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Research Article

Predicting Model for Epidemiological Risk Management of Citrus Pseudocercospora Leaves and Fruits Spot Disease in Cameroon

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ABSTRACT

The aim of this work was to construct, using regression analysis, models for predicting Pseudocercospora leaves and fruits spot disease (PLFSD) incidence for the epidemiological risk management on orange, grapefruits and Satsuma tangerine trees. Study was conducted in the large humid zone of Cameroon. Epidemiological and pedoclimatic data were collected from experimental orchards located in Foumbot, Njombé, Ekona and Nkolbisson and citrus growers plots located in Bokito, Boumnyebel, Kumba and Jakiri. Regression analyzes done for each citrus fruit species allowed us to describe the relationships between the epidemiological variables and the characteristic parameters of the sites. Citrus trees located in more acidic soils, with high silt and clay content show a higher incidence of the disease. On the other hand, trees in soils with a high sand content, rich in organic matter and organic carbon, phosphorus and exchangeable bases are less attacked by the PLFSD. The preponderance of the temperature effect on that of altitude has been proven and this climatic variable has proved to be the most important. Production system was ranked among the important variables on the different models and the agroforestry system showed a weak PLFSD development unlike the pure orchard. In sum, the use of low-susceptibility varieties on well-drained soils in agroforestry systems would significantly reduce PLFSD incidence.

Keywords: Epidemiology, risk factors, PLS regression, citrus, Pseudocercospora angolnsis.

INTRODUCTION

PLFSD caused by Pseudocercospora angolensis (Carvalho and Mendes; Crous and Braun) (Pretorius et al., 2003) is the most destructive citrus disease in tropical Africa (Yesuf, 2013). Since its discovery in Angola in 1952, its spread is increasing. After Sierra Leone, the disease has recently been reported in Ghana and is currently occurring in 23 African tropical countries and in Yemen (Kuate 1998, Harling et al., 2010, Yesuf 2013, Brentu et al. . This disease is a real scourge, not only because it causes losses of up to 100% of the fruit crop, but also because of quarantine and the ban on exporting to other production areas. to avoid the spread of the pathogen (Kuate et al., 1994b, Yesuf, 2002, Dagnew, 2014). This situation is detrimental to the already fragile economies of the small producers who supply most of the production on local markets. In addition, this disease represents a major threat to major citrus producing countries in which climatic conditions similar to those of the attacked countries are present (Chung & Timmer 2009, Jeger et al., 2017, Khanchouch et al., 2017).

Research in countries affected by this disease has focused on identifying the pathogen and describing the symptoms of the disease (Kirk, 1986, Pretorius et al., 2003). Subsequently, preliminary epidemiological studies have produced a distribution map of the disease (Corbaz, 1990). It has also been shown that the incidence of the disease increases with elevation in altitude. Cool (<20 ° C) and high relative humidity (> 60%) conditions were also very favorable for the development of the disease (Kuate et al., 1994a; Kuate et al., 1997; Hillocks 1998; Lawson et al., 2017). Varietal sensitivity studies to date have not

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found any resistant variety to PLFSD, however, different levels of susceptibility have been observed. Citrus genotypes according to three susceptibility groups (Bella-Manga et al., 1999, Diallo et al., 2011), the most sensitive citrus fruits are grapefruits and several tangerines, while orange and tangerine orange trees are moderately susceptible; lemons, limes, satsumas and tgrapefruit are not very susceptible (Seif & Hillocks 1999, Diallo 2003). Several studies on the efficacy of many fungicides have been carried out (Seif & Hillocks 1993, Yesuf 2007, Diallo 2003).

Chemical control remains the usually method against PLFSD in Cameroon and areas where the disease is prevalent (Kuate, 1998). The recommended molecules, doses, and number of treatments are standardized across countries and production areas (Kassahun et al., 2006, Yesuf, 2007, Pretorius and Holtz, 2008, Diallo et al., 2011, Lawson et al. , 2017). However, frequencies application and their cost are a barrier to their use by small citrus growers (Ndo 2007, Yesuf 2002). Moreover, this mode of fight is more and more discouraged because of its ecological impact; and most of the molecules used against P. angolensis are harmful to the environment (Alva et al., 1993, Houeto et al., 1995). It therefore appears necessary to develop an integrated and optimized method of control; and this requires a better understanding of the disease and the factors that favor it. In addition, there is variation in the severity and incidence of the disease in production ponds, sites and plantations (Ndo, 2011). These variations highlight the influence of factors like species/variety, climate, soil type or production system on disease development (Ndo et al., 2010).

Seif and Hillocks (1996), through regression analysis, developed several predictive models of PLFSD incidence. This analysis took into account only climatic factors (rainfall, temperature, relative humidity, wind speed ...). In addition, among the models obtained, some were invalidated because of the strong colinearities existing between the different variables. The other models selected had relatively low regression coefficients, suggesting that other factors influencing the development of the disease would be taken into account. The objective of this study is to construct, using regression analyzes, PLFSD incidence prediction models for the epidemiological risk management of three citrus varieties. Partially least squares (PLS) regression or partial least squares regression was used for this purpose.

MATERIAL AND METHODS

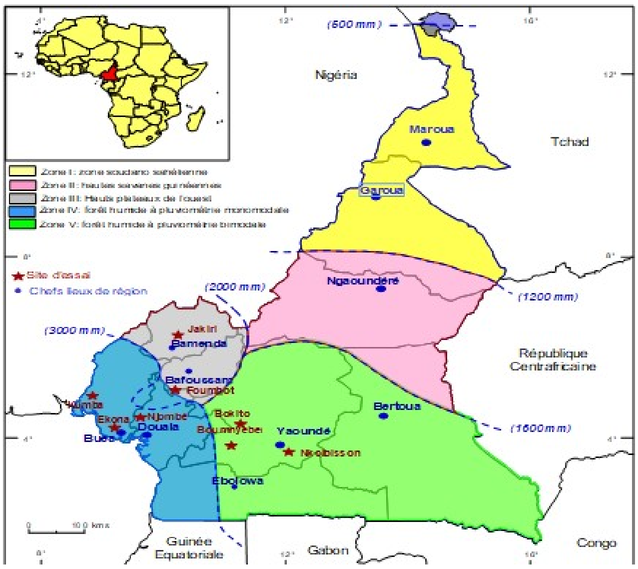
Study site

The study was conducted in the large humid zone of Cameroon in zones III, IV and V; which shows a great agroecological diversity (Ambassa-Kiki & Bernard, 2002). In the Western Highlands area (latitude 4°54' to 6°36' N, longitude 9°18' to 11°24' E, altitude: 1240-1800m above sea level asl) Jakiri and Foumbot sites were chosen. It is an area of mountains, with an average annual temperature of 19°C and an abundant average rainfall of monomodal type (1500-2500 mm).

