

Topography and Crop Management Are Key Factors for the Development of American Leaf Spot Epidemics on Coffee in Costa Rica

Jacques Avelino, Sandrine Cabut, Bernardo Barboza, Miguel Barquero, Ronny Alfaro, César Esquivel, Jean-François Durand, and Christian Cilas

First author and eighth authors: Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UPR Bioagresseurs de pérennes, Avenue Agropolis-TAA-31, Montpellier, F-34398 France; second author: Institut National de la Recherche Agronomique (INRA), UMR729 Analyse des Systèmes et Biométrie, Montpellier, F-34060 France; third, fourth, fifth, and sixth authors: Instituto del Café de Costa Rica (ICAFE), AP 37-1000, San José, Costa Rica; seventh author: Université de Montpellier II (UMII), Département de Mathématiques, Montpellier, F-34095 France.

Current address of J. Avelino: CIRAD/IICA-PROMECAFE, A.P.55, 2200, Coronado, San José, Costa Rica.
Accepted for publication 15 May 2007.

ABSTRACT

Avelino, J., Cabut, S., Barboza, B., Barquero, M., Alfaro, R., Esquivel, C., Durand, J.-F., and Cilas, C. 2007. Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. *Phytopathology* 97:1532-1542.

We monitored the development of American leaf spot of coffee, a disease caused by the gemmiferous fungus *Mycena citricolor*, in 57 plots in Costa Rica for 1 or 2 years in order to gain a clearer understanding of conditions conducive to the disease and improve its control. During the investigation, characteristics of the coffee trees, crop management, and the environment were recorded. For the analyses, we used partial least-squares regression via the spline functions (PLSS), which is a nonlinear extension to partial least-squares regression (PLS). The fungus developed well in areas located between $\approx 1,100$ and 1,550 m above sea level. Slopes were conducive to its development, but eastern-facing slopes were less affected than the others, probably because they were more exposed to sunlight, especially in the rainy season. The distance between planting

rows, the shade percentage, coffee tree height, the type of shade, and the pruning system explained disease intensity due to their effects on coffee tree shading and, possibly, on the humidity conditions in the plot. Forest trees and fruit trees intercropped with coffee provided particularly propitious conditions. Apparently, fertilization was unfavorable for the disease, probably due to dilution phenomena associated with faster coffee tree growth. Finally, series of wet spells interspersed with dry spells, which were frequent in the middle of the rainy season, were critical for the disease, probably because they affected the production and release of gemmae and their viability. These results could be used to draw up a map of epidemic risks taking topographical factors into account. To reduce those risks and improve chemical control, our results suggested that farmers should space planting rows further apart, maintain light shading in the plantation, and prune their coffee trees.

Additional keywords: altitude, climate, cropping practices, *Coffea arabica*.

Mycena citricolor is a basidiomycete fungus with a broad spectrum of hosts (26,39) and is confined to the American continent where it originated. It particularly attacks coffee trees, for which heavy yield losses have been reported in Central America (41, 43,46). It especially raises a problem in high-altitude zones where the best coffees in the region are produced (2). The disease, known as American leaf spot of coffee, occurs on branches, leaves, and fruits, causing leaf and fruit fall (43,46). On leaves, where the disease is most frequently seen, the pathogen usually causes light-brown, circular lesions 5 to 10 mm in diameter, with distinct edges and no surrounding chlorosis. The infection unit amounts to a mass of compact hyphae in the form of a pin, whose head, the propagule or gemma, becomes detached in contact with water (46). The production of carpophores is quite rare and occurs mostly on dead leaves at the end of the epidemic, which seems to suggest that any participation of basidiospores in epidemic development is negligible. There is little information available on conditions that are conducive to development of the fungus. It is known to develop particularly well in humid zones with little sunlight. Indeed, rainfall greatly contributes toward the release

and dissemination of propagules, and toward infection, which occurs only in the presence of liquid water (46).

It is difficult to control this disease. Specific varietal resistance seems to be absent because the fungus, of American origin, and the coffee tree, of African origin, did not evolve together (25). The broad host spectrum of the fungus also argues against any specific resistances. However, different degrees of susceptibility have been detected among commercial *Arabica* cultivars. Cultivars derived from the Timor Hybrid apparently are more susceptible than the others. Nonetheless, as soon as environmental conditions, especially humidity, approach optimum for the fungus, the differences in susceptibility lessen (43). Current control methods are fungicide based. Bordeaux mixture was used first, then lead arsenate, which subsequently was banned and replaced by triazoles such as cyproconazole (12,23,43). Alkalinization of fungicide solutions makes them more effective, probably because it helps to inhibit oxalic acid, a key factor in *M. citricolor* pathogenicity, which is particularly involved in digesting the cell walls of the host (29–31,43,44). Fungicides are usually expensive, which limits their use in a crisis period. Moreover, chemical control is not always very effective, particularly when extremely humid conditions prevail.

The purpose of this study was to help to identify factors conducive to the development of American leaf spot disease on coffee in order to improve its control. The study focused on

Corresponding author: J. Avelino; E-mail address: jacques.avelino@cirad.fr

doi:10.1094/PHYTO-97-12-1532

© 2007 The American Phytopathological Society

topographical factors and cropping systems. The fact that this disease is considered to be particularly serious in Costa Rica could have something to do with the cropping systems used in that country, which are among the most intensive in the world.

MATERIALS AND METHODS

Agrobiological context of the study. For almost 30 years, coffee grown in Costa Rica has produced some of the highest yields in the world (10,16,35). The generalized use of dwarf cultivars since the 1960s, primarily Caturra and Catuai, led to planting densities being multiplied three- or fourfold, and it was a decisive step toward nonmechanized intensification of the crop (10,16,35). Indeed, by changing cultivars, it became possible to apply other agricultural techniques that were well suited to those cultivars and typical of intensified Costa Rican coffee growing, such as reducing or removing shade trees, planting two or three coffee trees per planting hole, intensive input use, especially fertilizers and herbicides, and the introduction of elaborate pruning systems. Of course, depending on the knowledge of the farmers and their economic resources, those techniques are often only partially applied. Consequently, cropping systems have always been quite varied. Cropping system diversity increased even further recently, with the slump in coffee prices. Indeed, on some plantations, there have been obvious transformations in the cropping systems, notably with a return to moderate shading, usually providing suitable conditions for quality coffee production and prolonging the life span of the coffee trees (16).

In Costa Rica, Arabica coffee is grown at a wide range of altitudes (mainly between 600 and 1,900 m). Rainfall volumes and distribution are very variable, due to various oceanic influences affecting the country (strong Pacific influence in the west, with a very marked dry season, and strong Caribbean influence to the east, with a short dry season) (7). Therefore, there is substantial diversity in the production situations (11).

Methodological approach. The methodological approach chosen was a survey. It is a method that is particularly adapted to studying epidemics whose development depends on a large number of factors, particularly crop management. Thus, it has been used to study plant diseases in groundnut in West Africa (36), rice in Southeast Asia (37,38), apricot in France (42), and coffee in New Caledonia (22) and Honduras (3,4).

Sampling methods. Our study is based on a characterization of 57 commercial plots comprising 10 rows of 10 coffee trees each. Their area was between ≈ 100 and 300 m^2 , depending on the planting density. The plots were monitored between March 2002 and January 2004. The survey was conducted in locations particularly exposed to *M. citricolor*. Sampling was carried out in such a way as to cover a wide range of production situations (11), including most of the coffee-growing regions in Costa Rica. Several locations, which mainly differed in their altitude, were chosen in each region. Then, in each location, we selected commercial plots (usually three) that had different cropping systems (Fig. 1). In the first year, 48 plots were chosen by that approach in four regions (12 per region): Western Valley, Central Valley, Coto Brus, and Turrialba. In 2003, several growers did not want to participate in the study and their plots were excluded. The survey was continued on a sample of 43 plots: 34 plots already observed in 2002 (6, 10, 7, and 11 from the Western Valley, the Central Valley, Coto Brus, and Turrialba, respectively) and 9 new plots (3, 2, 1, and 3 from the Western Valley, the Central Valley, Turrialba, and Turrialba, respectively). The last was a region not sampled in 2002 (Fig. 1). Thus, the survey covered 91 plot-year individuals, 68 of which could be considered as pseudo-replications (34 replicated plots).

