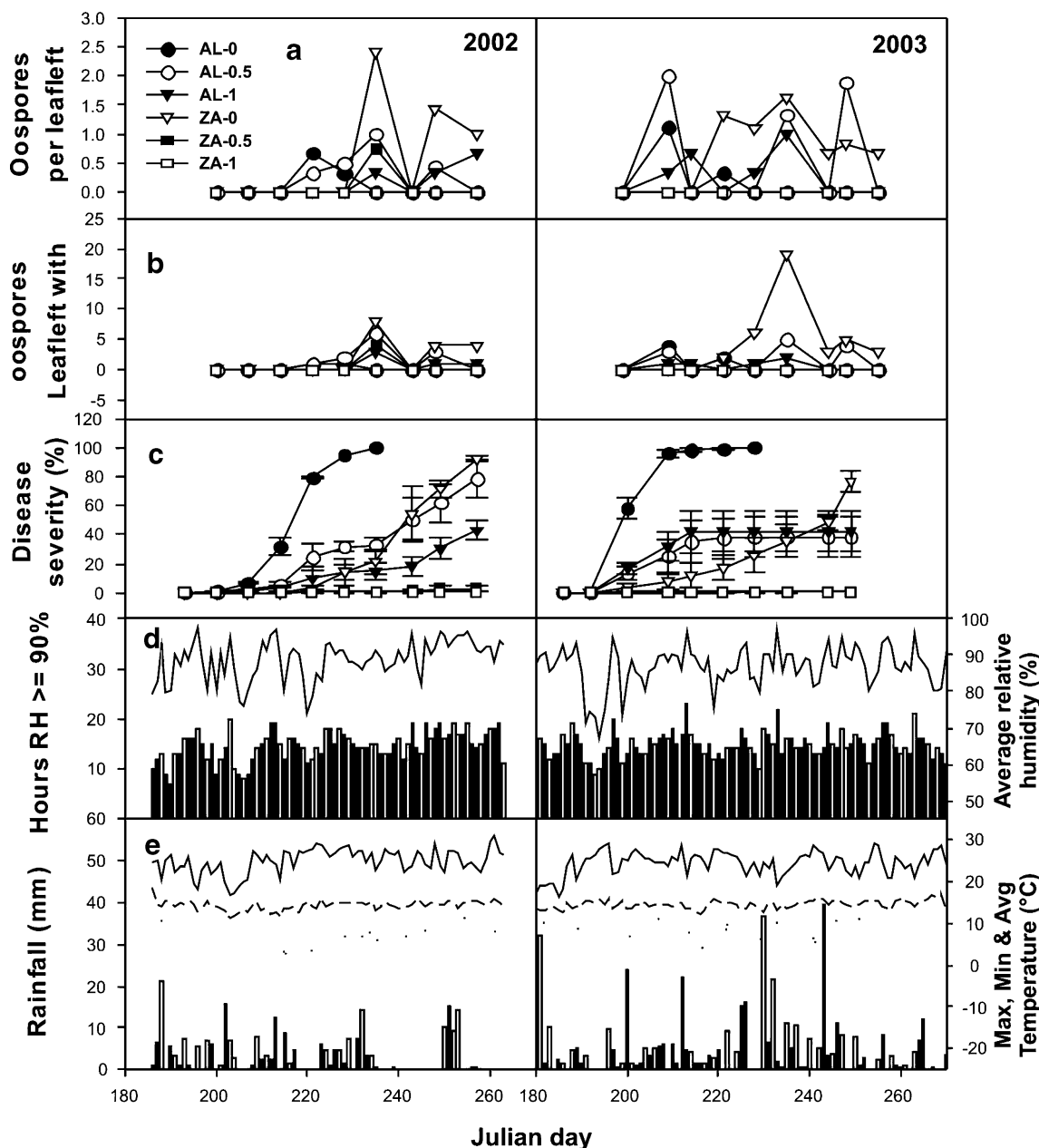


Fig. 1 Number of oospores formed per leaflet (a). Number of leaflets with oospores (b). Potato late blight disease progress curves (c) observed in the two potato cultivars Alpha and Zafiro included in this study with three protectant fungicide treatments (1.0×=chlorothalonil at 1.15 kg a.i. ha⁻¹): AL-0, Alpha without fungicide; AL-0.5, Alpha with half fungicide dose; AL-1, Alpha, with full fungicide dose; ZA-0,