In the Monomodal Rainfall Humid Forest Zone (latitude 2°6' to 6°12' N, longitude 8°48' to 10°30' E, elevation: 200-800 m asl), Njombé, Ekona and Kumba sites were chosen. It is a very humid and hot area, with average annual temperatures of around 22 to 29°C and a relative humidity of 85-95%. The average annual rainfall varies from 2500 to 4000mm or even 11000mm in some localities.

In the Humid Zone with Bimodal Rainfall (latitude 2°6'-4°54'/ 5°48' N, longitude 10°30'-16°12 'E, elevation 500-1000m asl), we have 2 rainy seasons (March-June and September-November) and 2 dry seasons (July-August, December-February). The climate is hot and humid, with temperatures of around 25° C and a average relative humidity of 75%. Nkolbisson, Bokito and Boumnyebel sites were chosen here. Figure 1 shows the localization of sites in the study area.

Figure 1: Map of test sites in the agro-ecological zones of Cameroon.



Experimental design and plots description of Two types of plots are involved in this test. The first type are IRAD experimental plots, implanted according to a well-established plan and protocol; and put in place for PLFSD studies. The second type concerns plots set up by small citrus growers. Their implementation follows the peasant strategies (Torquebiau, 2007). They are usually plots in which various species of fruit trees, cocoa or coffee trees, forest trees and annual or bisannual crops are in association. Table 1 presents a summary of the various parcels selected.

Table 1: Experimental design.

Experimental plots IRAD				
Sites	**Plots**	**species/ varieties**	**Number of stands**	**Type of plant association**
Foumbot	IRAD (2002)	orange trees, grapefruit trees, Satsumas.tangerine trees	15	Citrus orchard
Njombé			15	
Ekona	IRAD (2006)		15	Citrus orchard
Nkolbisson			15	

Producers plots

Sites	Plots	species / variety	Nombre de plants	Type of plant association
Bokito	Kédia	grapefruit trees., orange trees.	13	Cocoa agroforest with Citrus associated to other fruit trees and forest trees
	Abanda	orange trees.	6	
Jakiri	Quartier Shey	grapefruit trees, orange trees	12	Cocoa agroforest with Citrus associated to other fruit trees and forest trees
	Route Foumban	orange trees	6	
Kumba	Ekiliwindi	grapefruit trees, Satsumas tangerine trees, orange trees	18	Citrus orchard (grapefruit trees. tangerine trees and orange trees)
Boumnyebel	Mahol	grapefruit trees, orange trees	8	Citrus associated to other fruit trees

IRAD plots were set up in 2002 and 2006 following the same plan. Latter are located in Foumbot, Njombé, Ekona and Nkolbisson. Each parcel contains 14 varieties of citrus. Seedlings are derived from grafting on Citrus volkameriana. Plots are divided into 3 blocks with 2 trees of each variety per block. Tree planting distances are 7m x 7m and blocks are surrounded by a border of Grapefruits that are very PLFSD sensitive and that play "inoculum diffuser" role.

Citrus growers plots are located in Bokito, Boumnyebel, Kumba and Jakiri. These are two types of plots. The first type consists of agroforestry plots set up without a precise plan, with irregular spacings between the plants. Citrus are associated with other fruit trees, annual or bisannual crops, cocoa trees or coffee trees. In most of these plots, trees are grown from seedlings. Age and variety are not known exactly. These are 2 plots located in Bokito, 2 plots located in Jakiri and a plot located in Boumnyebel. Unlike the first 4 plots, which are composed of citrus seedlings, the Boumnyebel plot only has seedlings derived from a grafting on Citrus volkameriana. The Kumba plot is a citrus orchard.

Species Choice

This study takes into account differences sensitivity between citrus species. Grapefruits (apart from the Oroblanco variety) are classified as "very sensitive" with more than 70% of the leaves or fruits diseased. Orange trees, whatever the variety, are classified in the group of "sensitive" with 30 to 70% of the leaves or diseased fruits. Satsuma tangerines are classified as "less sensitive" (Bella et al., 1999, Kuate, 2003). In addition, these varieties was chose because of their better representation in sites.

Data collection

Disease intensity assessment

Data were collected once per season and per plot (September-October 2009, May-June 2010, January-February 2011, and May-June 2011). These 4 seasons represent 4 observation dates. They are not only rainy seasons (except the date 3) but also fruit seasons. These are also periods of high intensity of the disease (Kuate, up cit.). Citrus fruits selected from each plot were marked with a plaque with numbers and letters indicating the type of test, the site and the sequence number of the tree.

At each observation series, 10 shoots were chosen from each tree randomly. The choice was made to have a good symmetry representation of the tree (top, bottom, left, right, center). On each of the 10 shoots selected, the first 16 leaves were selected from the bottom to the top of the shoot. observations consisted of PLFSD lesions per leaf number counting.

Soil Sampling

Soil type has been identified as one of the determining factors in PLFSD development (Ndo et al., 2010), so it was necessary to characterize it in order to determine the elements that would play a predominant role. Soil samples were taken at 2 layers at different depths, namely 0-20 cm and 40-60 cm. These layers are assimilated to pedological horizons and designated as such. These data would make it possible to know the composition of the soil in the area explored by the roots. Indeed, citrus roots can reach up to 4 m deep, but more than 50% of them are between 60 and 70 cm deep (Noling, 2003). Sampling was done using an auger in the interval between the lines and near the marked trees. Several samples were taken per horizon at a plot level to form, after careful mixing, a composite sample per plot and per horizon. From these composite samples were extracted subsamples that were submitted to analysis at IRAD Laboratory of Soils, Plants, Waters and Fertilizers Analysis (LASPEE) in Yaounde.