Five coffee trees were marked in a zigzag inside each plot. When several coffee plants were planted in the same hole, they were considered as a single coffee tree. On each of those marked

coffee trees, three branches were identified at three different storeys. Some of the variables measured were evaluated on the marked trees and branches, as described below.

Disease predictors. The disease predictors measured or observed during the survey involved the environment (Table 1), crop management (Table 2), and certain characteristics of the coffee trees (Table 3).

The environmental variables were rainfall, topography, and soil (Table 1). Readings from 21 rain gauges installed near the study plots were used (generally one rain gauge for three plots). Rainfall was calculated for three periods of the year: (i) the start of the rainy season, from March to the first fortnight in June; (ii) the short dry period, corresponding to a period of lower rainfall inside the rainy season, from the second fortnight in June to the end of August; and (iii) the end of the rainy season, from September to December (Fig. 2). Annual rainfall also was calculated. The topography, which influenced temperature and humidity values in the plantation, was characterized by the altitude, slope inclination, and slope aspect. In addition, for each plot, we analyzed a composite soil sample taken just before the start of the rainy season near the five marked coffee trees (two subsamples per tree). The analyses focused on nutrients or soil characteristics essential for coffee growing (pH, K, Ca, Mg, organic matter, sands, and clays) (27). Nutritional factors also seemed to influence the development of the genus *Mycena* in general (33,45).

Crop management variables involved the structure of the plots, which was generally stable over time, and cropping practices which could change from one year to the next (Table 2). Data on the cultivar (Caturra or Catuai only), the number of weeding rounds (chemical or mechanical), the number of fertilizer applications (to the soil or to the leaves), and the number of soil improvement operations were obtained from the farmers. Although farmers were asked not to treat the study plots, some of them applied fungicides; consequently, fungicide use also was recorded. The distance between coffee trees in the planting row and the distance between rows were assessed by measuring the distances between the five marked trees and their neighboring trees. The shade percentage also was assessed on each marked coffee tree, with the help of a spherical densiometer used to measure forest overstory density (24). Two measurements were taken per year, at the beginning of the rainy season (May to June) and during the harvest (November). The means per plot and per year

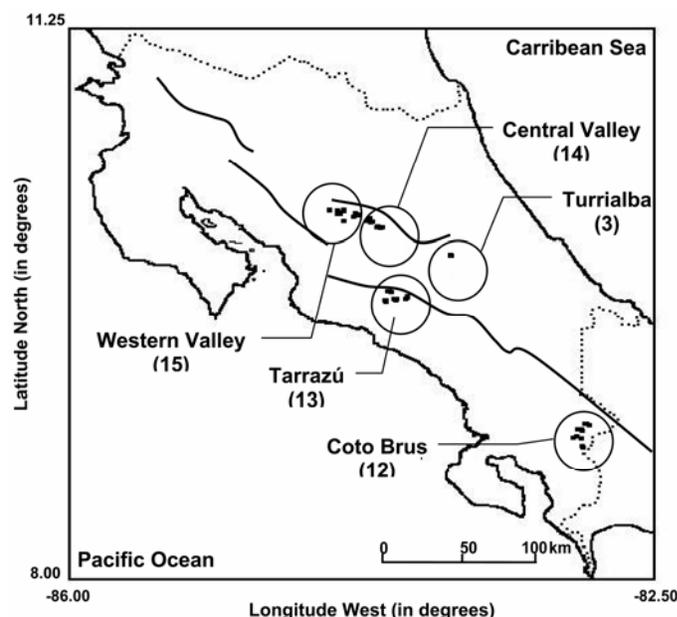


Fig. 1. Location of plots sampled during the survey in Costa Rica on American leaf spot disease, with their number per region in brackets.

were used for the analyses. The shade trees in the plot also were identified. Four categories of shade were established: only legumes, banana plants and legumes only, banana plants and other plants, and fruit or forest trees only. An additional category was established for plots without shade. Moreover, the number of coffee plants per planting hole was assessed for the five marked trees. Last, the pruning system applied also was noted. The pruning systems could be grouped into three categories depending on layout and the number of coffee trees on which the same pruning technique was applied. Pruning could be applied tree by tree, depending on the needs of each tree; by row, in accordance with a clearly established pruning plan; or by block of coffee trees, containing several rows, when all the coffee trees were exhausted. The types of pruning varied. However, cutting back, which consists of totally removing all branches and leaves, was a common practice. An additional category was established for plots without a pruning system.

The marked coffee trees were characterized by their age, trunk circumference at ground level (which is a sign of vigor), their height, and their architecture by counting the number of orthotropic stems (Table 3).

Disease variables. *M. citricolor* attacks were monitored on the marked branches. Five observations were carried out in general during each year: at the beginning of the rainy season (May to June), just before the harvest (August), at the beginning of the

harvest (September to October), during the harvest (November; observation not carried out in 2002 in the Central Valley), and toward the end of the harvest (December to January). On each inspection, the number of young diseased leaves and the number of lesions were counted. The criterion used to define the age of the leaves was a very short internode that forms during the dry season. Old leaves were located between the base of the branch and the short internode. Young leaves, from the current year, were located between the short internode and the tip of the shoot (1). Old leaves bore the residual inoculum of the earlier epidemic. That inoculum helped to trigger the subsequent epidemic on young leaves (43). Therefore, it was possible to separate different epidemics by identifying the age of the leaves. These observations led to the calculation of two variables: the annual maximum incidence (*I_{max}*, maximum percentage of diseased young leaves

TABLE 3. Description of variables characterizing the coffee tree

Variable	Unit	Range of values
Coffee tree age	year	2–23
Number of stems per tree ^z	...	1.6–10.2
Circumference of the coffee tree trunk at ground level ^z	cm	9.5–29.8
Coffee tree height ^z	m	1.20–2.81

^z Mean of five measurements per plot.

TABLE 1. Description of environmental variables

Variables	Unit	Range of values
Rainfall		
Beginning of the rainy season (from March to the first half of June)	mm	418–1,392
Short drought (from the second half of June to August)	mm	525–1,142
End of the rainy season (from September to December)	mm	904–2,385
Annual rainfall (from March to December)	mm	1,937–4,587
Topography		
Altitude	m	950–1,785
Slope inclination	%	2–70
Slope aspect	Degree (N = 0 or 360, S = 180)	0–355
Soil		
pH	...	4.3–6.6
K	Cmol (+)/liter	0.11–1.20
Ca	Cmol (+)/liter	0.33–14.6
Mg	Cmol (+)/liter	0.23–2.57
Organic matter	% of dry weight	3.48–16.10
Sands	% of dry weight	19.7–76.5
Clays	% of dry weight	5.5–62.4

TABLE 2. Description of crop management variables

Variables	Range of values for quantitative variables or categories for qualitative variables
Plantation structure	
Cultivar	Caturra or Catuai
Distance between coffee trees in the row ^w	0.73–1.40 m
Distance between rows ^w	1.10–2.26 m
Planting density ^x	3,334–10,629 coffee trees ha ⁻¹
Number of coffee plants per hole ^y	1–4
Shade percentage ^z	0–65
Shade type	Legumes only, bananas with legumes only, bananas with other plants, fruit or forest trees only, or no shade
Coffee tree pruning system	Per tree, per row, per plot, or no pruning
Cropping practices	
Annual number of leaf fertilizations	From 0 to 4
Annual number of soil fertilizations	From 0 to 4
Annual number of soil improvement operations	From 0 to 2
Annual number of fertilization operations	From 0 to 8
Annual number of chemical weedings	From 0 to 4
Annual number of mechanical weedings	From 0 to 3
Annual number of weedings	From 0 to 5
Annual number of fungicide sprayings	From 0 to 4

^w Mean of 10 measurements per plot.

^x Calculated from the distances between coffee trees in the row and from the distances between rows.

^y Mean of five holes per plot.

^z Mean of five evaluations repeated twice in the year.

in the year) and the annual maximum severity (S_{max} , maximum yearly value for the number of lesions:number of young leaves ratio).

