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Altitude, tree species and soil type are the main factors influencing the severity of *Phaeoramularia* leaf and fruit spot disease of citrus in the humid zones of Cameroon

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Abstract In Cameroon and most countries of tropical Africa where *Phaeoramularia* leaf and fruit spot disease of citrus is reported, a total yield loss of 50–100% is common. For effective control of this disease, it is imperative to understand how it originates, and which factors contribute to its development. To this end, the environmental and biological characteristics of each tree from 39 sites located in 13 citrus production basins in the humid zones of Cameroon were collected by means of a survey. Information was collected by interviewing heads of households, and by visual inspection of trees and their environment. The independent variable was severity of the disease while the dependent variables were environmental and biological characteristics. Climatic characteristics varied from one basin to another. The 13 citrus production basins were regrouped in five categories based on disease severity. Altitude, tree species and soil type were the main factors influencing the disease severity. Thus the higher is the altitude, the more important is the disease severity. Also, disease severity increased with increasing number of grape fruit, orange and pummelo trees. However, disease severity was lower on trees growing on volcanic soils as compared with the other soil types. Further analysis of these factors could lead to the development of a risk assessment model for *Phaeoramularia* leaf and fruit spot of citrus.

Keywords Citrus · Epidemiology · *Phaeoramularia angolensis* · Risk factors

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Introduction

In Cameroon, citrus plays an important role as a source of income for small-scale farmers, a nutritional requirement for the population and as forest regeneration trees (Kuate et al. 2006). Citrus is grown in areas generally referred to as "citrus production basins" with characteristics that favour their growth. These production basins are heterogeneous in terms of the citrus population, and biotic and abiotic characteristics of the environment. In many of them, citrus productivity is hindered by *Phaeoramularia* leaf and fruit spot disease (PLFSD). This disease caused by the fungus, *Phaeoramularia angolensis* (Carvalho & Mendes) Kirk, is a major limiting factor to citrus production in some 20 African countries and in the Yemen (Kirk 1986; Kuate 1998). In areas where the disease is reported, a total yield loss of 50–100% is common on susceptible varieties (Kuate et al. 1994). Citrus production is in quarantine in these countries. Because of traditional trade of plant material and the wind-borne dispersal of the fungal spores, this disease constitutes a potential threat (Seif and Hillocks 1993) for Brazil, Mediterranean countries, China, United States of America and other countries which furnish more than 70% of the citrus production.

PLFSD seriously affects leaves and fruits of most citrus species and their relatives, causing several spots and lesions of varying sizes (Brun 1972; Kuate et al. 1994). The susceptibility to *P. angolensis* varies within citrus species and cultivars. Among citrus species grown in Cameroon, grape fruits (*Citrus paradisi* Macf.), Tangerines (*C. reticulata* Blanco) and oranges (*C. sinensis* (L.) Osbeck) are very susceptible while lemons (*C. limon* (L.) Burm. F.) and Pummelo (*C. grandis* (L.) Osbeck), are less susceptible (Bella-Manga et al. 1999). Studies conducted in Cameroon to assess the incidence of the disease at different seasons in the humid forest zone and in the highlands (Kuate and Fouré 1988; Kuate et al. 1994, 1997) revealed that rainy seasons are conducive for disease development. Other studies have shown that leaf and fruit spot disease incidence increases with altitude and with cooler and more humid climatic conditions (Kuate et al. 2002). Furthermore, a series of fungicides have been screened and recommended for the control of PLFSD (Seif and Hillocks 1999; Diallo 2003; Kuate 2003; Yesuf 2007). However, the 'stereotyped' application rate makes treatment very expensive for farmers in developing countries and also raises environmental concerns that may result due to pollution. Taking into consideration the above constraints, it has become necessary to implement an integrated management approach to sustainably control this disease. Such an approach should take into account the fact that in these areas, citrus is grown in association with other perennial or annual crops. As a prelude, a better understanding of the conditions that favour the development of PLFSD in such a setting is imperative. This study was undertaken to determine the relationship between climatic (temperature, relative humidity, rainfall), environmental (altitude, soil type, cropping practices) and biological (variety, plant age) factors and PLFSD severity.

Materials and methods

Biophysical characterization of the sites

The study was conducted in three agro-ecological zones stretching from the northern limit of Cameroon's forest zone to the border with Equatorial Guinea and Gabon (Ambassa-Kiki 2000) (Fig. 1). The Western Highlands Zone (latitude 4°54′ to 6°36′ N, longitude 9°18′ to 11°24′ E, altitude: 1,240–1,800 m asl) encompasses the North–West and West regions. The only production basin of citrus here, Jakiri, is found in the North–West region. The zone enjoys a humid tropical climate ("Mountainous" type) with low mean temperatures (19°C) and abundant rains (1,500–2,500 mm) that occur in a monomodal configuration. The soils are loamy to clayey in texture.