Zafiro without fungicide; ZA-0.5, Zafiro with half fungicide dose; ZA-1, Zafiro with full fungicide dose, in two growing cycles (2002–2003). Climatic conditions: **d** bars hours/day with relative humidity ≥90%, lines daily average relative humidity (%). **e** bars daily rainfall (mm), lines maximum, minimum, and average temperature (°C)

infection rate (Table 2). The fourth group was made up of mild and short epidemics. Zafiro with the full fungicide dose during 2002 was included in this group (Table 2). The Mahalanobis squared distances among these four groups of epidemics were significant ($P < 0.0001$). Epidemics of the second group revealed the greatest number of leaflets with oospores. This group was characterized by having the longest epidemic duration with respect to the other groups (57 days; Table 2).

Effect of Climatic Variables on Oospore Formation

In the summer of 2002, the onset of epidemics occurred twenty days after emergence, while in 2003 it began eleven days after emergence. Rainfall in 2003 was more abundant than in 2002 (Fig. 1e). In 2002, there was a decrease in the minimum temperature of 4°C prior to the highest peak in oospore formation, which was also the case in 2003, when it decreased to 3°C (Fig. 1e); however, the average daytime

Table 1 Pearson's correlations coefficients for eight variables describing epidemic development from the 36 epidemics caused by *P. infestans*

Variables	Y_o	X_o	T_t	AUDPC	c	AUDPCs	b^{-1}
Y_{max}	0.4128	-0.2807	-0.3352	<u>0.8597</u>	0.532	<u>0.8496</u>	<u>0.8992</u>
Y_o		0.0885	-0.347	0.2728	-0.0045	0.3273	0.3188
X_o			-0.295	-0.4121	-0.2061	-0.3605	-0.3043
T_t				-0.2612	<u>-0.6874</u>	-0.5591	-0.5766
AUDPC					0.5095	<u>0.9305</u>	<u>0.8565</u>
C						<u>0.7400</u>	<u>0.8022</u>
AUDPCs							<u>0.9561</u>
b^{-1}							

The underlined values have relatively high positive correlation among the variables

Y_{max} Final severity as percentage of foliage affected, Y_o initial severity as percentage of diseased foliage, X_o time in days from planting to the appearance of the first symptoms, T_t total duration of the epidemics in days, AUDPC area under the disease progress curve, c shape of the curve, AUDPCs standardized AUDPC in the time of the total duration of the epidemic in days, b^{-1} estimator of the apparent infection rate