Another evaluation of *M. citricolor* attacks was carried out on the final annual inspection of the plots. All the coffee trees of the study plots (100 coffee trees per plot in general) were scored according to an attack intensity scale where 0 = healthy plant; 1 = plant hardly attacked, an attack usually located on part of the tree, with little defoliation; 2 = moderately attacked plant, a virtually generalized attack with few lesions per leaf and moderate defoliation; and 3 = severely attacked plant, generalized attack with numerous lesions per leaf and severe defoliation). A synthetic index (I_a), between 0 and 1, which reflected attack intensity on a plot scale, was calculated as follows:

$$I_a = \frac{\sum_{i=0}^3 i \cdot n_i}{3 \cdot N}$$

where N is the number of coffee trees in the plot and n_i the number of coffee trees scored i on the attack intensity scale.

I_{max} , S_{max} , and I_a were first described (skewness and kurtosis) and compared (Pearson correlation coefficient) in order to choose the disease variable to be modeled.

Statistical analyses. The modeling technique used was partial least-squares regression via spline functions (PLSS) (13). It offers an additive nonlinear extension (18) to the linear partial least-squares regression (PLS) model (17,40,47,48).

PLS helps to reduce the drawbacks of principal components regression. In fact, the latter regresses responses on the summaries of predictors provided by the principal components of greatest variance, which are not necessarily the most explanatory. On the other hand, PLS regression constructs principal components of the space of the predictors by maximizing the quality of their relationship with the variable to be explained. In addition, PLS is efficient when few observations are available compared with the number of predictors, and when the latter are linked to each other, which is often the case in survey studies. PLS is particularly used in the fields of chemistry and agrifood matters (17,19,40,47,48). It has been used in crop protection, for example, to model leaf wetness from microclimatic data (6), and to assess how certain forest stand characteristics affect the development of the pine processionary caterpillar (40).

PLSS can be viewed as linear PLS regression of the response Y onto the coding matrix B whose columns are constructed by transforming the predictors X through B -spline basis functions (13); in short, $PLSS(X, Y) \equiv PLS(B, Y)$.

The PLSS model falls within the category of additive models (18), $Y = f_A^1(X_1) + \dots + f_A^p(X_p) + \varepsilon$, where f_A^i is a spline (i.e., a piecewise polynomial function which captures the influence of predictor X_i on the response Y through the construction of A components). Note that all the variables are standardized. The splines used here are of first or second degree for quantitative predictors, which gives piecewise linear or quadratic functions respectively. They are of degree 0 for qualitative predictors, thus giving piecewise constant functions. The connection points between the different pieces are called the "knots;" they are located within the range of the predictor values. Along with the degree and number A of components in the model, they form part of the tuning parameters of the method. If no knot is used and the degree is 1, the PLS linear regression model is found.

One of the advantages of local polynomials is that the model is quite robust when faced with a problem of extreme values of the predictors, because the B -spline basis functions used in PLSS vanish outside an interval of knots (13). One limitation is that a spline function f_A^i is null outside the range of the predictor X_i , meaning that prediction through PLSS is efficient only when the observation to be predicted lies within the range of the training sample. PLSS also inherits some advantages of PLS as regards problems of data scarcity and multicollinearity among predictors.

The quality of PLSS models is measured by two indicators. The percentage of variance of the response explained by the principal components is a goodness-of-fit criterion. The prediction residual sum of squares (PRESS), obtained by cross-validation, is a goodness-of-prediction criterion. Cross-validation consists of removing a certain number of individuals in blocks from the data set, predicting their value from the model constructed with the remaining data, and comparing the predictions obtained with the real values. The operation is repeated until all the individuals have been removed and predicted. The smaller the PRESS is, the greater is the goodness-of-prediction. A value >1 means that the prediction given by the model is no better than the prediction of the response by its mean. In such a case, the model is rejected.

In addition, cross-validation can be used to determine the number A of principal components to be kept in the model. Indeed, the PRESS value is a function of A . The number chosen is the one that gives the lowest PRESS value. In our study, in order to determine A , a campaign of cross-validation experiments was conducted by using different percentages of left-predicted observations (1, 5, 10, 20, and 25%). The observations were selected following their natural position in the matrix of data (i.e., according to the sampling year as the first criterion of organization, and the region they belonged to as the second criterion. The mean curve was adopted to evaluate the PRESS value.

The other PLSS tuning parameters, the degree of the splines and the number and location of knots, need to be carefully chosen for each predictor. The fact that no automatic procedure is available for selecting those parameters can be considered as a drawback as well as an advantage. On the one hand, manual selection of parameters is laborious; however, on the other hand, it enables the user to take account of prior knowledge of the data at hand in the model-building stage. In our study, tuning parameters were chosen for the continuous variables by taking an ascending approach (13). The categorical variables with few levels always were transformed by splines of degree 0. In the ascending approach, starting from linear transformations of continuous variables, the degree of the splines and number of knots is gradually increased while improving the goodness-of-prediction by cross-validation. There is no absolute rule for stopping the ascending approach. The usual rule consists of finding a balance between the complexity of the model on the one hand (i.e., the number of components, the degree of the splines, and the number

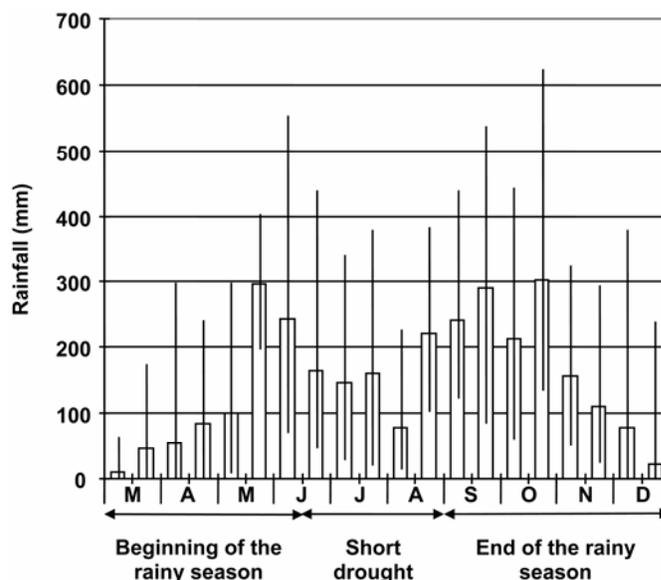


Fig. 2. Average rainfall distribution in Costa Rica in 2002 and 2003. Data for 21 rain gauges grouped by fortnightly periods. The vertical lines indicate minimum and maximum values.

of knots; the higher the degree and the larger the number of knots, the more difficult interpreting the model becomes), and the goodness-of-fit and the goodness-of-prediction on the other hand.

In the final phase of the model-building stage, called the “backward pruning step,” the predictors whose influence on the response is judged negligible can be eliminated. That influence is evaluated for each predictor by the range of the variable transformed by its spline function: the smaller the range, the more the effect of the predictor is considered negligible. Elimination is accepted if it does not increase the PRESS value.

For these analyses, we used the PLSS regression program written by Durand (*personal communication*) in the R language

(32), which is available on the website of the Mathematics Department of the University of Montpellier II, France.

One of the first uses of an additive spline extension similar to PLSS (15) was on sensory analysis data (19). This approach proved to be useful because it revealed nonlinear relationships between the sensory characteristics of different products and dependent hedonic variables.