The Monomodal Humid Forest Zone (latitude 2°6′ to 6°12′ N, longitude 8°48′ to 10°30′ E, altitude: 200–800 m asl, excluding Mount Cameroon at 4,095 m) includes the South–West region with four productions basins (Kumba, Muyuka, Ekona and Idenau), and the Littoral region with three production basins (Pouma, Njombé and Edéa). It enjoys a variant of the equatorial climate which is very humid and hot. The mean annual temperature ranges from 22°C to 29°C, and the relative humidity is as high as 85–90%. Rains are heavy (average: 2,500–4,000 mm/year, excluding Debundscha with 11,000 mm) with a monomodal

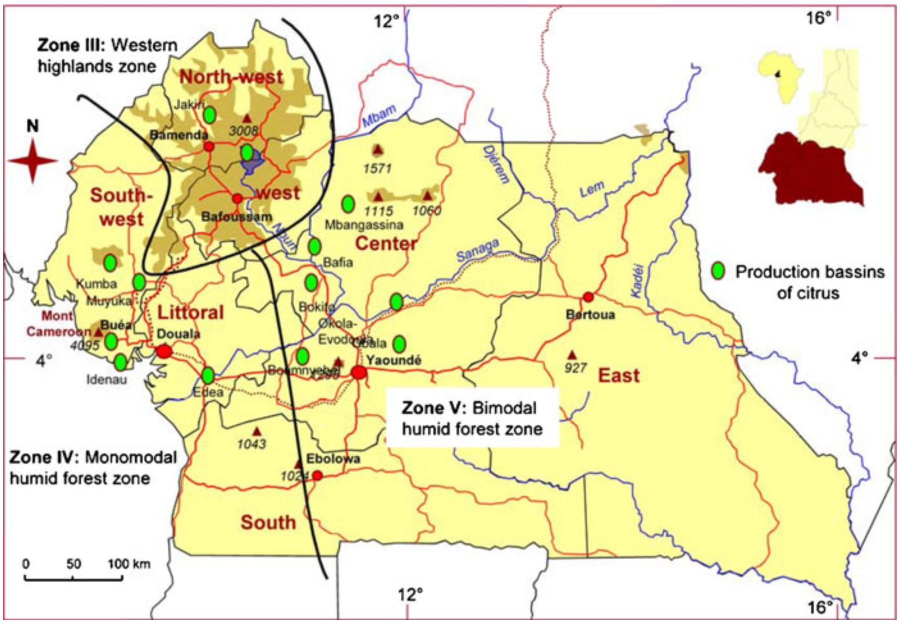

Fig. 1 Citrus production basins in three contrasting humid agro ecological zones of Cameroon

pattern. The soils are sandy to sandy clay or clayey in texture.

The Bimodal Humid Forest Zone (latitude 2°6′ to 4°54′/5°48′ N, longitude 10°30′ to 16°12′ E, altitude: 500–1,000 m asl) comprises the Centre, East and South regions. But only the Centre region was involved with the study with five production basins i.e.: Boumnyebel, Mbangassina, Bokito, Obala and Okola. This zone has a sub-equatorial climate, hot and humid. The mean temperature is 25°C and the relative humidity averages 75%. The average annual rainfall ranges from 1,300 mm and 2,500 mm and is of bimodal pattern. The soils are sandy loam, sandy clay loam or clay in texture.

Field study

In this study carried out in 2005 and 2006, 13 production basins (cf. preceding paragraph) were selected based on differences observed in their biophysical characteristics, the composition of the citrus population, or the cropping practices in use, after cluster analysis of these preliminary data. However, due to the difficult access to some parts of the Western Highlands Zone, only one production basin could be selected while 7 and 5 were chosen in the Monomodal and Bimodal Humid Forest Zones, respectively. Within each production basin, three sites were selected and citrus farms that represented the diversity of the site were sampled.

A survey was conducted off-season (June–July) in 2005 and 2006. In 2005, 38 sites were sampled and observations made on 1,512 trees belonging to 126 households. In 2006, the same sites were sampled again, plus an additional site from Bokito production basin, and observations made on 1,904 trees belonging to 206 households. The parameters evaluated whenever possible were climatic (temperature, relative humidity, rainfall), environmental (altitude, soil type, farming practices) and biological (variety, plant age, etc.) factors. As regards the climate, weather data covering 20 years were collected from the Department of National Meteorology in Douala, Cameroon. In general, sites belonging to the same production basin or basins located in the same agro-ecological zone shared the same climatic data because of the prevailing situation of the meteorological network. The other kinds of data were obtained during interviews with the heads of households and by visual inspection of the trees and their environment. This concerns mainly the surroundings of the citrus farm (forest, savannah), altitude (with six altitude classes defined based on their frequency during the survey: 1–200 m, 201–400 m, 401–600 m, 601–800 m, 1,201–1,400 m, and 1,601–1,800 m), tree localization (tree with companion crops such as food crops, coffee or cocoa

Table 1 List of variable describing citrus trees and their environment

Parameter	Variable	Modality
Climate	Annual rainfall	mm
	Relative humidity	%
	Temperature	°C
Location	Altitude	10–1,684 m asl
	Vegetation	1: Forest
		2: Savannah
		3: Galery forest
	Soil texture or stoniness	1: Clay
		2: Gravelly soil
		3: Sand
		4: Clayish
		5: Sandy clay
		6: Clayish sand
		7: Dark volcanic soil
		8: Brown volcanic soil
		9: Brown clay
	Farming system (FS)	1: Tree-dominated home garden in front of the house
		2: Tree-dominated home garden behind the house
		3: Citrus orchard
		4: Associated fruits orchard
		5: Citrus intercropped in cocoa farm
		6: Citrus intercropped in coffee farm
		7: Citrus intercropped with food crop
		8: Citrus in a fallow
		9: Other situations
	Trees situation	1: Under shade
		2: Semi shade situation
		3: Under direct sunlight
	Nature of water source around the tree	1: Stream
		2: River
		3: Swamp
		4: Ocean
Tree characteristic	Species	1: Orange tree
		2: Tangerine
		3: Grape fruit
		4: Pummelo
		5: Lemon
	Variety	29 varieties
	Age	1: < 10 years
		2 :10–25 years
		3: >25 years
	Type	1: Sow plant
		2: Graft
		3: Sucker