Results obtained made it possible to know granulometric or texture composition, pH, essential nutrient contents (N, P, K ...), as well as the cation exchange capacity (CEC) of the soils of the plots. Comparative standards proposed in the literature for better soil characterization have been used (Landon, 1984, Landon, 1991, Shepherd & Walsh, 2002) .For Bokito and Jakiri sites with more than one plot, averages were calculated per site, so that only data per site and not per plot.

Climatic and geographical data

For each site, the average climate data (temperature, relative humidity, rainfall) for the last 20 years were obtained from the nearest meteorological station. These meteorological data were obtained at the of National Meteorology Direction located in Douala. It centralizes data from stations across the country.

Altitudes and geographic coordinates (latitude, longitude) were obtained at the foot of each selected tree using a GPS (Global Positioning System).

Statistical analyses

Variables of the disease

Only data on leaves were used for this study. Three variables allow us to evaluate the disease intensity. For severity, parameters used are the average number of lesions per leaf for each tree (Nles) and the average number of lesions per sick leaf and per tree (Nlesm). The Nlesm allows to know the degree of real severity or conditional severity on organs that exhibit symptoms. The incidence of the disease is given by the proportion of diseased organs (Pom). The Pom is calculated by the ratio:

$$Pom = \left(\frac{Total\ diseased\ leaves}{Total\ \ observed\ leaves}\right) * 100$$

With regard to the production system, percentages that represent the rate of trees in an agroforestry system or in a pure orchard have been calculated. The means by variety of the 3 variables (Nles, Nlesm, Pom) were also calculated on the 4 dates. This calculation made it possible to have for each site an average per variable and per citrus variety; so as to have a table by variety, presenting the sites and their characteristics (intensity of disease, altitude, climate, soil, production system). It is a question of knowing for each variety, how evolves the disease according to sites parameters.

PLS Regression Analysis

Several explanatory variables used are correlated with each other. We thus note correlations greater than 80% between mean altitude, temperature and rainfall. It is the same between soil parameters where we find correlations around 70% (clay, sand and silt, organic matter and organic carbon ...). This strong collinearity between independent variables guided our choice towards PLS regression. Indeed, a "traditional" multiple regression analysis would have led to an uncertain estimation of the regression parameters and models that are difficult to interpret (Johansson & Nilsson, 2002, Olav et al., 2011). PLS regression objective is to understand and describe the complex relationships between dependent variables (Y) and independent variables (X) in the absence of a theoretical model (Tenenhaus et al., 2005, Palomino & Carrascal, 2007). It is used when the number of X variables is very important compared to the Y variables or generally when the number of independent and/or dependent variables is high (Tenenhaus et al., 1995, Tenenhaus, 1998). The purpose of this study is to describe for each citrus variety the relationships that exist between disease variables and parameters that characterize the sites. PLS regression was performed with XLSTAT 2011.3.02 software. The variables explained (Y) are the variables of the disease (Nles, Nlesm and Pom), that are 3 variables. The explanatory variables (X) are the parameters of climate, soil, altitude and cropping system (for which we note the percentage of trees in an agroforestry system or in a pure orchard), i.e. 22 variables. This analysis was carried out on orange trees, grapefruits, tangerine trees.

PLS Regression starts with a canonical analysis that generates components (th). The first component t1 is constructed as a linear combination of the explanatory variables xj. This linear combination seeks to group the variables xj and to best explain y variable. When the explanatory power of these variables xj is low, a second component t2 is constructed as a linear combination of xj not correlated with t1 and well explaining the residuals y1. The components are represented on axes. The construction of the second axis makes it possible to extract the information that does not appear in the first component t1. This procedure can be continued by using the y residues each time. The number of th components is determined by cross-validation. Cross-validation is performed with Q2 indices that measure the marginal contribution of each PLS th component to the predictive power of the model. Contribution of the th component is significant if Q2h ≥ 0.0975 or if at least one Q2hk ≥ 0.0975.

All variables are centered-reduced to have data independent of the unit, the units of measurement of climate variables and soil being very different. Two other measures of validity of the model are generated: (i) R^2X which is the proportion of the variance of the independent variables x explained by th components in the model; (ii) R^2Y which is the proportion of the variance of the dependent variable explained by the th components in the model. This last parameter corresponds to the multiple correlation coefficient R2 (Johansson & Nilsson, 2002, Tenenhaus et al., 2005). Correlations between different variables and components also guide the choice of components. Stronger correlations are, better variables are represented in the model. Relative influence of each explanatory variable in the model can be expressed as the importance of the variable in projection (Variable Importance in Projection) or VIP. Variables with high VIP (> 0.8) are the most important in the construction of Y (Tenenhaus et al, 2005).

RESULTS

Environmental factors effect on orange trees behaviour. Number of th components choice and their degree of explanation of the variables

Results of the cross-validation allow to retain the components 1 and 2 to explain Nles, Nlesm and Pom variables. Indeed, $Q^2 2 \geq 0.0975$ (Table 2). All disease variables can be explained by the model because at the level of the component 2, all the Q^2 are ≥ 0.0975. The first component (t1) explains 58% of x and 10% of y, while the 2nd component explains 11% of x and 73% of y (Table 3).

Table 2: Model quality index (Q^2) by component and variable explained on orange trees.

Espèce	Composante	Q^2			
		Nles	Nlesm	Pom	Total
Orange trees	1	-21.676	-21.621	-23.382	-22.226

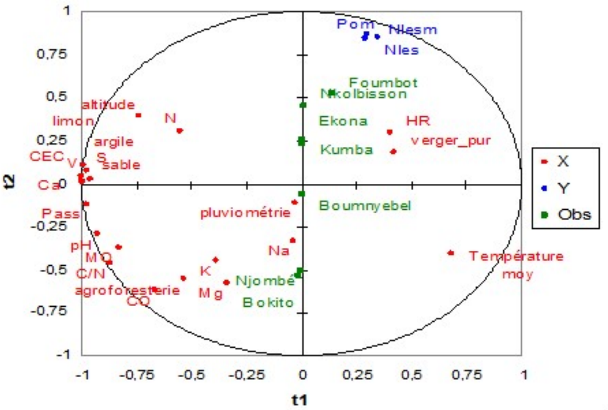
	2	0.297	0.281	0.384	0.321
	3	-0.192	-0.225	-0.281	-0.232
Grapefruit trees	1	-31.726	-31.075	-35.983	-32.928
	2	0.309	0.240	0.484	0.344
	3	-0.091	-0.335	-0.052	-0.192
Satsumas tangerine trees	1	-18.297	-27.825	-21.750	-22.624
	2	0.011	0.218	0.046	0.094
	3	0.229	-0.189	0.294	0.183

Table 3: Explanatory power of the th components of the PLS regression model on orange trees. grapefruit trees and satsumas tangerine trees.