RESULTS

Epidemic dynamics. The development of *M. citricolor* was highly dependent upon rainfall (Fig. 3). A reduction in rainfall

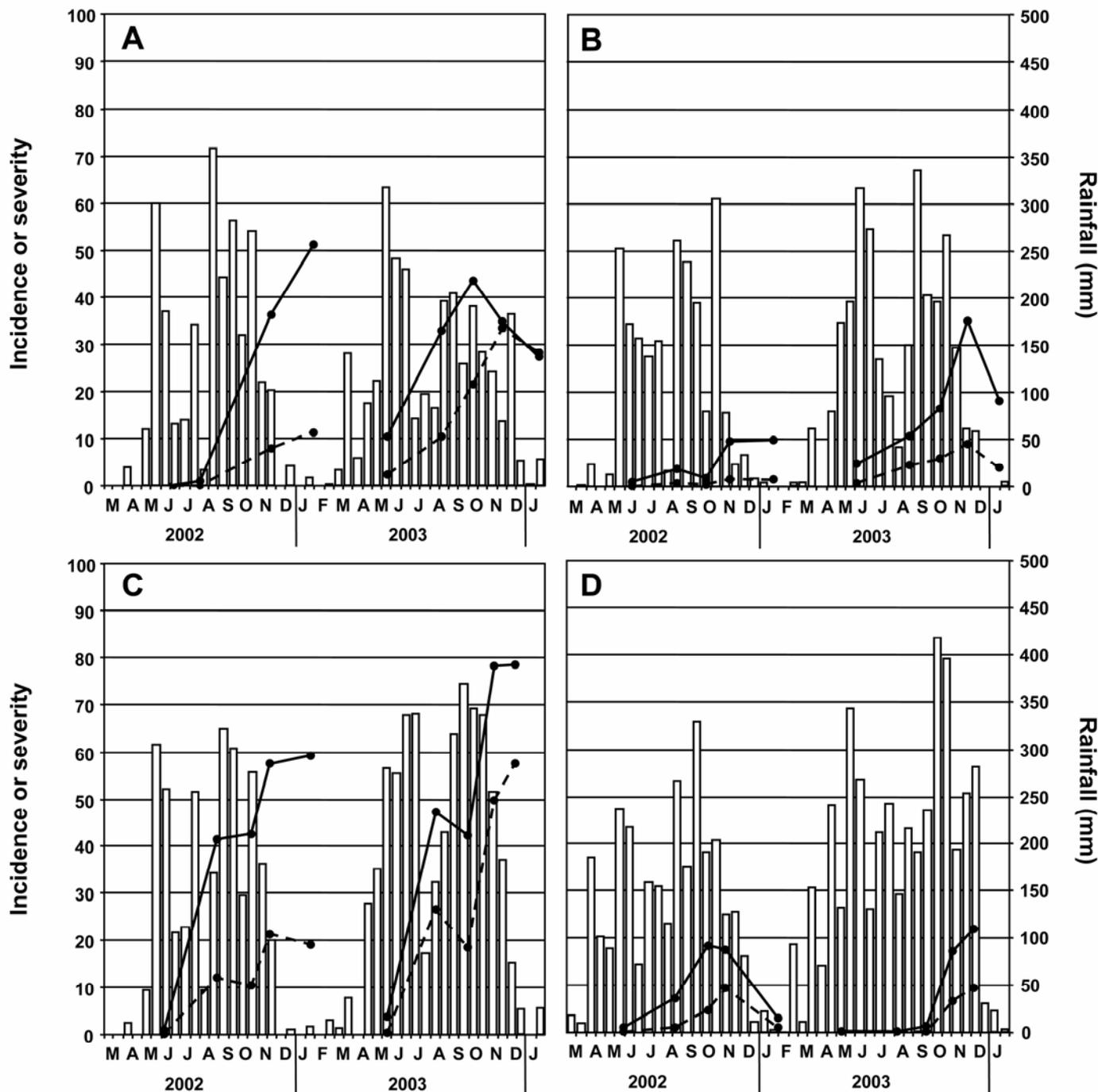


Fig. 3. Variation in incidence (percentages of infected young leaves, continuous lines) and severity (number of lesions for 10 young leaves, dotted lines) of American leaf spot disease of coffee depending on rainfall (histograms). **A.** Plot in Central Valley, 1,400 m above sea level, south-facing slope, 17% slope, distance between coffee rows 1.3 m, no shading. **B.** Plot at Tarrazú, 1,500 m above sea level, northeast-facing slope, 40% slope, distance between coffee rows 2.2 m, 26% shading. **C.** Plot in Western Valley, 1,375 m above sea level, west-facing slope, 32% slope, distance between coffee rows 1.7 m, no shading. **D.** Plot at Coto Brus, 1,002 m above sea level, west-facing slope, 35% slope, distance between coffee rows 2.2 m, 9% shading.

was reflected in a deceleration in the epidemic progress, or even a decrement. Sometimes, the slowing down occurred after the short dry season (Fig. 3B and C) and at the end of the rainy season (Fig. 3A, B, and D). It should be noted that the incidence (i.e., the percentage of diseased young leaves) and the severity (i.e., the number of lesions in relation to the number of young leaves) evolved in a similar way.

Ia did not vary in the same way, between the two study years, for all 34 replicated plots. For 13 of them, scattered in the four sampled regions, there was a clear increase of *Ia* (+0.20 on average); for 3 plots (two regions), a decrease happened (-0.14 on average); and for 18 plots (four regions), *Ia* was quite similar (-0.01 on average). In this last case, *Ia* had very low values in general (<0.05 on average for both years). These different behaviors for plots, sometimes close geographically, suggested that local factors, such as cropping practices, and not regional factors alone, such as altitude, affected *Ia*. Moreover, the observed variations between the two study years suggested that variables different from site covariates had an effect on the epidemics.

Description of variables characterizing the epidemic. The attack intensity index (*Ia*), the annual maximum incidence (*I_{max}*), and the annual maximum severity (*S_{max}*) were correlated: the coefficients of linear correlation were all very high and highly significant (Table 4).

Ia displayed a distribution that was more spread out: the positive dissymmetry was less strong and the kurtosis was greater than for *I_{max}* and *S_{max}* (Table 4). Therefore, *Ia* seemed to provide greater discrimination between attack levels. In addition, the evaluation of incidence and severity seemed to be less precise at the end of the year, due to the small numbers of leaves present at that time of year in several plots.

The main predictors of *Ia* by PLSS regression. For the reasons given above, we chose to model the response *Ia* by PLSS regression. The predictors retained in the model, the polynomial degrees, and the number and the location of knots, are indicated in Table 5.

The similar shape of the PRESS curves obtained for the different experiments achieved by using different percentages (1, 5, 10, 20, and 25%) of left-predicted observations and the fact that those curves were close to the mean curve gave evidence of the robustness of the model (Fig. 4). The lowest PRESS value for the mean curve corresponded to the optimum dimension 7 (PRESS = 0.60). This value, which is quite high, suggests that the prediction of the response was not very good. That might be explained by

the existence of a large number of null *Ia* values that were poorly predicted by the model (Fig. 5). Another possible reason was the presence of two qualitative predictors with a small number of categories (four and five) (Table 5) that made it difficult to predict the continuous variable *Ia*. However, 64.2% of the variance of *Ia* was reconstituted by the first seven components of the model. The goodness-of-fit was reflected in a standard error of 0.14 (Fig. 5) for observed values of *Ia* ranging from 0.00 to 0.90 (Table 4).

Although the model could not predict *Ia* well, it did, given the goodness-of-fit, detect the main predictors and the nature of their links to *Ia*. The hierarchy of predictors was established in accordance with the range of their values transformed by their respective spline function. The most influential variables were related to plot

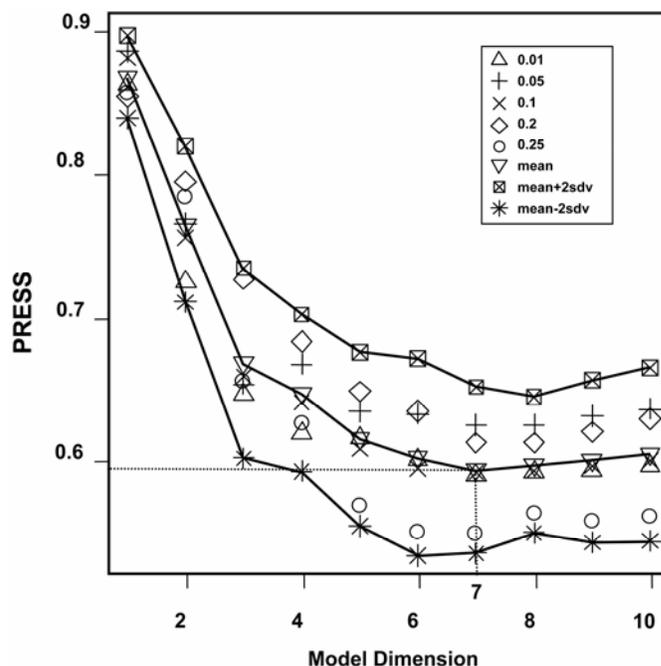


Fig. 4. Prediction residual sum of squares (PRESS values) of the partial least-squares regression via the spline functions model depending on the number of components. Cross-validation was carried out by using different percentages of left-predicted observations (1, 5, 10, 20, and 25%). The mean curve was used to determine the optimum number of components of the model.