Table 1 (continued)

Parameter	Variable	Modality
Management pattern	Chemical application	1: Fungicide
		2: Insecticide
		3: No chemical application
		4: Fungicide and insecticide mixture
Phenological state	Presence or absence of leaves /fruits	1: Presence
		2: Absence
	Stages of leaves/fruits	1: Young leaves/fruits;
		2: Matured leaves/fruits
Severity	Severity on leaves or fruits (SPLSD/SPFSD)	0: Absence of disease symptoms
		1: Percent of surface of organ with disease symptoms <25%
		2: 25% > Percent of surface of organ disease symptoms <50%
		3: Percent of surface of organ disease symptoms >50%

trees, in orchards, under a canopy or exposed to direct sunlight, etc.), soil texture (cf. above), data on crop management practices (generally supply by the farmers), species, variety (when it was possible) and age of trees, tree type (grafted, seed derived…), insecticide or fungicide spraying, presence of a source of water (could be noticed by the observer), location of trees (under a canopy or in full sunlight). The phenological state of trees was also noticed (presence of leaves and fruits and their stages). Epidemiological data were collected by noting the severity of disease on a scale varying from 0 to 3 where 0 represented no disease symptoms on leaves or fruits, and 3 represents more than 50% of the surface of the organs considered (fruits or leaves) covered by the disease symptoms. The overall parameters and variables evaluated are summarized in Table 1.

Statistical analysis

An analysis of variance was performed to compare the different production basins, and within each basin to compare the different sites where epidemiological data (severity) were collected. Treatment means were compared using the Student-Newman-Keuls test at the 5% level of probability. The analysis was done with the GLM (General Linear Model) procedure of the SAS (Statistical Analysis System) software, version 8.0.

Based on PLFSD severity, the 13 citrus production basins were regrouped in categories by average distance between clusters. Agronomic, physiological, and environmental data collected from the survey were evaluated by stepwise logistic regression analysis using the SAS (Statistical Analysis System) software, version 8.0.

To segment the database and predict risk factors for the development of PLFSD, the Classification and Regression Trees (CART) analysis method was used (Breiman et al. 1984). As the response is a quantitative variable, it is a non parametric regression method which allowed explaining a dependent variable Y as a function of several quantitative or qualitative predictors (Nakache et al. 1996). The results obtained with these methods are presented in trees (Guyot et al. 2010). It consists of dividing a set of observations into two groups, obtained by grouping the modalities of an explanatory variable into two modalities, such that the two resulting groups are the most different with respect to the variable to be explained (in this case disease severity on leaves or fruits). The most explanatory variable is the one that separates the initial population into two sub-populations, which themselves can be subsequently divided according to the same principle by the same variable or other explanatory variables (Avelino et al. 2004). The segmentation therefore orders variables according to their discriminatory power. The quality of each division is accessed by applying the Student t test on the means obtained. Each segment of the tree which represents a sub-population is a node of the tree. In each node are the mean, the standard

Table 2 *Phaeoramularia* leaf and fruit spot disease (PLFSD) severity on leaves and fruits between 2005 and 2006 in different sites of citrus production basins in humid zones of Cameroon