	Orange trees		**Grapefruit trees**	**Satsumas tangerine trees**			
	t1	**t2**	**t1**	**t2**	**t1**	**t2**	**t3**
Altitude	0.545	0.156	0.597	0.061	0.159	0.518	0.400
Relative humidity	0.162	0.089	0.184	0.157	0.148	0.046	0.005
Mean temperature	0.461	0.161	0.505	0.104	0.301	0.718	0.025
Rainfall	0.001	0.012	0.000	0.013	0.145	0.502	0.272
Clay%	0.991	0.012	0.991	0.007	0.998	0.017	0.001
Silt%	0.959	0.006	0.959	0.010	0.968	0.028	0.017
Sand%	0.986	0.000	0.986	0.003	0.964	0.001	0.044
Organic matter	0.687	0.141	0.687	0.177	0.748	0.054	0.117
CO	0.446	0.377	0.446	0.313	0.509	0.139	0.242
N	0.305	0.093	0.305	0.112	0.925	0.087	0.017
C/N	0.755	0.210	0.754	0.211	1.000	0.009	0.002
Pass	0.954	0.014	0.953	0.012	0.875	0.013	0.045
K	0.153	0.198	0.153	0.050	0.012	0.214	0.651
pH	0.856	0.085	0.855	0.078	0.847	0.009	0.073
Ca	0.998	0.000	0.998	0.000	0.366	0.128	0.320
Mg	0.116	0.334	0.116	0.135	0.027	0.254	0.570
Na	0.001	0.111	0.001	0.006	0.004	0.001	0.985
S	0.998	0.000	0.997	0.000	0.561	0.072	0.224
CEC	0.922	0.001	0.921	0.007	0.980	0.009	0.011
V	1.000	0.002	1.000	0.000	0.992	0.001	0.000
Agroforest plots	0.284	0.307	0.566	0.222	0.029	0.268	0.242
Orchard	0.179	0.034	0.201	0.212	0.017	0.384	0.481
R^2x	0.580	0.106	0.599	0.086	0.526	0.158	0.216
Variable y	t1	t2	t1	t2	t1	t2	t3
Nles	0.122	0.731	0.115	0.791	0.131	0.639	0.075
Nlesm	0.085	0.716	0.108	0.767	0.045	0.881	0.000
Pom	0.089	0.754	0.101	0.865	0.096	0.733	0.053
R^2y	0.099	0.733	0.108	0.808	0.091	0.751	0.043

When we look at the correlations between variables and their th components, we note that altitude and almost all soil variables have strong negative correlations with the t1 component. t2 component has strong positive correlations with disease variables (Figure 2). These variables are best explained by the two components. It is noted that rainfall and sodium (Na) are poorly represented in the center of the correlation circle. These variables are very weakly correlated to both components. They are also very weakly disease explanatory because they have low R^2 on both components (Table 2). The saturation rate (V) is the best explained variable on t1 component. When the observation sites are projected in the plan, we find that Foumbot, Nkolbisson, Ekona and Kumba sites are closer to disease variables. These are sites that have the most severe disease attacks. On the other hand, the sites of Bokito and Njombé are the furthest away from the variables of the disease. They have low severities. The site of Jakiri is placed outside the plane, it is an extreme site which is located at higher altitude, but with low severity of the disease. There are also strong positive correlations between soil variables, agroforestry system and disease variables.

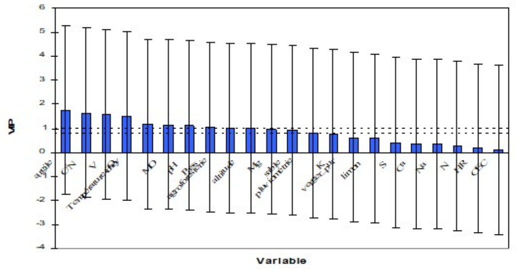
Figure 2: Position of sites in the correlation circle of climate. soil and disease variables with t1 and t2 components on orange trees.



Importance of variables in the model

Only 13 variables out of 22 enter the model construction with a VIP> 0.8 (Figure 3). On the graph, they go in order of importance of the clay rate to the rainfall. It is noted that the texture of the soil, its composition in organic matter, its pH are important, so average temperature, altitude as well as agroforestry system are among the most important factors.

Figure 3: Representation of the most important factors (VIP >0.8) that build the model of the influence of environmental factors on the development of PLFSD on orange trees (95% confidence interval).



Parameters of the regression model

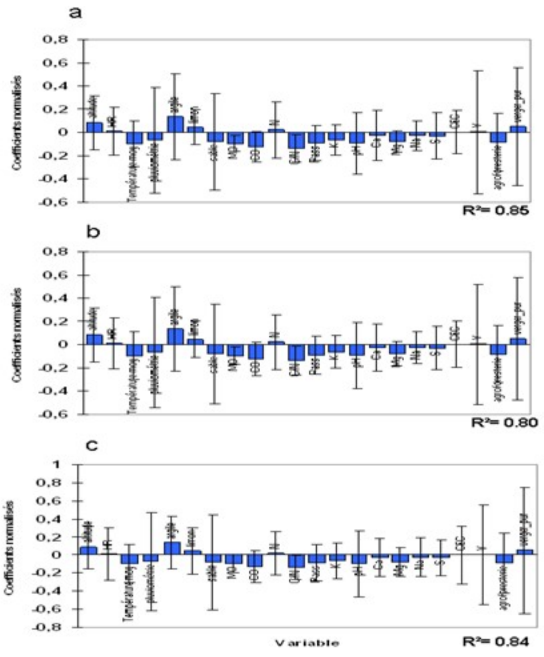
For each of disease variables, constants and regression coefficients are shown in Table 4. It is noted that these coefficients are mostly negative and therefore of the same sign as correlations. Some variables, which are not very important in the model, however, have high correlation coefficients (Na, N, K). On standardized data, VIPs have the highest regression coefficients (Figure 4). Normalized regression coefficients are small (<0.2). The trends are the same for three disease variables. Regression coefficients of the variables C/N, clay, organic carbon (CO), organic matter (OM), pH, temperature, assimilable phosphorus (Pass) and agroforestry are the highest.

