Original article

Distribution of *Pseudocercospora* fruit and leaf spot, *Phytophthora* foot rot and scab diseases and their effect on Citrus tree decline prevalence in the humid zones of Cameroon

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Summary

Introduction – **Declining Citrus trees (CTD) is a major constraint causing Citrus production depletion in Cameroon. It has been established that some pests and diseases are the major sources of that phenomenon. Improving knowledge of the contributing factors to the occurrence of CTD is key to the control of this.** *Materials and methods* – **A study based on the presence of fungal diseases caused by *Pseudocercospora angolensis*, *Elsinoe* spp., and *Phytophthora* spp., was carried out in 39 sites of Citrus producing basins of Cameroon. The aim of the study was to determine environmental factors favouring CTD occurrence in an effort to reducing their devastating effects on three common species of Citrus. Disease symptoms were noticed by examining tree's organs (leaves, fruits, branches, trunk). A regression analysis was applied with the General Linear Model to select the main factors involved in CTD development. The prevalence of CTD was treated as dependent variable. Independent variables were Citrus species, soil texture, vegetation, rainfall, temperature, relative humidity, altitude, observation year, prevalence of PLFSD, Citrus scab and *Phytophthora* diseases.** *Results and discussion* – **CTD was found in all the sites, with a mean prevalence of 25%. Citrus species, soil texture, vegetation, rainfall, *Pseudocercospora* fruit spot disease, scab, and *Phytophthora* foot rot disease were the main factors associated with CTD prevalence.** *Conclusion* – **The importance of CTD was confirmed. It has been shown that PLFSD and *Phytophthora* diseases were much more likely to contribute to tree decline contrary to Citrus scab. However, further analysis could pave the way for the development of a risk assessment model for CTD.**

Keywords
Citrus spp., declining trees, *Elsinoe* spp., epidemiology, *Phytophthora* spp., *Pseudocercospora angolensis*

Significance of this study

What is already known on this subject?

- It is known that fungal diseases like fruit and leaf spot disease caused by *Pseudocercospora angolensis*, Citrus scab disease caused by *Elsinoe* spp., and *Phytophthora* diseases of Citrus caused by *Phytophthora* spp. are those which cause the major damages on Citrus in Cameroon. However, high treatment cost is a major concern for smallholders, who constitute approximately 80% of Citrus producers.

What are the new findings?

- The study showed that Citrus tree decline has become a threat faced by producers. Prevalence of Citrus tree decline (CTD) in Citrus production basins of Cameroon is one of the new findings; also the link between fungal diseases and CTD is highlighted in this study. The involvement of other factors such as Citrus species, rainfall and soil type on the development of CTD has been demonstrated.

What is the expected impact on horticulture?

- These findings will lead to the development of a risk assessment model of CTD.

Introduction

Citrus fruits are economically important. In monetary value, they represent the greatest group of fruits in the international trade (Gibbon, 2003). Citriculture occupies an important place in Africa in general (Olife *et al.*, 2015). In Cameroon in particular, fruit production constitutes an important source of revenue for many households (Temple, 2001). The fresh fruit market is the main output of the Citrus production due to lack of conservation and transformation facilities. Citrus fruits are one of the main vitamin sources, minerals and fibers, thus they contribute to the health of populations in the country. Citrus production mostly involves orange, grapefruit, lemon and tangerine (Ndo *et al.*, 2010). Citrus are generally grown in home gardens, but in larger scale in family farm holdings in the main producing basins in Cameroon. They are usually grown in association with cocoa and coffee, in most cases in a complex agroforestry system (Kuate *et al.*, 2006a). The development of Citrus is favoured by tropical climate, also favourable to the growth of several pathogens. Hence, the pro-eminent constraint faced by farmers in these regions is largely referring to these biotic factors (Mariau, 1999).

The occurrence of Citrus tree decline (CTD) was noted in several agroecological zones in Cameroon. The origin of declining Citrus had not yet been well established, but the main hypothesis is that CTD may be due to abiotic factors (soil

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type, climate, altitude, *etc.*), or biotic factors (pests, diseases, tree species, *etc.*) or their combinations. Diseases caused by fungi, viruses, bacteria, nematodes and other insects occur frequently on perennial crops in the tropics (Mariau, 1999). They cause damages on any organ of trees, from roots to leaves and fruits. Fungal diseases like fruit and leaf spot disease caused by *Pseudocercospora angolensis*, Citrus scab disease caused by *Elsinoe* spp., and *Phytophthora* diseases of Citrus caused by *Phytophthora* spp., are those which cause the major damages on Citrus in Cameroon (Kuate, 1998; Nelson, 2008; Dewdney *et al.*, 2012). While the first two pathogens attack leaves and fruits and cause spots and scabies respectively, the identification of which has been the subject of several studies (Ndo *et al.*, 2010; Kuate, 1998; Timmer *et al.*, 1996; Ndo, 2007; Dagnew *et al.*, 2014), *Phytophthora* spp. attacks the root systems and trunks, of Citrus trees at any age causing *Phytophthora* foot rot, root rot, brown rot, or gummosis (Graham and Timmer, 1992; Cacciola *et al.*, 2008; Futch and Graham, 2013; Graham and Timmer, 2014).