Citrus production basin	Site (n 2005–n 2006)	Mean PLFSD±Sd severity on leaves		Mean PLFSD±Sd severity on fruits		Mean altitude
		2005	2006	2005	2006	–
Boumnyebel	Makaï (43–23)	0.1±0.3[a]	0.0±0.2[a]	0.9±0.9[a]	0.5±0.9[a]	376
	Minse, Maloung (16-34)	0.0±0.0[a]	0.0±0.2[a]	1.2±1.1[a]	0.3±0.6[a]	376
	NkonMadog (16-46)	0.1±0.4[a]	0.2±0.4[a]	0.8±0.9[a]	0.8±1.0[a]	376
Mbangassina	Mbangassina (34–50)	0.0±0.0[a]	0.1±0.2[a]	0.1±0.4[a]	0.3±0.4[a]	450
	Goura (28–40)	0.0±0.0[a]	0.2±0.4[a]	0.3±0.5[a]	0.3±0.5[a]	440
	Etam Nyat (43–56)	0.1±0.3[a]	0.1±0.4[a]	0.4±0.6[a]	0.4±0.6[a]	480
Bokito	Beni (75–13)	0.0±0.0[a]	0.5±0.5[a]	0.5±0.7[a]	0.2±0.4[b]	407
	Ndomdjengué (92–116)	0.0±0.2[a]	0.1±0.3[b]	0.8±0.8[a]	0.6±0.6[a]	450
	Ombessa (0–57)	–	0.1±0.3[b]	–	0.3±0.5[b]	571
Obala	Nkolmelen (55–52)	0.0±0.0[a]	0.2±0.4[a]	0.7±0.5[a]	0.4±0.6[b]	559
	Nkoltomo, Efok (63–51)	0.0±0.2[a]	0.1±0.3[a]	0.4±0.7[b]	0.3±0.5[b]	538
	Batschenga (62–56)	0.0±0.2[a]	0.1±0.3[a]	0.5±0.7[ab]	0.7±0.6[a]	516
Okola	Nkol akok (38–21)	0.0±0.0[a]	0.0±0.0[a]	0.3±0.6[b]	0.1±0.2[a]	573
	Nguibassal (70–59)	0.0±0.2[a]	0.1±0.3[a]	0.2±0.5[b]	0.2±0.4[a]	474
	Ekekam (63–61)	0.1±0.2[a]	0.2±0.4[a]	0.6±0.7[a]	0.2±0.5[a]	526
Pouma	Seppè (44–34)	0.0±0.0[a]	0.1±0.3[ab]	0.4±0.6[a]	0.2±0.4[a]	56
	Nkonga (39–34)	0.1±0.3[a]	0.2±0.4[a]	0.3±0.6[a]	0.3±0.6[a]	200
	Tjiedikoï (19–34)	0.0±0.0[a]	0.0±0.0[b]	0.5±0.5[a]	0.4±0.8[a]	205
Njombé	Njongo (115–259)	0.1±0.2[a]	0.2±0.4[a]	0.1±0.3[c]	0.1±0.4[a]	96
	Loum chantier (49–39)	0.0±0.1[a]	0.2±0.4[a]	0.4±0.6[b]	0.2±0.4[a]	200
	Nlohé (56–91)	0.1±0.2[a]	0.2±0.4[a]	0.7±0.7[a]	0.1±0.5[a]	289
Edéa	Edéa (16–17)	0.0±0.0[a]	0.0±0.0[b]	0.2±0.4[a]	0.0±0.0[a]	91
	Kopongo (38–34)	0.1±0.3[a]	0.2±0.4[a]	0.2±0.6[a]	0.1±0.2[a]	91
	Beon (21–53)	0.1±0.3[a]	0.1±0.3[ab]	0.1±0.3[a]	0.2±0.4[a]	91
Jakiri	Jakiri (36–105)	0.3±0.4[a]	0.2±0.4[ab]	1.0±0.7[b]	1.0±0.9[ab]	1,684
	Kifue (40–15)	0.3±0.5[a]	0.4±0.5[a]	1.6±1.0[a]	1.2±0.9[a]	1,684
	Waïnamah (37–45)	0.2±0.4[a]	0.2±0.4[b]	1.1±0.7[b]	0.7±0.7[b]	1,684
Kumba	Barombi–kang (36–50)	0.3±0.5[a]	0.2±0.4[a]	0.4±0.6[b]	0.6±0.9[b]	221
	Ekiliwindi (34–33)	0.0±0.9[b]	0.2±0.4[a]	0.2±0.4[b]	1.4±0.8[a]	450
	Mabonji–Tantcha (20–18)	0.0±0.0[b]	0.2±0.4[a]	0.8±0.9[a]	0.4±0.8[b]	xz
Muyuka	Bombé (23–50)	0.1±0.2[a]	0.2±0.4[a]	0.0±0.2[a]	0.1±0.3[a]	55
	Banga (38–18)	0.0±0.2[a]	0.0±0.0[b]	0.1±0.3[a]	0.2±0.4[a]	44
	Yoké–Malendé (27–34)	0.1±0.3[a]	0.0±0.0[b]	0.0±0.0[a]	0.1±0.2[a]	33
Ekona	Mautu (22–21)	0.0±0.0[b]	0.1±0.4[a]	0.3±0.7[b]	0.2±0.4[b]	200
	Ekona town (35–46)	0.4±0.5[a]	0.2±0.4[a]	0.5±0.8[b]	0.4±0.8[b]	380
	Buea(11–36)	0.1±0.3[b]	0.2±0.4[a]	1.1±0.8[a]	0.9±1.1[a]	1,400
Idenau	Idenau (25–24)	0.3±0.5[a]	0.4±0.5[a]	0.0±0.0[a]	0.0±0.0[a]	36
	Bakingili (15–34)	0.3±0.5[a]	0.2±0.4[ab]	0.1±0.3[a]	0.1±0.4[a]	10
	Limbé (9–44)	0.0±0.0[a]	0.1±0.3[b]	0.0±0.0[a]	0.0±0.2[a]	17

Phaeoramularia leaf and fruit spot disease severity was rated using a scale of 0 = no disease to 3 = >50% of organs area covered with lesions. Inside each production basin numbers fallowed by a different letter are significantly different according to the Student-Newman-Keuls test at $P < 0.05$. n2005: number of citrus trees sampled

Sd Standard deviation

deviation, and the number of individuals and the percentage of the total population concerned. This analysis was done using the SPSS Answer Tree version 3.1, 2002.

Results

PLFSD severity

The disease was present in all citrus production basins. However, the severity varied with basin and site (Table 2). The highest severities were found in Jakiri (western highlands) and the lowest in Idenau (low altitude humid forest with monomodal rainfall pattern).

In two out of 13 basins (Boumnyebel and Mbangassina) significant differences were not found between the sites (Table 2). In most of these basins, sites and altitude were also different. Based on PLFSD severity, the classification of citrus production basins in 2005 and 2006 yielded five groups (Fig. 2). The first group was composed of Boumnyebel, Kumba and Ekona. In this group nearly all the production basins are situated in the monomodal humid forest zone and altitudes varied between 200 m and 450 m. The second group comprised Bokito and Obala with altitude varying between 407 m and 571 m, situated in the bimodal humid forest zone. The third group composed of Mbangassina, Okola and Pouma, occurred for its majority in the bimodal humid forest zone. The fourth group cosisted of Njombé, Edea, Muyuka and Idenau, with very low altitude (10–289 m), and all the basins situated in the monomodal humid forest zone. At last, the fifth group consisted solely of Jakiri situated in the western highlands zone (altitude: 1,684 m asl).