Table 4: Regression coefficients of environmental variables for each parameter of PLFSD on orange trees.

Variable	Coefficients de régression								
	Oranger			Grapefruit trees			Satsumas		
	Nles	Nlesm	Pom	Nles	Nlesm	Pom	Nles	Nlesm	Pom
Constant	8.099	12.647	86.259	5.596	7.954	60.246	-0.392	2.263	-2.870
Altitude	0.000	0.001	0.004	0.000	0.001	0.003	0.000	0.000	0.002
Relative humidity	0.008	0.012	0.081	0.040	0.056	0.383	0.020	0.026	0.520
Mean temperature	-0.100	-0.151	-1.006	-0.097	-0.135	-0.934	-0.046	-0.109	-1.290
Rainfall	0.000	-0.001	-0.004	0.000	0.000	-0.001	0.000	0.000	0.000
Clay %	0.034	0.052	0.346	0.049	0.069	0.473	0.002	0.014	0.085
Silt%	0.019	0.029	0.193	0.054	0.075	0.516	0.016	0.028	0.436
Sand %	-0.022	-0.034	-0.224	-0.043	-0.059	-0.409	-0.007	-0.016	-0.187
Organic matter	-0.083	-0.125	-0.832	-0.109	-0.151	-1.043	-0.021	-0.083	-0.671
CO	-0.478	-0.722	-4.806	-0.492	-0.686	-4.730	-0.038	-0.146	-1.209
N	0.227	0.343	2.283	0.642	0.895	6.170	-0.091	0.031	-1.915
C/N	-0.003	-0.005	-0.035	-0.004	-0.006	-0.040	0.000	-0.001	-0.005
Available soil P	-0.004	-0.007	-0.045	-0.006	-0.008	-0.055	-0.001	-0.001	-0.019
K	-0.185	-0.279	-1.858	-0.173	-0.241	-1.664	-0.014	-0.049	-0.423
pH	-0.310	-0.468	-3.116	-0.433	-0.604	-4.160	-0.038	-0.081	-1.046

Ca	-0.008	-0.012	-0.079	-0.009	-0.012	-0.086	-0.006	-0.014	-0.174
Mg	-0.515	-0.778	-5.183	-0.566	-0.789	-5.440	-0.052	-0.145	-1.514
Na	-15.028	-22.702	-151.168	-0.811	-1.130	-7.789	11.736	14.939	299.38
S	-0.008	-0.013	-0.083	-0.009	-0.012	-0.085	-0.004	-0.010	-0.122
CEC	0.000	0.001	0.004	0.020	0.027	0.189	0.006	0.009	0.146
V	0.000	0.000	-2.166	0.000	0.000	-0.001	-0.002	-0.005	-0.061
SAF	-0.065	-0.098	-0.650	-0.226	-0.314	-2.166	-0.054	-0.228	-1.794
Verger pur	0.057	0.087	0.576	0.130	0.181	1.250	0.074	0.148	2.026

Figure 4: Representation of the most important factors (VIP >0.8) that build the model of the influence of environmental factors on the development of PLFSD on orange trees (95% confidence interval).

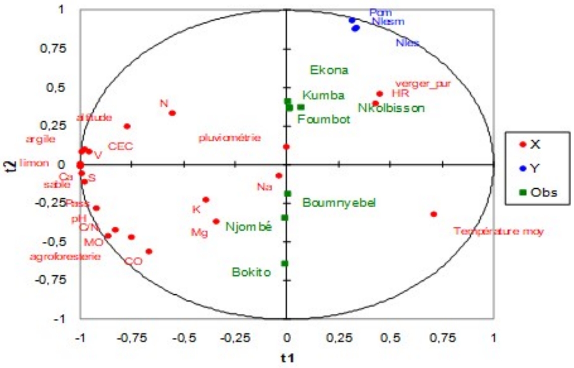


From constants and regression coefficients we can write the equation of the model for each of disease variables. The equation of the model summarizes the importance of the different variables opposite the PLFSD. Considering only VIPs, the disease incidence equation (POM) was obtained with the reduced centric variables. On orange trees, we have: Pom = 86,259 + 0,004 altitude - 1,006 average temperature - 0,004 rainfall + 0,346 clay rate - 0,224 sand content - 0,832 organic matter content - 4,806 organic carbon content - 0,035C / N - 0,045 phosphorus assimilable -3,116 pH - 5,183 magnesium content - 2,166 soil saturation rate - 0,650 percentage of trees in agroforestry system.

Environmental factors effect on grapefruit behaviour. Number of th components choice and their degree of explanation of the variables. Components 1 and 2 are used to explain the variables Nles, Nlesm and Pom. Indeed, the cross validation shows that $Q^2 2 \geq 0.0975$ (Table 2). All disease variables can be explained by the model, because at the level of the component 2, all the Q^2 are ≥ 0.0975. The first component (t1) explains 60% of x and 11% of y, while the 2nd component explains 9% of x and 81% of y (Table 3).

Rainfall and sodium levels are placed in the center of the correlation circle (Figure 5). They are very weakly correlated with t1 and t2, their R^2 are also very weak (Table 3). These variables are therefore weakly disease explanatory. Saturation rate (V) is the best explained variable on the t1 component. There are strong and negative correlations between the t1 component and several soil variables. Correlations are strong and positive between the t2 component and variables disease.

Figure 5: Position of sites in the correlation circle of soil climate and disease variables with components t1 and t2 on grapefruit trees.



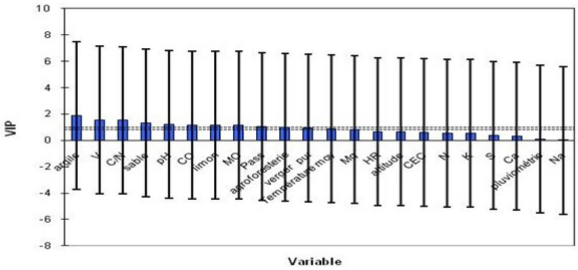
Projection of the observation sites on the plane formed by the two components makes it possible to note that the sites of Foumbot, Nkolbisson, Ekona and Kumba are closer to the variables of the disease. These are the sites with the most severe attacks. On the other hand, the sites of Bokito and Njombé are the furthest away from the variables of the disease. The site of Jakiri is placed outside the plane. This site is extreme, it is located at very high altitude and has low severity of the disease.