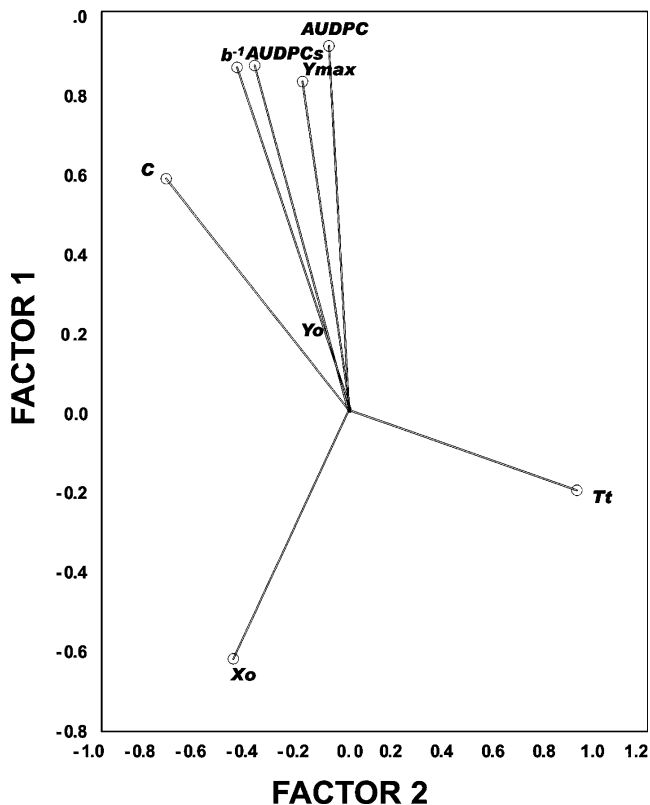


Fig. 2 Biplots of approximate correlation and relative importance of variables measured for the 36 late blight epidemics. The variance explained in this biplot was 77%. Varimax rotated biplot of the first and second factors. Longer vectors fairly parallel to an axis represent variables that are best represented by the two factors biplotted. Small vectors represent variables that are not well-represented by the plane and may be associated with any of the remaining factors not plotted. Variables with small angles between vectors and vectors in the opposite direction are positively and negatively correlated, respectively. T_t is the total duration of the epidemic in days; X_o is the time in days from planting to first symptoms; Y_o is the percentage of initial disease severity; Y_{max} is the percentage of final severity; AUDPC is the area under the disease progress curve; AUDPCs is the standardized AUDPC scaled by the time of the total duration of the epidemic in days; c is the shape of the curve; and b^{-1} is the estimator of the apparent infection rate. These last two values were estimated using the Weibull model

temperature remained constant in this period in both years. In both growing seasons, rainfall had a high and significant correlation with the average number of oospores per leaflet ($r > 0.7$; $P = 0.0811$).

Discussion

Oospore formation was favored by a slow, gradual and long epidemic, promoted by suitable weather conditions and fungicide management of the crop at suboptimal rates. Most oospore infected leaflets were found in epidemics with a disease severity above 25%. Cohen et al. (2000) in Israel and Stromberg et al. (2001) in Sweden determined that oosporulation occurred with disease severities over 50%, mostly towards the final stage of the epidemic. In our study, this early oospore differentiation was indicative of the presence of both mating types since the beginning of the infection. Slow disease progress and long epidemic duration promoted abundant formation of oospores in leaflets, with a maximum oospore formation peak around 35 to 42 days after the onset of the epidemic in 2002 and 2003 respectively. Flier et al. (2001) reported lower oospore formation in tissue with multiple lesions in Toluca, attributing it to the fact that there is little time for its formation because the leaf decays rapidly once infected. In plots where the epidemic was very explosive, there were fewer leaflets with oospores, and those that did have oospores generally had a smaller number of oospores. The rapid increase in disease severity in these plots was due to the susceptibility of the host plant and to the management of the disease. Cv. Zafiro (resistant) showed small lesions, with little sporulation and slow disease development, which apparently allowed for enough time as the lesions coalesced for the formation of oospores. The lesions in cv. Alpha (susceptible) were large, with abundant sporulation, where generally a single lesion covered the available leaf tissue in a relatively short time. A smaller amount of leaflets with

Table 2 Classification of *P. infestans* epidemics based on three explanatory variables selected by multivariate analysis best describing the epidemics (b^{-1} , Y_0 , and T_i) and number of total oospores per epidemic

Group of epidemic ^a	Epidemic ^b	Treatment	b^{-1}	Y_0	T_i	Total oospore formation ^c
1	1	AL-0	0.01871	0.01	35	3
1	2	AL-0	0.01871	0.01	35	0
1	3	AL-0	0.01846	0.01	35	0
1	19	AL-0	0.02681	0.00001	36	4
1	20	AL-0	0.02698	0.00001	36	2
1	21	AL-0	0.02705	0.00001	29	2
2	4	AL-0.5	0.0108	0.001	57	13
2	5	AL-0.5	0.01288	0.01	57	4
2	6	AL-0.5	0.01191	0.0001	57	0
2	7	AL-1	0.00955	0.0001	57	6
2	8	AL-1	0.00936	0.01	57	0
2	9	AL-1	0.00931	0.0001	57	0
2	10	ZA-0	0.0126	0.00001	57	31
2	11	ZA-0	0.01258	0.00001	57	11
2	12	ZA-0	0.01187	0.0001	57	0
2	22	AL-0.5	0.00442	0.0001	57	16
2	23	AL-0.5	0.01054	0.00001	57	14
2	24	AL-0.5	0.01	0.00001	57	1
2	25	AL-1	0.00502	0.0001	57	1
2	26	AL-1	0.00994	0.00001	57	5
2	27	AL-1	0.01229	0.00001	57	1
2	28	ZA-0	0.01176	0.00001	57	26
2	29	ZA-0	0.0117	0.00001	57	13
2	30	ZA-0	0.01246	0.0001	57	16
2	31	ZA-0.5	0.00242	0.00001	57	0
2	32	ZA-0.5	0.00242	0.00001	57	0
2	33	ZA-0.5	0.00242	0.00001	57	0
2	34	ZA-1	0.00167	0.00001	57	0
2	35	ZA-1	0.00167	0.00001	57	0
2	36	ZA-1	0.00167	0.00001	57	0
3	13	ZA-0.5	0.00371	0.0001	50	9
3	14	ZA-0.5	0.00208	0.0001	50	0
3	15	ZA-0.5	0.00539	0.0001	50	0
4	16	ZA-1	0.00178	0.00001	43	0
4	17	ZA-1	0.0006	0.0001	43	0
4	18	ZA-1	0.00358	0.00001	43	0