Categorising variables associated with PLFSD

Altitude, tree species, soil textural class and vegetation were the main variables associated with the severity of PLFSD during the 2-year survey either on leaves or on fruits ($P<0.05$) (Table 3) and the ranking order did not vary with the year of observation. These three variables were always significantly associated with disease severity, contrary to vegetation in some cases.

The heterogeneity in the disease severity with principal factors can be seen on Figs. 3, 4, 5 and 6. Disease severity varied with altitude (Fig. 3): the higher the altitude, the more severe the disease was. The highest severity (with >25% of organ surface covered with lesions) was found between 1,601 m and 1,800 m asl, while the lowest was found between 1 m and 200 m asl. However between 401 m and 800 m asl, disease severity was almost as important as in class 1,201–1,400. Disease severity on leaves (SPLSD) was always higher than on fruits (SPFSD).

Disease severity was significantly higher on grape fruits than on the other species. Least squares means (LSMean) analysis with tree species on SPLSD and SPLFSD showed that grape fruit LSMean was 2.03, followed by orange (1.64), lemon (1.51), tangerine (1.49), and pummelo (1.47). Although the disease was present on all species, its severity varied among citrus tree species (Fig. 4).

Within the nine soil textural classes found in the humid zones of Cameroon, the highest disease severity either on leaves or on fruits occurred on trees situated on sandy soils (Fig. 5). No significant difference was found in SPLSD and SPLFSD between sandy and sandy clay soils, and between dark clay and brown clay ($P>0.05$) in one group. However, there was a significant difference between this group and another group of soil textural classes made up of the other five classes ($P<0.05$).

The highest disease severity was found on trees situated in semi-forested land (Fig. 6). With respect to vegetation, PLFSD was present on citrus situated in all the three types of vegetation. However, no significant difference was observed in SPLSD and SPLFSD between the three vegetation types.

Prediction of risk factors for the development of PLFSD

The previous analyses brought out several variables that are closely linked to disease severity. In the segmentation analysis, the entire variables measured were used to confirm the variables that best explained the disease severity. The segmentation trees obtained with 2005 and 2006 data show that disease severity respectively on leaves and fruits in the two answer trees are mostly explained by altitude (Fig. 7). The higher the altitude, disease severity is more important. Tree species is also a risk factor, and in lower altitudes, disease severity is higher in presence of grape fruit. In presence of other species (orange,

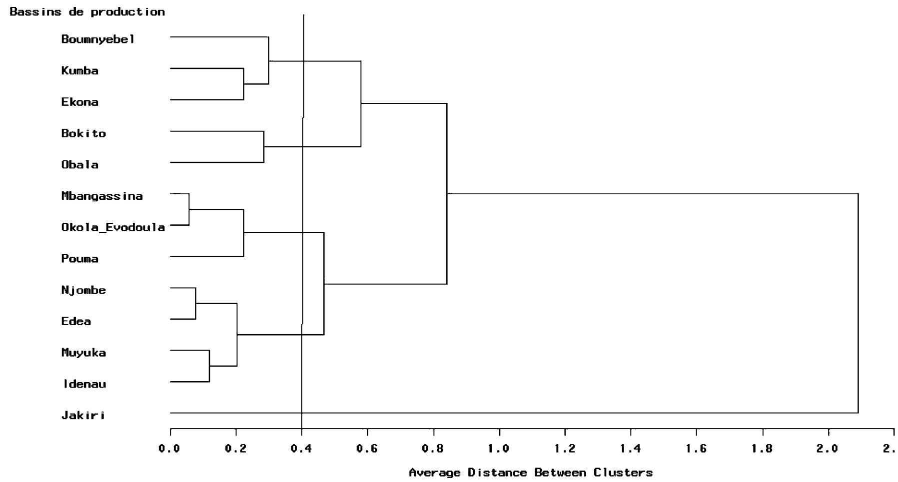

Fig. 2 Classification of citrus production basins of Cameroon by clustering *Phaeoramularia* leaf and fruit spot disease severity in 2005 and 2006

pummelo, lemon and tangerine), high disease severity on leaves (a) is noticed on trees treated with fungicide and insecticide mixture. In addition, when trees are treated with fungicide and insecticide mixture, higher temperature (>26°C) increases the severity of disease on leaves (a). However, in presence of orange trees, tangerine, lemon and pummelo, disease severity on fruits (b) increases with lower temperature (≤26°C).

Table 3 Variables significantly associated with *Phaeoramularia* leaf and fruit spot disease of citrus selected from data collected between 2005 and 2006 in the humid zones of Cameroon

Year	Symptoms	Factor	d. f.	F value	Pr > F
2005	Leaves spot	Altitude	1	134.4	<.0001
		Tree species	4	32.4	<.0001
		Soil type	8	10.8	<.0001
		Vegetation type	2	3.0	0.0482
	Fruits spot	Altitude	1	147.1	<.0001
		Tree species	4	39.7	<.0001
		Soil type	8	6.3	<.0001
		Vegetation type	2	8.4	0.0002
2006	Leaves spot	Altitude	1	143.7	<.0001
		Tree species	4	40.2	<.0001
		Soil type	8	2.2	0.0233
		Vegetation type	2	10.4	<.0001
	Fruits spot	Altitude	1	274.4	<.0001
		Tree species	4	57.7	<.0001
		Soil type	8	2.9	0.0037
		Vegetation type	2	8.5	0.0002

Discussion

Prediction of risk factors for the development of PLFSD

Our results show that PLFSD epidemics depend mainly on altitude, tree species, soil texture, vegetation, temperature, rainfall and management pattern. This is in line with the finding that the epidemic risk results from regional and local risk factors, the latter including production situation (Avelino et al. 2006). These factors are not independent, for example in Jakiri, there is an abundance of orange trees which are surrounded by semi forested land and the altitude of this zone is high. The relationships between these three factors have an impact on disease severity, the highest disease severity is found in this citrus production basin.