Importance Variables in the model

Number of most important variables that go into the construction of the model (VIP> 0.8) is 13 (Figure 6). On the graph, they go in order of importance of the rate of clay at magnesium (Mg). It is noted that the texture of the soil, its organic matter composition, its pH as well as the culture system are important. Among the climatic factors, only the average temperature is found in VIPs. Altitude is also not among the most important factors

Figure 6: Representation of the most important factors (VIP >0.8) that build the model of the influence of environmental

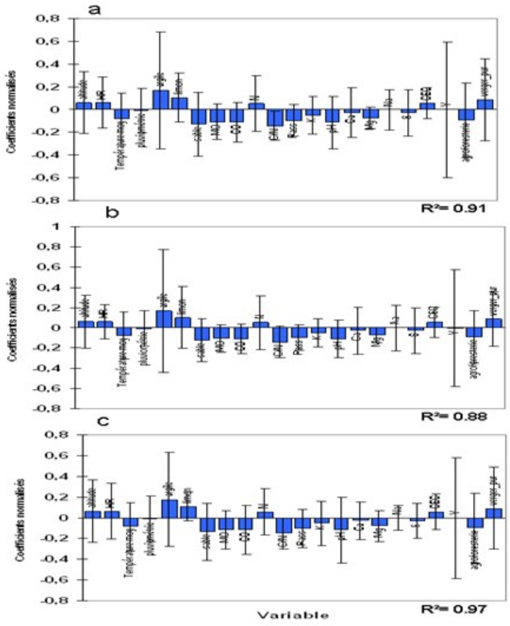
factors on the development of PLFSD on grapefruit trees (95% confidence interval).



Regression model Parameters of the

On grapefruits, for each of the disease variables, the constants and regression coefficients in centric-reduced data are shown in Table 4. It can be seen that constants are lower than on orange trees. Regressions coefficients are the same signs as on orange trees. Na and N variables, which are not VIPs, have the highest center-reduced regression coefficients. On standardized data, VIPs have the highest regression coefficients (Figure 7). Normalized regression coefficients are small (<0.2). They range from 0.001 for sodium (Na) to 0.16 for clay. The trends are the same for the three disease variables. Regression coefficients of the variables clay, sand, pH, organic carbon (OC), organic matter (OM), pH, and silt are the highest. These variables are also the first most important in the model. The saturation rate (V) is among the VIPs but has a very low regression coefficient (0.002-0.003).

Figure 7: Normalized regression coefficients of explanatory variables (altitude. climate. soil) for variable Nles (a). Nlesm (b) and Pom (c) of PLFSD on grapefruit trees (95% confidence interval).

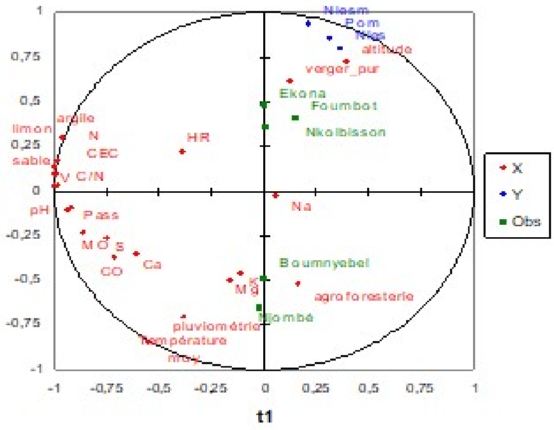


Considering only VIPs, incidence disease equation (Pom) was obtained with the reduced centric variables. On grapefruits, we have: Pom = 60.246 - 0.934 average temperature + 0.085 clay content - 0.409 sand content +0.516 silt rate -1.043 organic matter content - 4.730 organic carbon content -0.040C / N - 0.055 available phosphorus - 4.160 pH - 0.001 soil saturation rate - 2.166 percentage of trees in agroforestry systems + 1.250 percentage of trees in pure orchards

Environmental factors effect of on satsumas behaviour. Choice of the number of th components and their degree of explanation of the variables. Results of the cross-validation allow to retain the components 1, 2 and 3 to explain Nles, Nlesm and Pom variables. Indeed, $Q^2 3$ and $Q^2 4$ are ≥ 0.0975 (Table 2). All disease variables can be explained by the model, because at the level of the component 3, Q^2Nles = 0.229 and Q^2pom = 0.294; at component 2 Q^2Nlesm = 0.218. The first component (t1) explains 53% of the x and 9% of the y, while the 2nd component explains 16% of the x and 75% of the y. Third component explains 22% of x and 4% of y (Table 3).

For correlations, we'll observe only components t1 and t2 which explain about 80% x and y variables. Na level is placed at the center of the correlation circle (Figure 8). This variable is very weakly correlated with t1 and t2. Its R^2 is very weak on t1 and t2, but very high on t3 (Table 4). C/N ratio is the best explained variable on t1 component and this correlation is negative. Altitude and disease variables have strong positive correlations with t2 component.

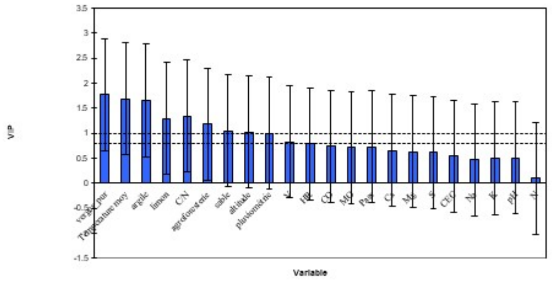
Figure 8: Position of sites in the correlation circle of climate. soil and disease variables with t1 and t2 components on satsumas tangerine trees.



Observation sites jrojection on plane formed by the two components makes it possible to note that Foumbot, Nkolbisson and Ekona sites are closer to disease variables. These sites are those with the most severe attacks. On the other hand, sites of Boumnyebel and Njombé are the furthest away from disease variables. Kumba and Jakiri sites are placed outside the plane.