TABLE 4. Description of variables characterizing the *Mycena citricolor* epidemic

Variable	Pearson correlation coefficient ^z			Range of values	Fischer skewness coefficient	Pearson kurtosis coefficient
	<i>Ia</i>	<i>I_{max}</i>	<i>S_{max}</i>			
<i>Ia</i>	1	0.87**	0.82**	0.00–0.90	1.29	3.78
<i>I_{max}</i>	...	1	0.90**	0.0–78.1	1.80	5.85
<i>S_{max}</i>	1	0.00–6.11	2.89	11.94

^z *Ia* = index of attack intensity, $\sum_{i=0}^3 \frac{i \cdot n_i}{3 \cdot N}$ where *N* is the number of coffee trees in the plot and *n_i* the number of coffee trees scored *i* in the severity scale (text provides more details). *I_{max}* = annual maximum incidence (maximum percentage of diseased young leaves in the year), *S_{max}* = annual maximum severity (maximum value in the year for the number of lesions: number of young leaves ratio), and ** = level of significance, *P* < 0.01.

TABLE 5. Selected degrees and knots for the predictors retained in the final partial least-squares regression via the spline functions model

	Altitude	Slope exposure	Slope inclination	Distance between rows	Shade percentage	Coffee tree height	Annual no. of fertilization operations	Shade type ^y	Coffee tree pruning system ^y	Short drought ^z
Degree	2	2	2	2	1	1	1	0	0	1
Knots	...	90, 180, 270	1.5, 2.5, 3.5, 4.5	1.5, 2.5, 3.5	...

^y For the qualitative predictors, the degree is 0 and the number of knots is *n* – 1, where *n* is the number of categories. The knot location is chosen in order to separate the categories.

^z From the second half of June to August.

topography (altitude, slope aspect, and slope inclination). However, it cannot be completely excluded that some site-covariates, such as topography, appeared slightly more important than they truly were because of the presence of pseudo-replications in the data set. Nonlinear relationships appeared clearly between altitude and slope aspect on the one hand and *Ia* on the other (Fig. 6). Altitudes between 1,100 and 1,550 m seemed to be more favorable to *M. citricolor* development. The optimum altitude appeared to be located at approximately the midpoint (1,368 m). Slopes were conducive to fungus development (mainly >20%), but eastern-facing slopes were clearly less affected than the others. Variables in the model that characterized crop management or the coffee tree (distance between rows, shade percentage, coffee tree height, annual number of fertilizer applications, type of shading, and pruning system) were less influential (Figs. 6 and 7). Coffee tree rows that were well separated from each other (particularly >1.9 m), and a large number of fertilizer applications (more than three per year) were less suitable conditions for *M. citricolor*. Conversely, tall coffee trees (especially >1.9 m) and shading (mainly >11%) seemed to provide more suitable conditions for the fungus. The most favorable shade for the fungus was that provided by fruit trees or forest species. An absence of pruning also acted in favor of the fungus. The most severe pruning system, by blocks, was the most unfavorable. Rainfall during the short dry season was the final variable to be entered into the model (Fig. 7). The wetter that period, the more intense were the epidemics.

Regional effect on *Ia*. Introducing a region variable into the model did not greatly improve it. The goodness-of-fit was slightly better (66.5% of the variance of *Ia* was explained by the first seven components of the model compared with 64.2% in the previous model), but the goodness-of-prediction decreased (optimal mean PRESS = 0.62 compared with 0.60 in the previous model). The region factor was not one of the most important predictors in the new model; it was ranked ninth, ahead of shading type and rainfall in the short dry season, thus indicating that the region variable was a minor predictor. In the results

shown in Table 6, the regional effect on *Ia*, which was significant, probably is confounded partly with altitude effects, because major regional differences existed for that factor. The regions also differed for other factors but in a less pronounced way: slope inclination, height of the coffee trees, or even the type of shading. Although the χ^2 test was not performed because some of the expected values were under five, the high value of χ^2 suggests a strong relation between region and the type of shading.

DISCUSSION

By showing that numerous factors of different types affect the development of American leaf spot of coffee, our study illustrated the complexity of the *M. citricolor*–*Coffea arabica* pathosystem. That complexity was increased by the existence of nonlinear relationships between variables, which were captured by PLSS. Greater complexity would have arisen if interactions had existed between the variables, as is the case in other pathosystems (8,37, 42). However, using the latest version of PLSS, which took into account all the bivariate interactions (14), we verified that there was no interaction between the variables used in the model. However, we cannot rule out having omitted some complex interactions involving more than two variables, or variables that were not used in the model.

Our study revealed the importance of topographical factors in the development of *M. citricolor* epidemics. The fungus appeared to be favored particularly by regions between $\approx 1,100$ and 1,550 m above sea level, where temperatures were cool without being excessively cold, which probably contributed to shortening the latency period. This observation explains the good results with artificial inoculations when the incubation temperature for inoculated leaves was 20°C (29–31). It also indicated why *M. citricolor* is a major constraint for coffee growing at altitudes where the best coffee in Costa Rica are produced. Although the effect of altitude on the fungus already had been reported in Central America (2), the effect of slope exposure had never been documented. Eastern-facing slopes were much less attacked, probably because, in the tropics, those slopes benefit from longer exposure to sunshine, which reduces leaf wetness duration and, thus, the rate of successful infections (46). Indeed, even during the rainy season, which can last for almost 10 months, the morning climate is generally sunny, with banks of cloud forming in late morning or early afternoon. Conversely, the other slopes, particularly western-facing slopes, received less sunshine because of cloudiness. The effect of the slope inclination also could be interpreted by a shading effect in the plots, which was potentially greater when the slopes were steeper. Our results tallied with those obtained on certain diseases of forest species in temperate or cold climates. In fact, topography is a factor that strongly influences parasite development or the expression of the diseases they cause (5,9, 28,34). To our knowledge, our study is one of the first references on the subject concerning a tropical cultivated species. The topographical effects we found suggested more complex effects linked to the position of the plot on the hillside and the influence of the adjacent relief on that plot. This point is particularly important for oak decline caused by the genus *Armillaria* (5) or white pine blister rust caused by *Cronartium ribicola* (9). In the case of *M. citricolor*, it is likely that the position of a plot at the bottom of a deep valley mostly remaining in the shade, regardless of the direction of the slope on which it is sited, would be conducive to disease development. Including that variable (i.e., plot position) might make it possible to improve the quality of the model. Given these results, consideration could be given to mapping epidemic risks, taking into account topographical factors that affect the disease, as has been produced for the Swiss needle cast disease (34).