Segmentation trees showed that at higher and lower altitudes, the effect of altitude is dominant whereas at medium altitude, other factors could have an impact on disease severity. To study this impact, it is necessary to do experimentation in such zones and consider all these factors. This work was done in field conditions where the number of trees per species was not the same. Abundance of some tree species was also not even in all the studied zones. This is the case of grape fruit trees very sensitive species, which were abundant in low altitude zones (Pouma, Edea, Muyuka, Ekona, and Idenau) and rare in high and medium altitude zones (Jakiri, Obala, Mbangassina). To quantify the effect of such a factor, it will be better to analyse the same number of trees per species and per citrus production basin.

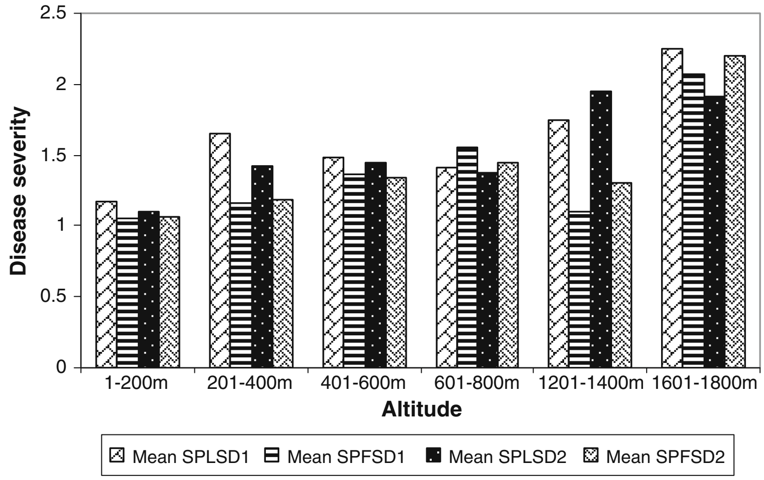

Fig. 3 Altitudinal differences in *Phaeoramularia* leaf and fruit spot disease severity on citrus leaves and fruits in 2005 (*1*) and 2006 (*2*) in the humid zones of Cameroon

In addition it will be important to include factors such as the genetic background of the pathogen to ensure a uniform host-pathogen system.

Concerning the management pattern of disease, the high severity on leaves noticed on trees treated with fungicide and insecticide mixture can be explained by an inappropriate treatment timing: treatments by farmers are usually done while when leaves and fruits are already attacked and therefore have no impact on disease severity.

Severity of PLFSD

PLFSD is one of the most important diseases limiting the production of citrus in areas where disease is prevalent. Predicting disease severity based on some predetermined factors is critical for managing PLFSD especially in developing countries where a high production cost is unsustainable. In this study, we have demonstrated that altitude, tree species and soil type are the major factors that influence the severity of PLFSD of citrus. In addition, we observed that disease was present where it was not expected, basins with similar characteristics exhibiting very different levels of disease, and that there was more disease on leaves than on fruits. These facts indeed raise questions, for instance which kind of predictive model should be used and on which scale.

Although the disease was present in all the production basins, the severity varied among basins and site, contrary to the previous founding that disease can only be found on susceptible varieties situated on sites above 200 m (Kuate et al. 1994). Differences in severities observed in the different sites of each production basin could be due to the fact that basins were not homogeneous in terms of citrus species present, farming systems, soil type, altitude, vegetation and other ecological factors. Disease severity observed in the 13 production basins was categorized into four categories: very high (Jakiri),

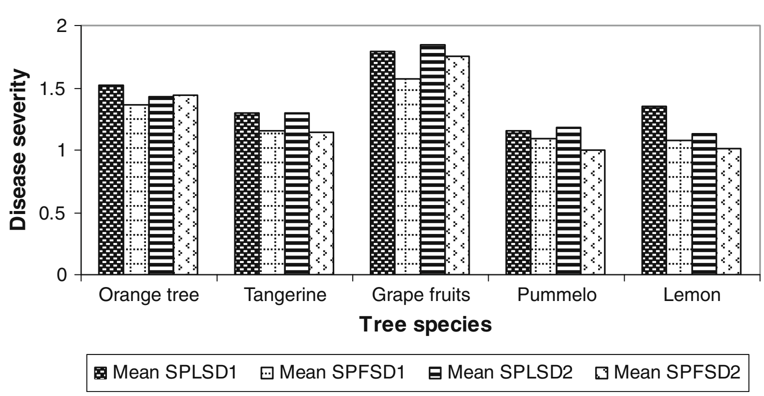

Fig. 4 Heterogeneity in *Phaeoramularia* leaf and fruit spot disease severity on leaves and fruits of citrus species in 2005 (*1*) and 2006 (*2*) in the humid zones of Cameroon