Importance of variables in the model. The number of most important variables that go into the model construction (VIP> 0.8) is 11 (Figure 9). On the graph, they are in order of importance of pure orchard system at relative humidity (RH). It is noted that soil texture, cropping system, climate and altitude are important.

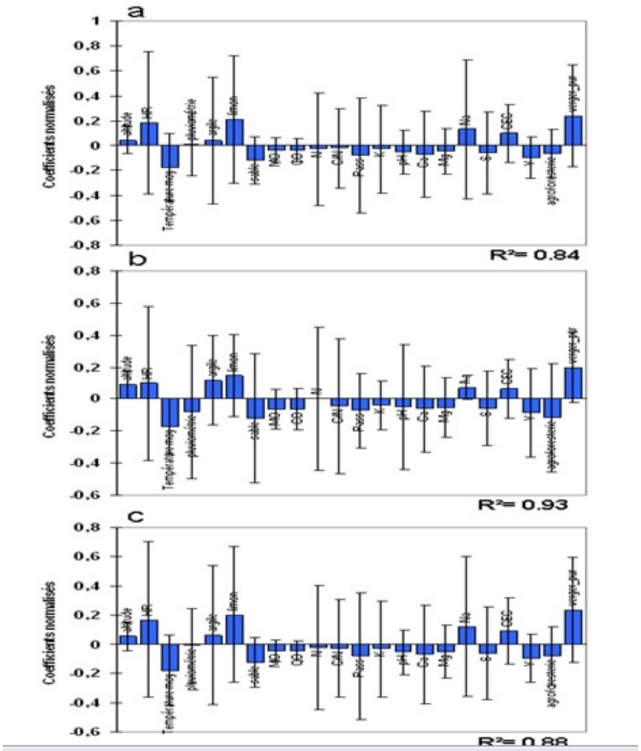
Figure 9: Representation of the most important factors (VIP >0.8) that build the model of the influence of environmental factors on the development of PLFSD on tangerine trees Satsuma (95% confidence interval).



Parameters of the regression model

On Satsumas, for each of the disease variables, constants and regression coefficients in centered-reduced data are shown in Table 4. It is found that the constants are lower than on orange and grapefruits. Regression coefficients are of the same signs as for the 2 previous varieties. Non-VIP variable Na has the highest center-reduced regression coefficients. On standardized data, VIPs have the highest regression coefficients (Figure 10). generally, normalized regression coefficients are small (<0.2). However, some variables such as silt ratio and pure orchard system are distinguished from others with regression coefficients ≥ 0.2. The coefficients vary from 0.004 for nitrogen (N) to 0.24 for the pure orchard system. Trends are the same for the three disease variables. VIPs (relative humidity, average temperature, pure orchard system) have the highest regression coefficients.

Figure 10: Normalized regression coefficients of explanatory variables (altitude. climate. soil) for PLFSD variable Nles (a). Nlesm (b) and Pom (c) on satsumas (95% confidence interval).



Considering only VIPs, the disease incidence equation (Pom) was obtained with the reduced centric variables. On satsumas, we have: Pom = - 2,870 + 0,002 altitude - 1,290 mean temperature - 0,0003 rainfall + 0,520 Relative Humidity + 0,473 clay rate - 0,187 sand rate + 0,436 silt rate - 0,005 C / N - 0,061 rate Soil saturation - 1.794 percentage of trees in agroforestry systems + 2.026 percentage of trees in pure orchards

DISCUSSION

Soil factors

The variables related to soil texture (clay rate, sand content and silt rate) are very important in disease development on orange, grapefruits and satsumas. The clay and silt rates are positively correlated with disease variables, while the sand ratio is negatively correlated. The higher the rate of clay and/or silt, the higher the disease incidence. On the other hand, the higher the rate of sand, the less the trees are attacked. Silt regression coefficients are larger than those of the sand rate and the clay rate for the satsumas model. On the other hand, those of clay and sand are more important on grapefruits. On orange trees, silt is not an important variable in the disease equation. These results seem to be in contradiction with those obtained by Ndo et al. (2010), which show that trees located in sandy soil are more attacked by the disease. It should be remembered however that, during this study, characteristics soil were described only after observation. Nevertheless here, soil analyzes were carried out in laboratory, which makes it possible to think that these results are more reliable.

Soil organic matter also plays a very important role in the disease level. On orange trees, organic matter, organic carbon and C/N ratio are classified among the most important factors of the model, on grapefruits it is only organic carbon. On satsumas, organic carbon and the C/N ratio are important. These 3 variables have negative regression coefficients, they are negatively correlated to the disease. Trees on soils rich in organic matter and organic carbon are less attacked by the PLFSD.

Soil pH is important in the disease regression equation. Its regression coefficient is negative and is negatively correlated with disease variables. more acidic soils favor the development of the disease on orange tree and on grapefruits. On Satsuma, this variable is not important. Studies have shown the importance of the role soil pH plays in the development of other plant diseases. On Coffea arabica, the incidence of orange rust, which attacks the leaves, also increases with a decrease in pH (Lamouroux et al., 1995; Avelino, 1999).

Other soil variables such as magnesium content in orange trees, available phosphorus in orange and grapefruits, and saturation levels for all varieties are important variables. All these variables are negatively correlated to the disease with negative regression coefficients. The more soil is rich in phosphorus and exchangeable bases, the less the trees that are there are sick.

These conditions which favor a decrease in the disease intensity are similar to the optimal conditions necessary for citrus growth. Indeed, citrus fruits grow best on well-drained soils (more sandy and less clayey). A pH varying between 5.5 and 7 (optimum 6-7) is necessary for the good development of citrus (Walali Loudyi et al., 2003). They need inputs of nitrogen, phosphorus, potassium and also micro nutrients such as magnesium, copper, zinc. The hypothesis is that optimal soil conditions for tree development could make them less sensitive or less vulnerable to PLFSD. This hypothesis is contrary to that emitted in the framework of the pathosystem Hemileia vastatrix-Coffea arabica where the media that favor the development of the coffee plant are favorable to the development of orange rust (Lamouroux et al., Top cit., Avelino et al., 2004).