Epidemic risks due to topographical effects might be increased or decreased depending on certain crop management variables. A

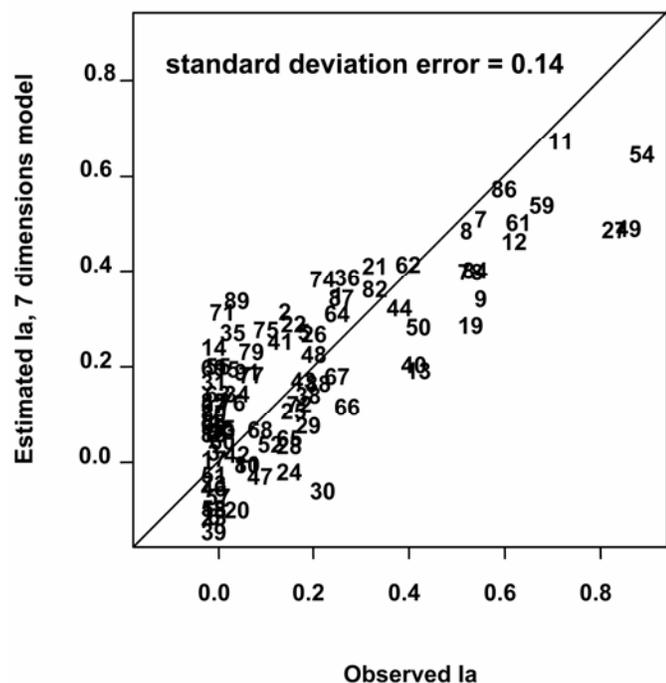


Fig. 5. Comparison of observed values and values predicted by the partial least-squares regression via the spline functions model for the American leaf spot attack intensity index (*Ia*). *Ia* = 0 if all coffee trees are healthy, *Ia* = 1 if all coffee trees are severely attacked and defoliated. More details of *Ia* calculation are described in the text.

way of reducing the risk is to reduce the percentage of shade provided by species planted with coffee trees. In fact, shade trees intercept radiation, reduce wind speed, and probably increase the leaf wetness duration (20), which helps to forestall gemmae desiccation and promote infection (46). The effect of shade is particularly marked when provided by fruit trees or forest species, probably because such trees are only lightly pruned or not pruned at all during the year, unlike legumes or even banana plants, which are pruned regularly. Shade also may be reduced by

increasing the distance between coffee planting rows, which reduces intraspecific shading, with effects similar to those already mentioned for the shade provided by cultivated species grown with coffee trees. In addition, that practice probably reduces the possibilities of infection from row to row. Indeed, Wellman (46) reported quite short (<60 cm) horizontal distances for gemmae dissemination by splashing. Coffee tree height is also a factor that influences fungus development: taller trees cast more shade. Introducing a pruning system whose immediate effect would be to

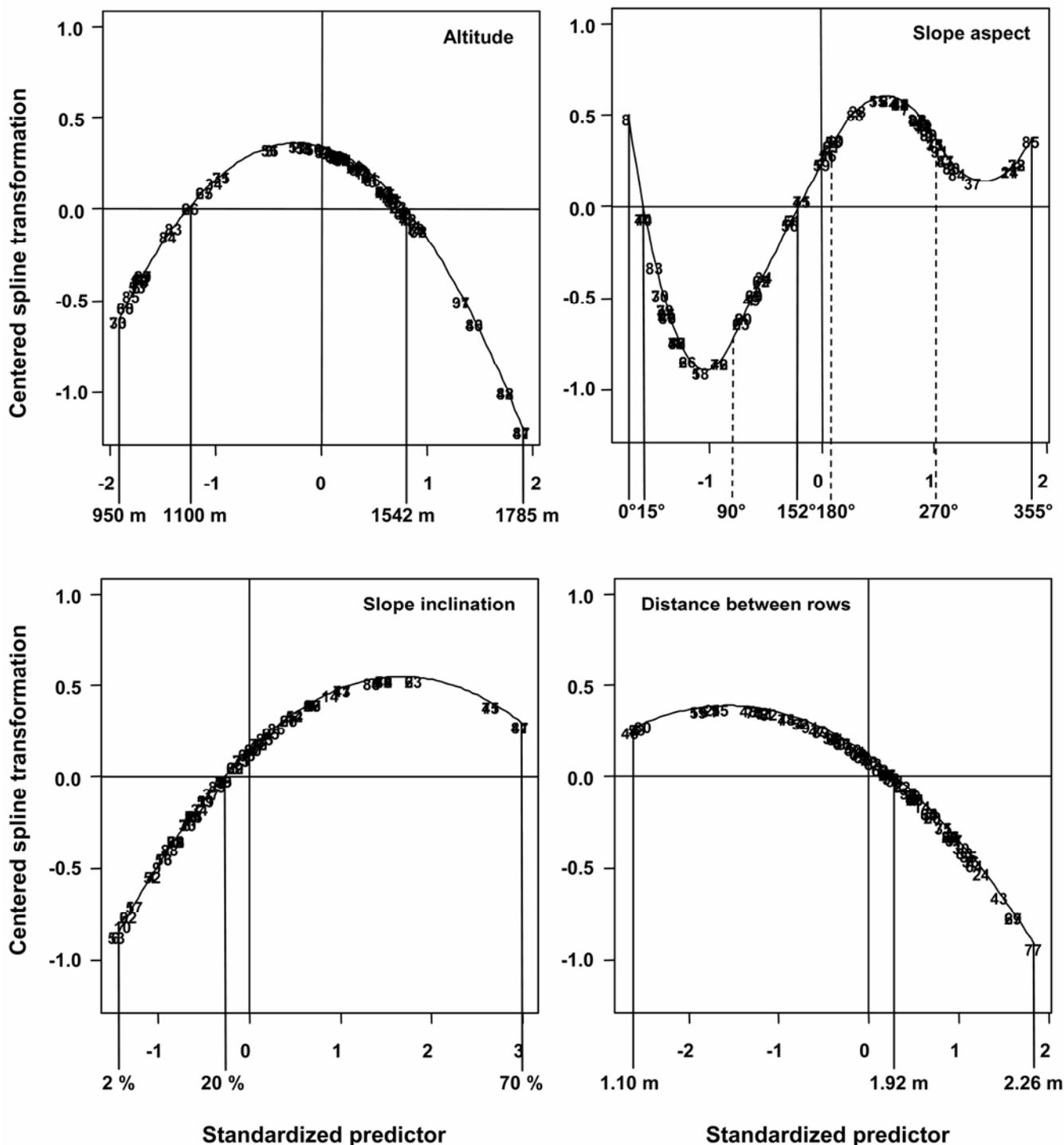


Fig. 6. Representation of the largest first 4 predictors (out of 10) transformed by the spline functions of the partial least-squares regression via the spline functions model to predict the American leaf spot attack intensity index (*Ia*). Predictors are standardized and the transformed values are centered. The predictors are presented in decreasing order of influence from left to right and top to bottom, according to the range of the transformed values. The dashed lines indicate the location of knots. The solid lines indicate the minimum, maximum, and some critical values for each quantitative predictor. Tables 1 and 2 provide details about the predictors. The meaning of *Ia* is described in the text.