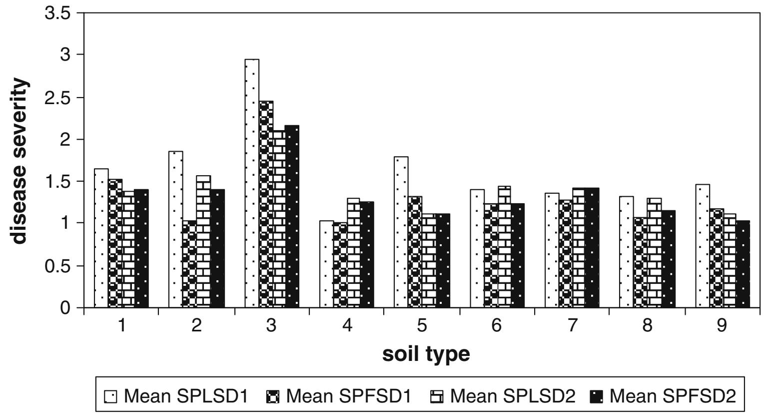

Fig. 5 Heterogeneity in *Phaeoramularia* leaf and fruit spot disease severity of citrus amongst soil types in the humid zone of Cameroon

high (Boumnyebel, Bokito, Obala, Ekona), middle (Okola, Kumba, Pouma, Mbangassina) and low severity (Idenau, Muyuka, Njombé, Edea). With this classification, we observed that basins could have in common the same altitude, the same soil type, the same species for instance but present very high differences in PLFSD severity. The differences observed showed that other factors not considered in the present study are involved in the development of PLFSD. Severity on leaves is almost always more important than on fruits. This can be due to the lack of fruits at the moment of certain observations. Disease severity either on leaves or on fruits in each citrus production basin developed in the same way in 2005 and in 2006.

Factors involved in the development of PLFSD

Biotic factors (tree species and varieties, trees age, leaves and fruits stages, vegetation type, production system) and abiotic factors (altitude, mean temperature, relative humidity, rainfall, soil texture) were recorded during the survey. As with other diseases of perennial crops (Agostini et al. 2003; Avelino et al. 2004; Mouen Bedimo et al. 2007), this study shows that numerous factors of different types affect the development of PLFSD of citrus, illustrating the complexity of *Phaeoramularia angolensis*-citrus pathosystem. Among those factors, altitude, soil texture, tree species and vegetation type are the most important. The effect of these factors on disease severity was the same irrespective of the observation year.

Altitude

The highest severities on fruits and leaves were found at higher altitudes (Jakiri situated at 1,684 m asl.) and the lowest at lower altitudes (Idenau situated between 10 m and 36 m asl.). In addition, some differences were found

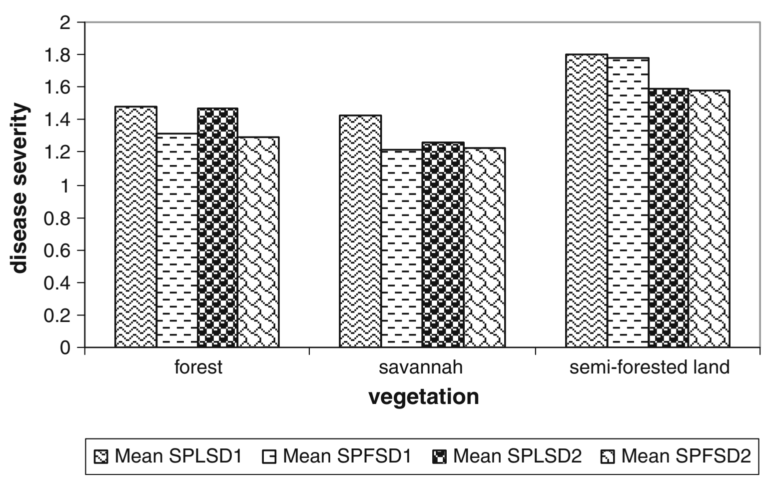

Fig. 6 Differences in *Phaeoramularia* leaf and fruit spot disease severity of citrus amongst the major vegetations in the humid zone of Cameroon