In all regression equations, nitrogen and potassium levels did not appear as important variables. These elements are however with phosphorus the most important for the development of plants and often represent the basic inputs in any soil fertilization. Regression and correlation coefficients are generally negative for potassium and positive for nitrogen. Thus, although not very important for disease development, it can still be said that the higher the nitrogen level of a soil, the higher the intensity of the disease. As for potassium, it's the opposite. It is noted that of all the elements of the soil, only the nitrogen level is positively correlated with the variables of the disease.

Climatic factors and altitude

The importance of mean temperature is emphasized in all regression equations on all varieties. This variable is still one of the first VIPs in the different models and is the most important climatic variable. It has a high and negative regression coefficient. The correlation is negative with all the disease variables. The lower the temperature, the more trees are attacked. This variable is more important on orange and satsumas than on grapefruits. On grapefruits, it is the only important climatic variable.

Rainfall is one of the important variables in the development of the disease on orange and satumas, but not on grapefruits. The regression coefficient for this variable is negative. Regression coefficient of humidity is positive. Relative humidity is one of the most important variables only on satsumas.

The altitude is ranked among the VIP on orange and on satsumas. Its regression coefficient is positive. The same is true of its coefficient of correlation with the disease variables. The higher the altitude, the higher the disease incidence on trees. The different regression models show a greater importance of temperature with respect to altitude and other climate variables.

In most previous studies on citrus PLFSD, altitude appears to be one of the main factors influencing the development of the disease (Seif & Hillocks 1993, Diallo 2001, Kuate 2002, Ndo et al., 2010). Temperature is often mentioned as a less important factor or correlated with altitude. PLS regression allowed a better estimate of the temperature in the disease equation. Indeed, a simple elevation in altitude can’t have an influence on the disease if it’s not accompanied by a drop in temperature. On the other hand, a drop in temperature even without altitude elevation may favor an increase in the incidence of the disease.Ekona site verifies this hypothesis. In fact, the average altitude in Ekona is 442.1 m, the average temperature is 23.7°C. disease incidence on orange, grapefruits and satsumas is not significantly different from Foumbot (mean temperature 23 ° C, altitude 1010.5 m). The difference between the average temperature of Foumbot and that of Ekona is not very great, but the difference between the altitudes is considerable.

The importance of rainfall on the disease development is apparent in a number of studies conducted on this disease. Increased rainfall has been shown to increase the disease incidence (Kuate 1998, Diallo 2003). Flowering delayed by watering in dry season has also significantly reduced diseases attacks (Kuate & Fourré, 1988). The importance of this variable on grapefruits was not detected. By cons, on orange and satsumas, it is very important in the model. However, regression equations show that disease incidence decreases with an increase in rainfall. This result seems to be contrary to those obtained in previous studies. It reveals, however, the dual role that rainfall can play. Indeed, studies have shown that for spores that are scattered at short distances, the rain, at the same time as it promotes the release of spores and their deposition on sensitive plants, can also when it is violent or prolonged to cause the leaching of spores already deposited and thus be unfavorable to the progression of the disease (Sache, 2000, Tucker et al., 2001). In the case of PLFSD, this aspect should be better studied in the laboratory and by field experiments.

Production system

The two production systems (agroforestry and pure orchard) were ranked among the important variables on the different models. On orange trees, only the agroforestry system is retained as VIP. On grapefruits and satsumas, both variables are important. The correlation coefficients are negative for the agroforestry system and positive for the pure orchard (Ndo et al., 2010). This suggests, all other conditions being equal, that the intensity of the disease is lower on trees in agroforestry systems than on trees in pure orchards. The assumption here is that trees and other associated crops can act as a windbreak. The inoculum being dispersed much more by wind (Sif 1996, Seif & Hillocks 1998, Kuate 1998), its evolution becomes more difficult in mixed crops system than in a system where all species are hosts of the same pathogen. Moreover, because of its biodiversity and its structural characteristics such as shading and spatial structure, the spread of diseases is reduced in agroforestry systems (Akoutou et al., 2017).

Agroforestry system seems to be less favorable for disease development. In this system citrus fruits are in association with other plants. These plants form pathosystems with other pathogens that need to be considered to optimize the production of the system. In this context, it is necessary to highlight the host range of pathogens that attack these plants. In the PLFSD case, no other hosts are known apart from citrus. In setting up these systems, the spatial arrangement of citrus fruits should aim at the isolation of susceptible varieties. On the other hand, we know in this type of system the existence of pests with a wide range of hosts. Mirids, for example, attack cocoa trees and kolatiers (Babin, 2009). Fruit flies attack citrus fruits and other fruit trees (mango trees, guavas, etc.) (Ndzana et al., 2008). It therefore seems important to develop a reasoned agroforestry system that can allow production optimization and integrated pests management.

CONCLUSION

Chemical control remains the only known effective control method against citrus PLFSD. However, the high number of treatments recommended, the cost and availability of chemicals are a barrier to their use by small citrus growers. In addition, this method of control does not allow to respect the standards of ecologically clean products. The search for alternative solutions to this means of struggle, or at least that would contribute to its reduction, is therefore indispensable. This study was done to contribute to the management of the PLFSD epidemiological risk on citrus varieties by constructing models for predicting the incidence of the disease using regression analyzes. With regard to soil, results show that the higher the rate of clay and/or silt, the greater the disease incidence. On the other hand, the higher the rate of sand, the less the trees are attacked. Trees on soils rich in organic matter and organic carbon are less attacked, and more acidic soils favor the development of the disease. More soil is rich in phosphorus and exchangeable bases, the less the trees that are located there are sick. For geo-climatic factors, the importance of mean temperature is emphasized in all regression equations and on all varieties. This variable is still one of the first VIPs in the different models and is the most important climatic variable. The correlation is negative with all the variables of the disease and the lower the temperature, the more the trees are attacked. The higher the altitude, the higher the incidence of disease on trees. Agroforestry and pure orchard production systems were ranked among the important variables on the different models. Thus, all conditions being equal, the intensity of the disease is lower on trees in agroforestry systems than on trees in pure orchards. These results make it possible to make some recommendations to producers: (i) the use of low-sensitive varieties in high altitude and low temperature zones (Tangerine Beauty, (ii) the planting of citrus under shade, (iii) the citrus plantation in agroforestry plots in which citrus fruits alternate with trees of different families.This work should be continued in order to arrive at the definition of a control strategy that will take into account the risk factors and will minimize the use of fungicides.

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