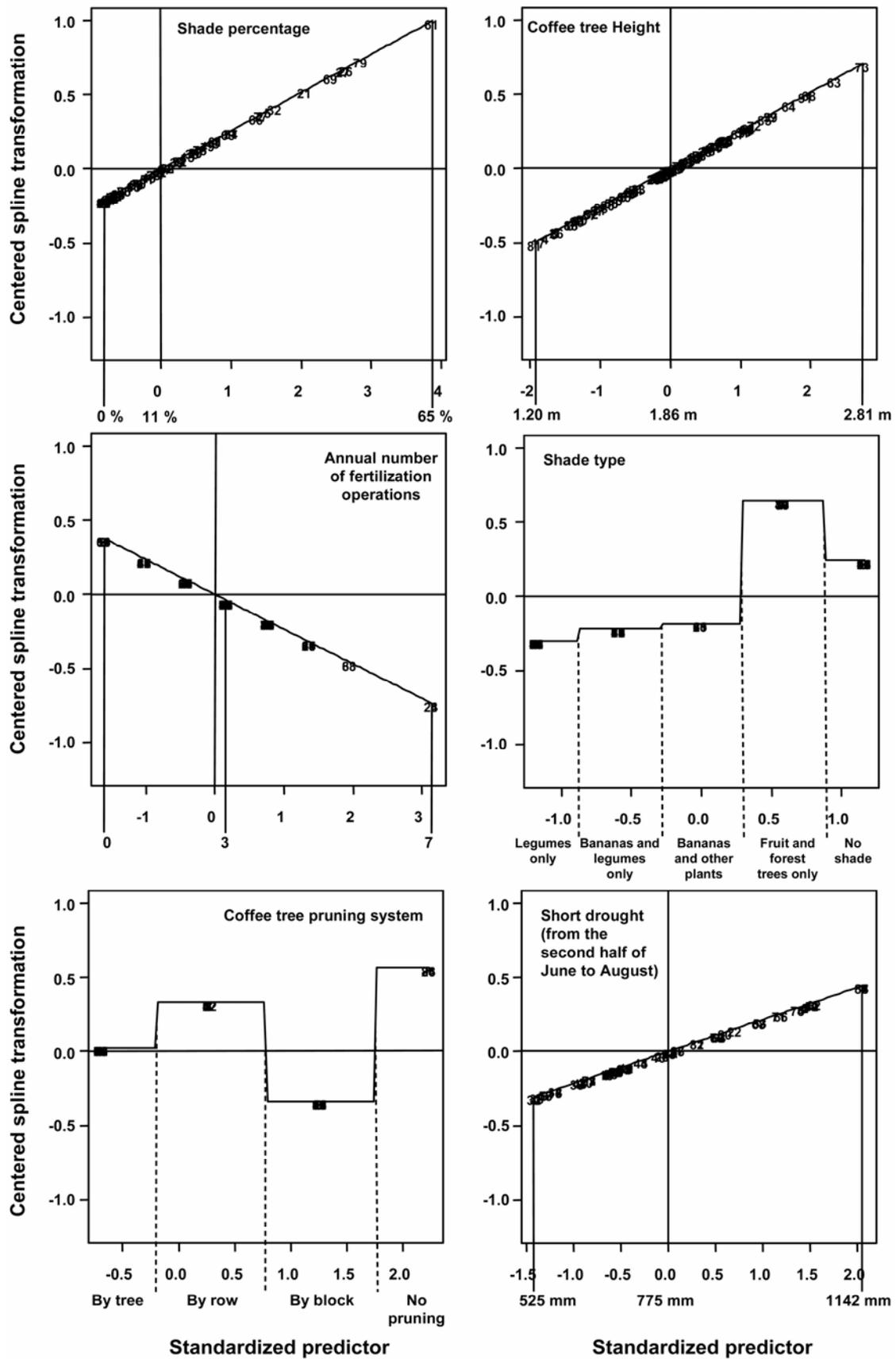


Fig. 7. Representation of the final 6 predictors (out of 10) transformed by the spline functions of the partial least-squares regression via the spline functions model to predict the American leaf spot attack intensity index (*Ia*). Predictors are standardized and the transformed values are centered. The predictors are presented in decreasing order of influence from left to right and top to bottom, according to the range of the transformed values. The dashed lines indicate the location of knots. The solid lines indicate the minimum, maximum, and some critical values for each quantitative predictor. Tables 1, 2, and 3 provide details about the predictors. The meaning of *Ia* is described in the text.

TABLE 6. Regional differences for *Ia* and for the predictors retained in the partial least-squares regression via the spline functions model (means for quantitative variables or percentage of plots in the main category for qualitative variables)^w

Region	<i>Ia</i>	Altitude (m)	Slope inclination	Distance between rows	Shade percentage	Coffee tree height	Annual no. of fertilization operations	Short drought ^x	Slope exposure	Shade type ^y	Coffee tree pruning system
Western Valley	0.34 a	1,402 b	19 b	1.76	9	1.68 b	2.2	869	40% west	53% NS	40% by block
Central Valley	0.16 b	1,426 b	18 b	1.77	8	1.81 ab	3.1	741	43% west	53% NS	50% by tree
Coto Brus	0.16 b	1,071 c	25 ab	1.95	17	1.97 a	2.1	760	33% east	36% LO	77% by tree
Tarrazú	0.14 b	1,582 a	32 a	1.70	12	1.97 a	3.0	755	46% north	64% LO	87% by tree
<i>F</i> value	3.6*	47.4**	3.0*	2.7	1.5	3.9*	1.9	0.9
χ^2 Value (Df) ^z	11.0 ^{NP} (9)	34.3 ^{NP} (12)	17.7 ^{NP} (9)

^w For quantitative variables, analysis of variance and Newman-Keuls mean comparison tests were performed; for qualitative variables, χ^2 values were calculated; * and ** = level of significance at $P < 0.05$ and 0.01 , respectively. Data followed by the same letter are not significantly different according to the Newman-Keuls test ($P < 0.05$). For slope exposure, quantitative data were categorized as follows: North is [325, 360], [0, 45], East is [45, 135], South is [135, 225], and West is [225, 325]. The Turrialba region is not included in this analysis because only three plots were sampled, and only in 2003.

^x From the second half of June to August.

^y NS = no shade and LO = legumes only.

^z NP indicates χ^2 test was not performed because some of the expected values were < 5 .

eliminate inoculum and reduce tree height consequently helps to reduce epidemic risks. Pruning by blocks appears to be particularly effective. Indeed, when cutting back is carried out, it can lead to the complete removal of all foliage over a large area, which can be unfavorable to fungus development. However, if there is too long an interval between pruning rounds for this type of system, and if trees are allowed to grow tall, it could lead to an increase in epidemic risks, as explained earlier. Fertilization appears to be unfavorable to epidemic development, most probably because good nutritional conditions might lead to a dilution of the disease, through an increase of host foliage that is faster than the epidemic (21). Defoliation caused by the disease also may appear to be less severe when plots are well fertilized.

Rainfall in the short dry period in the middle of the rainy season seems to play a strong role in epidemic development. That period is characterized by a succession of rainy days followed by days without rain. Dry spells probably affected the production and release of gemmae, and certainly had an effect on their viability. Wellman (46) reported that detached gemmae kept dry for 3 days and then moistened failed to germinate.

Our study enabled us to make immediate recommendations for coffee growers in Costa Rica to more effectively control American leaf spot disease. Our results suggest that growers should apply planting densities, pruning systems, and shading practices that suit the altitude, aspect, and inclination of the slopes, which are factors that largely determine the epidemic risk. In zones with a high epidemic risk due to their topographical characteristics, an attempt should be made to increase the distances between coffee planting rows, and to reduce shade and avoid shade cast by fruit trees and forest species. These practices should facilitate chemical control, or even improve the expression of any partial resistance. Caution is called for when using forest species in plantations at risk. This call for caution is aimed primarily at all types of certification claiming to be sustainable development that propose, with every good intention, transforming coffee systems while often overlooking their impact on the parasite component of the system.

ACKNOWLEDGMENTS

We thank the INRA-CIRAD joint fund, which co-funded this study with ICAFE via the project entitled "Towards a decision support system for controlling parasites on Arabica coffee," call for proposals 2001-mathematics applied to agricultural sciences.