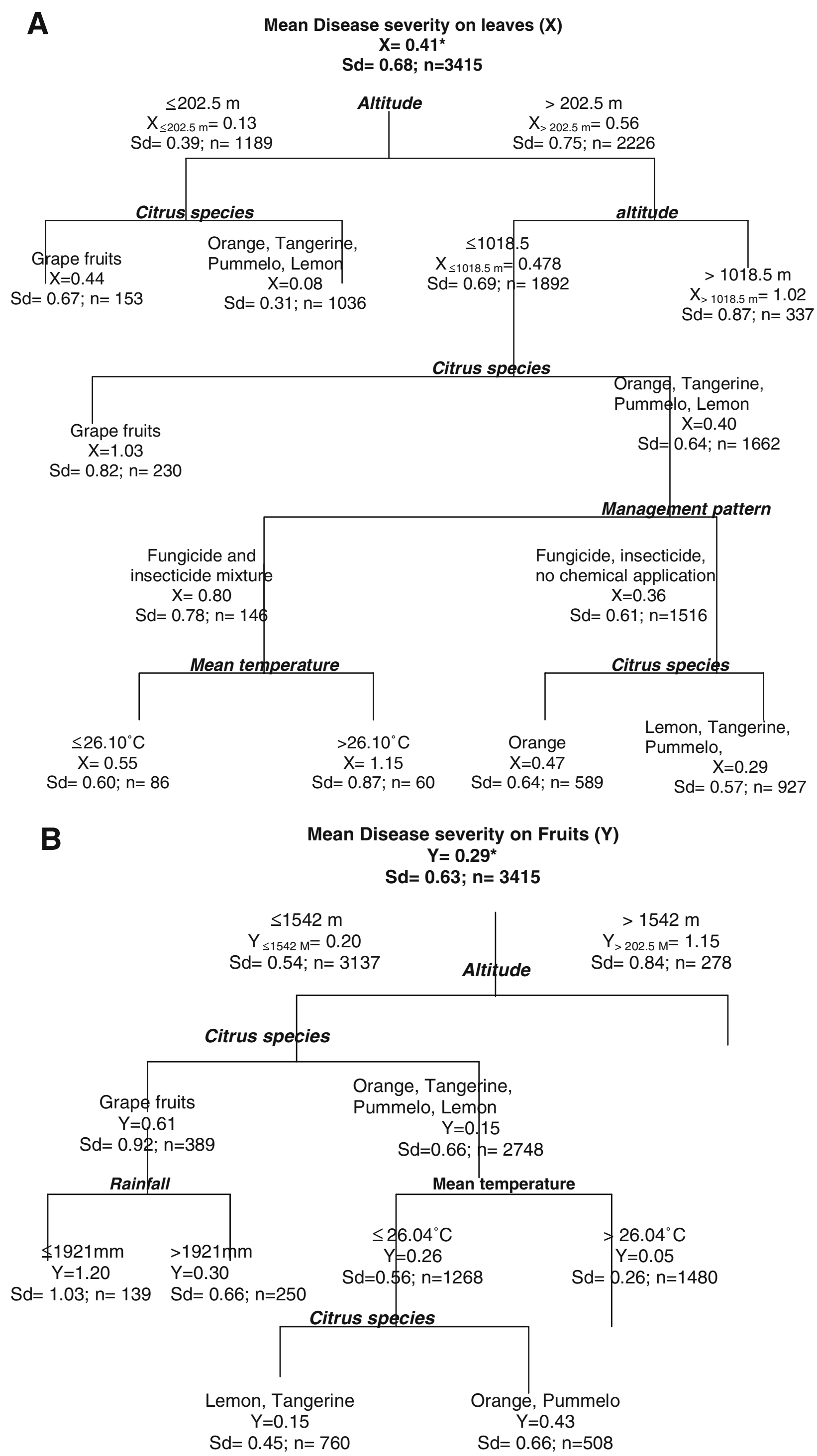

Fig. 7 Risk factors associated with the severity of *Phaeoramularia* leaf and fruit spot disease in the humid zone of Cameroon. **A** Altitude, citrus species, citrus variety and tree type as main factors responsible for disease severity on leaves. **B** Altitude, citrus species, rainfall, mean temperature and citrus variety as the main factors responsible for disease severity on fruits

**Classification of disease severity*
0: Absence of disease symptoms
1: Percent of surface of organ with disease symptoms<25%
2: 25%> Percent of surface of organ disease symptoms <50%
3: Percent of surface of organ disease symptoms >50%

between sites situated in the same basin but at different altitudes ($P<0.05$) This was the case for Ekona where in one of the sites situated at 1,400 m asl., the mean severity index on fruits was 2.1 while in the two other sites situated between 200 m and 380 m asl, the mean severity index on fruits was 1.3. Those results show as in previous studies on PLFSD the importance of altitude in the development of this disease (Kuate 1998). In production basins and sites situated in medium altitude, disease severity presented very significant differences ($P<0.05$). In some of them the severity was high (Boumnyebel, Bokito and Obala) while in the others it was very low (Mbangassina and Okola). All those basins are situated around 500 m asl.

Citrus species

(Bella-Manga et al. 1999) classifed species according to their sensibility to PLFSD. In this classification, grape fruit trees (*Citrus paradis*i Macf.) and some tangerines (*C. reticulata* Blanco) were in the very sensitive group, whereas oranges (*C. sinensis* (L.) Osbeck) and other tangerines were in the medium group and lemon (C. limon (L Burm. F.) and pummelo (*C. grandis* (L.) Osbeck) were in the less sensitive group. Results obtained in this study confirm this classification, even though differences observed are not statistically significant. Disease severity observed on grape fruit trees was the highest, followed by severity on orange trees. The number of trees observed was not the same per species and the distribution of species observed was not equitable in all the basins. Orange trees were the most abundant in almost all the production basins. It is known that, the degree of sensitivity of species equally depends on geographical zones (Kuate et al. 1994; Diallo 2001) and probably on the genetic background of the pathogen.

Soil texture

Highest disease severity is observed on trees situated on sandy soils followed by sandy clay, dark clay and brown clay soils with no significant differences. Soils with sand and clay are very suitable for the development of citrus (Walali Loudyi et al. 2003). Trees situated on such soils perform very well and so could be threatened by *Phaeoramularia angolensis*. This potential threat to citrus production needs to be confirmed by a more precise analysis of soil composition.

Vegetation

Forest, savannah and semi forested land are the three types of vegetation surrounding citrus orchards in the humid zones of Cameroon. Disease severity was higher on trees situated in semi forested land, but differences observed between the three types of vegetation were not statistically significant. Semi forested land are mostly situated on high and medium altitude sites (Jakiri, Bokito). Orange trees are abundant on those sites. All those factors could lead to a higher disease severity in those zones.

This research has shown relationships between *Phaeoramularia* leaves and fruits spot disease severity and altitude, citrus species and varieties, soil texture, vegetation and rainfall. These relationships can be exploited to develop a predictive model for *Phaeoramularia* leaves and fruits spot disease severity. Prediction accuracy, however, could be improved by taking into account experimentation done with risk factors in the different citrus production basins; in addition, the number of trees per species per basin should be equitably chosen to avoid any interdependence and take into account the diversity of the host-pathogen system. Additional years of data collection should improve this survey and contribute to the determination of more robust predictive risk factors.

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