b^{-1} Estimator of the apparent infection rate, Y_0 initial severity as percentage of diseased foliage, T_i , total duration of the epidemics in days, *AL-0* Alpha without fungicide, *AL-0.5* Alpha with half dose of fungicide, *AL-1* Alpha (complete dose), *ZA-0* Zafiro without fungicide, *ZA-0.5* Zafiro with half dose of fungicide, *ZA-1* Zafiro with complete dose of fungicide

^a Groups of epidemics were determined using cluster analysis performed on scores of three principal components obtained after three successive principal component analyses

^b Epidemics from 1 to 18 correspond to 2002; epidemics from 19 to 36 correspond to 2003

^c Number of oospores formed during the progress of the epidemics

coalescent lesions were recovered in the treatments of this cultivar.

The management of the host influenced the progress of the epidemic. The epidemics of the treatments with either half or full fungicide dose on the susceptible cv. Alpha were similar to the epidemics on the more resistant cv. Zafiro without chemical protection (Fig. 1). Management of cv. Alpha with fungicides allowed for slower, but

sustained epidemics with more gradual disease progress, giving time for the formation of oospores. It is clear that the fungicide only influenced the progress of the epidemic, without apparent effect on oospore formation (Groves and Ristaino, 2000). Hanson and Shattock (1998b) mention that in places with equal frequency of both mating types, a minimum abundance of oospores is to be expected; however, Fernández Elguezabal (1993) found in vitro that

the amount of oospores is directly proportional to the level of resistance of the variety. Turkensteen et al. (2000) established that the resistance level did not directly influence oospore formation, concluding that only 59% of the variability in oospore production is attributed to the degree of resistance of the variety. Although the number of oospores was low in our plots, the results show that their production was not directly linked to the genetics of the host as described by Fernández Elguezal (1993) and Turkensteen et al. (2000), but rather that the type of epidemic. Our results thus show that slow and sustained epidemics that allow for multiple infections on leaflets result in higher production of oospores.

High relative humidity also favors oospore formation, whether this is caused by either rainfall or irrigation (Cohen et al. 2000). In our plots rainfall was a climatic factor with a high correlation with oospore formation; however, this factor is clearly also positively correlated with advancing disease progress (Grünwald et al. 2002). The Valley of Toluca is characterized by an average monthly temperature of 12–17°C and annual precipitation of between 800 and 900 mm, most of which occurs during the potato growing season (Grünwald et al. 2000). The amount of oospores produced naturally was very low compared with that produced when leaves of Alpha were incubated in vitro (data not shown), which could have been due to one or various factors: our experiment was conducted under natural infection conditions, with the native, genetically diverse population of *P. infestans* (Grünwald and Flier 2005; Grünwald et al. 2001). However, it is to be expected that not all isolates of opposite mating type will produce oospores as they can be sexually incompatible due to unknown incompatibility factors (Erwin and Ribeiro 1996; Flier et al. 2001). Furthermore, despite the fact that both mating types coexist in the Valley of Toluca in a 1:1 ratio (Gallegly and Galindo 1958; Grünwald et al. 2001), this does not necessarily imply that both mating types have to be uniformly distributed within a field. In 1998, Smirnov found that only 25% of the leaf tissue with double lesions presented both types of compatibility (unpublished).

In conclusion, four types of epidemics were identified and two main factors were involved in oospore formation. Factors involved in oospore formation were type of epidemic as influenced by fungicide management and rainfall. Oospore formation was favored by slow, and sustained epidemics, which are common in commercial potato fields in the developing world in spite of many fungicide applications. Thus, oospores will invariably form where populations of *P. infestans* are sexual and disease management will have to address the fact that oospores will be incorporated into the soil and serve as a source of primary inoculum for new epidemics in the following crop season.

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