LITERATURE CITED

- Avelino, J., Muller, R. A., Cilas, C., and Velasco, H. 1991. Développement et comportement de la rouille orangée du caféier (*Hemileia vastatrix* Berk. et Br.) dans des plantations en cours de modernisation plantées de variétés naines dans le Sud-Est du Mexique. *Café Cacao Thé* 35:21-42.
- Avelino, J., Toledo, J. C., and Medina, B. 1993. Développement de la rouille orangée (*Hemileia vastatrix*) dans une plantation du sud-ouest du Guatemala et évaluation des dégâts qu'elle provoque. Pages 293-302 in: Proc. 15th Int. Sci. Colloquium on Coffee, Montpellier, ASIC, Paris.
- Avelino, J., Willocquet, L., and Savary, S. 2004. Effects of crop management patterns on coffee rust epidemics. *Plant Pathol.* 53:541-547.
- Avelino, J., Zelaya, H., Merlo, A., Pineda, A., Ordoñez, M., and Savary, S. 2006. The intensity of a coffee rust epidemic is dependent on production situations. *Ecol. Model.* 197:431-447.
- Bruhn, J. N., Wetteroff, J. J., Jr., Mihail, J. D., Kabrick, J. M., and Pickens, J. B. 2000. Distribution of *Armillaria* species in upland Ozark Mountain forests with respect to site, overstory species composition and oak decline. *Eur. J. Forest. Pathol.* 30:43-60.
- Chtioui, Y., Francl, L. J., and Panigrahi, S. 1999. Moisture prediction from simple micrometeorological data. *Phytopathology* 89:668-672.
- Coen, E. 1983. Climate. Pages 35-46 in: Costa Rican Natural History. D. H. Janzen, ed. The University of Chicago Press, Chicago.
- Colbach, N., Meynard, J. M., Duby, C., and Huet, P. 1999. A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Prot.* 18:451-461.
- Dahir, S. E., and Carlson, J. E. C. 2001. Incidence of white pine blister rust in a high-hazard region of Wisconsin. *North. J. Appl. For.* 18:81-86.
- De Graaff, J. 1986. *The Economics of Coffee*. Pudoc, Wageningen, The Netherlands.
- De Wit, C. T. 1982. La productivité des pâturages sahéliens. Pages 22-35 in: La productivité des pâturages sahéliens—une étude des sols, des végétations et de l'exploitation de cette ressource naturelle. F. W. T. Penning de Vries and M. A. Djiteye, eds. Pudoc, Wageningen, The Netherlands.
- Diaz Moreno, J., and Napoles V. M. 1961. Comportamiento de varios fungicidas en el combate de *Mycena citricolor* del cafeto en Ecuador. *Turrialba* 11:93-97.
- Durand, J. F. 2001. Local polynomial additive regression through PLS and splines: PLSS. *Chemom. Intell. Lab. Syst.* 58:235-246.
- Durand, J. F., and Lombardo, R. 2003. Interactions terms in nonlinear PLS via additive Spline transformation. Pages 22-29 in: *Between Data Science and Applied Data Analysis*. Proc. 26th Annu. Conf. Gesellschaft für Klassifikation, University of Mannheim. M. Schader, W. Gaul, and M. Vichi, eds. Springer-Verlag, Heidelberg, Germany.
- Durand, J. F., and Sabatier, R. 1997. Additive splines for PLS regression. *J. Am. Stat. Assoc.* 92:1546-1554.
- Fernández, C. E., and Muschler, R. G., 1999. Aspectos de sostenibilidad de los sistemas de cultivo de café en América central. Pages 69-96 in: *Desafíos de la Caficultura en Centroamérica*. B. Bertrand and B. Rapidel, eds. IICA, San José, Costa Rica.
- Frank, I. E., and Friedman, J. H. 1993. A statistical view of some chemometrics regression tools. *Technometrics* 35:109-135.
- Hastie, T., and Tibshirani, R. 1990. *Generalized Additive Models*. Chapman and Hall, London.
- Huon de Kermadec, F., Durand, J. F., and Sabatier R. 1997. Comparison between linear and non linear PLS methods to explain overall liking from sensory characteristics. *Food Qual. Pref.* 5/6:395-402.
- Jaramillo-Robledo, A., and Gómez-Gómez, L. 1989. Microclima en plantaciones a libre exposición solar y bajo sombrío. *Cenicafé* 15:65-79.
- Kushalappa, A. C., and Ludwig, A. 1982. Calculation of apparent infection rate in plant diseases: development of a method to correct host growth. *Phytopathology* 72:1373-1377.

22. Lamouroux, N., Pellegrin, F., Nandris, D., and Kohler, F. 1995. The *Coffea arabica* fungal pathosystem in New Caledonia: interactions at two different spatial scales. *J. Phytopathol.* 143:403-413.
23. Leandro, G., and Soto, C.A. 1980. Evaluación de fungicidas para el combate de *Mycena citricolor* y *Cercospora coffeicola* en café. *Agron. Costarric.* 4:41-45.
24. Lemmon, P. E. 1957. A new instrument for measuring forest overstory density. *J. For.* 55:667-668.
25. Leppik, E. E. 1970. Genes centers of plants as sources of disease resistance. *Annu. Rev. Phytopathol.* 8:323-344.
26. Lodge, D. J., and Cantrell, S. 1995. Fungal communities in wet tropical forests: variation in time and space. *Can. J. Bot.* 73(Suppl. 1):S1391-S1398.
27. Miranda Arauz, A. 1989. Consideraciones para uniformar los resultados de un análisis químico de suelo—separata curso regional sobre nutrición mineral del café 1988. IICA, Guatemala.
28. Nevalainen, S. 2002. The incidence of *Gremmeniella abietina* in relation to topography in southern Finland. *Silva Fenn.* 36:459-473.
29. Rao, D. V., and Tewari, J. P. 1987. Production of oxalic acid by *Mycena citricolor*, causal agent of the American leaf spot of coffee. *Phytopathology* 77:780-785.
30. Rao, D. V., and Tewari, J. P. 1988. Suppression of the symptoms of American leaf spot of coffee with calcium hydroxide. *Plant Dis.* 72:688-690.
31. Rao, D. V., and Tewari, J. P. 1989. Occurrence of magnesium oxalate crystals on lesions incited by *Mycena citricolor* on coffee. *Phytopathology* 79:783-787.
32. R Development Core Team 2004. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
33. Rosling, A., Lindahl, B. D., Taylor, A. F. S., and Finlay, R. D. 2004. Mycelial growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. *FEMS Microbiol. Ecol.* 47:31-37.
34. Rosso, P. H., and Hansen, E. M. 2003. Predicting Swiss needle cast disease distribution and severity in young Douglas-fir plantations in coastal Oregon. *Phytopathology* 93:790-798.
35. Samper K. M. 1999. Trayectoria y viabilidad de las caficulturas centro-americanas. Pages 1-68 in: *Desafíos de la Caficultura en Centroamérica*. B. Bertrand and B. Rapidel, eds. IICA, San José, Costa Rica.
36. Savary, S. 1987. Enquête sur les maladies fongiques de l'arachide (*Arachis hypogaea*) en Côte d'Ivoire. II. Epidémiologie de la rouille de l'arachide (*Puccinia arachidis*). *Neth. J. Plant Pathol.* 93:215-231.
37. Savary, S., Castilla, N. P., Elazegui, F. A., and Teng, P. S. 2005. Multiple effects of two drivers of agricultural change, labour shortage and water scarcity, on rice pest profiles in tropical Asia. *Field Crops Res.* 91:263-271.
38. Savary, S., Willocquet, L., Elazegui, F. A., Teng, P. S., Du, P. V., Zhu, D., Tang, Q., Huang, S., Lin, X., Singh, H. M., and Srivastava, R. K. 2000. Rice pest constraints in tropical Asia: characterization of injury profiles in relation to production situations. *Plant Dis.* 84:341-356.
39. Sequeira, L. 1958. The host range of *Mycena citricolor* (Berk. & Curt.) Sacc. *Turrialba* 8:136-147.
40. Tenenhaus, M. 1998. La régression PLS, Théorie et Pratique. Edition Technip, Paris.
41. Tewari, J. P. 1984. The American leaf spot of coffee. *Univ. Alberta Agric. For. Bull.* 7:66-68.
42. Thébaud, G., Sauvion, N., Chadœuf, J., Dufils, A., and Labonne, G. 2006. Identifying risk factors for European stone fruit yellows from a survey. *Phytopathology* 96:890-899.
43. Wang, A., and Avelino, J. 1999. El ojo de gallo del cafeto (*Mycena citricolor*). Pages 243-260 in: *Desafíos de la Caficultura en Centroamérica*. B. Bertrand and B. Rapidel, eds. IICA, San José, Costa Rica.
44. Wang, A., and Tewari, J. P. 1990. Role of oxalic acid in pathogenesis by *Mycena citricolor* (Agaricales, Hymenomycetes), causal agent of the American leaf spot of coffee. *Cryptogam. Bot.* 1:396-398. not cited
45. Weitz, H. J., Ballard, A. L., Campbell, C. D., and Killham K. 2001. The effect of culture conditions on the mycelial growth and luminescence of naturally bioluminescent fungi. *FEMS Microbiol. Lett.* 202:165-170.
46. Wellman, F. L. 1950. Dissemination of *Omphalia* leaf spot of coffee. *Turrialba* 1:12-27.
47. Wold, H. 1975. Soft modelling by latent variables; the nonlinear iterative partial least squares approach. Pages 117-144 in: *Perspectives in Probability and Statistics (Papers in Honor of M. S. Bartlett)*. J. Gani, ed. Academic Press, London.
48. Wold, H. 1984. PLS Regression. Pages 581-591 in: *Encyclopaedia of Statistical Sciences*, Vol. 6. N. Johnson and S. Kotz, eds. John Wiley, New York.