Currently, control measures rely mainly on the use of chemicals (Seif and Hillocks, 1999; Diallo, 2003; Kuate, 2003; Yesuf, 2007; Tyson *et al.*, 2001). However, there is a growing concern for human safety and the environmental protection (Suhr *et al.*, 2003). High treatment costs is also a cause of concern for small farmers. This therefore calls for a search of environmentally friendly control methods, with regard to human health as well. Integrated management approach to sustainably control diseases has been recently explored. In the case of declining Citrus trees, such an approach should take into account the fact that one or several factors or diseases may be involved. For effective control of declining Citrus trees, it is therefore important to have a holistic understanding on its occurrence. For instance, which factors are responsible for the development of CTD? The hypothesis is that the development of CTD is linked to the involvement of biotic and abiotic factors. In this study, we carried out a survey in 39 Citrus production sites from humid zones of Cameroon in order to collect information and to understand the factors underlying the prevalence of CTD.

Materials and methods

Sites characterization

The study was carried out in three agro-ecological zones, stretching from the northern limit of Cameroon's forest zone to the border with Equatorial Guinea and Gabon (Figure 1). These were the Western highlands, the Mount Cameroon area and its surroundings, and the humid forest zone.

(i) The Western Highlands (latitude 4°54' to 6°36'N, longitude 9°18' to 11°24'E, altitude: 1,240–1,800 m a.s.l.) encompasses the North-West and West Regions. The climate is "Mountainous" type with low annual mean temperatures of 19 °C, and abundant rainfall of 1,500 to 2,500 mm annually that occurs in a monomodal configuration. The soils are loamy to clayey in texture.

(ii) The Mount Cameroon area and it surroundings, also known as the monomodal humid forest zone (latitude varied from 2°6' to 6°12'N, and longitude from 8°48' to 10°30'E, altitude: 200 to 800 m a.s.l., put aside Mount Cameroon with 4,095 m), includes the South-West and the Littoral region. The climate is a variation of equatorial climate with mean annual temperature ranging from 22 to 29 °C, relative humidity as high as 85–90%, and abundant rainfall that occurs in a monomodal pattern (average: 2,500 to 4,000 mm year^{-1}, with the exception of Debundscha with 11,000 mm). The soils are sandy to sandy clay or clayey in texture.

(iii) 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 a.s.l.) encompasses the Centre, East and South regions. The climate is a sub-equatorial climate, with mean temperature of 25 °C and relative humidity averaged 75%, with a bimodal rainfall pattern (average annual: 1,300 to 2,500 mm). The soils are sandy loam, sandy clay loam, or clay in texture.

Field survey

In total, 13 production basins were selected, based on differences observed in their biophysical characteristics and the composition of the Citrus population. In this line, seven production basins, *viz.*, Kumba, Muyuka, Ekona, Idenau, Pouma, Njombé, and Edéa were selected in the monomodal humid forest zone; five, *viz.*, Boumnyebel, Mbangassina, Bokito, Obala and Okola were selected in the bimodal humid forest zone; and one production basin, *viz.*, Jakiri, was selected in the western highlands. It should be recalled that Jakiri represented the only accessible basin in the highlands. Within each production basin, three sites were selected and Citrus farms representing the diversity of the site were sampled.

Each site in this study represented a geographical space with climatic and soil conditions rather homogeneous and thereby can ensure a homogeneous production. The site was composed by many villages. The selection of studied sites

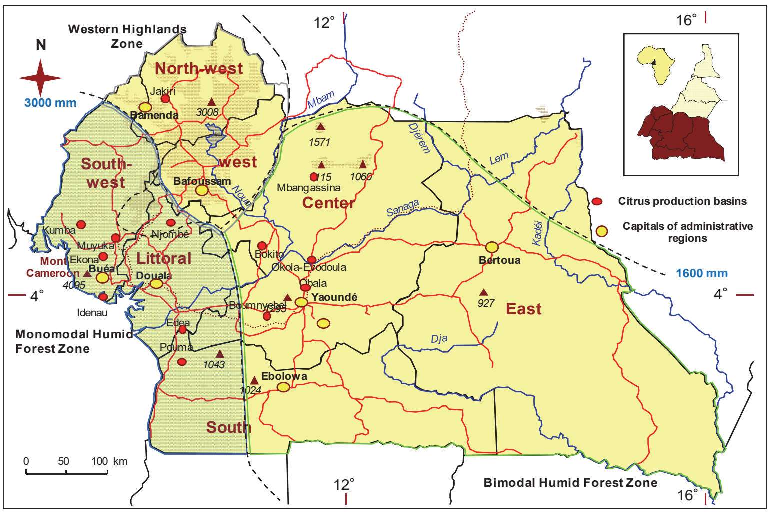

FIGURE 1. Citrus production basins in three contrasting humid agro-ecological zones of Cameroon.

TABLE 1. List of parameters describing Citrus trees environment.

Parameter	Variable	Modality
Climate	Annual rainfall	Mm
	Relative humidity	%
	Temperature	°C
Location	Altitude	10–1,684 m a.s.l.
	Vegetation	1: Forest 2: Savannah 3: Gallery 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

and Citrus farms was made on the base of the diversity in Citrus production basins.

Observations have been made during three consecutive years, and data were collected between June–July of each year during the producing phase. Three Citrus species were observed, *viz*., *Citrus limon* (L.) (lemon), *Citrus sinensis* (L.) (orange), and *Citrus paradisi* (Macf.) (grapefruit). Annual observations were made on an average of 611 orange trees, 277 lemon trees and 263 grapefruit trees.

Climatic data covering 20 years were collected from meteorological stations in production basins. Other data were obtained by measuring or by visual inspection of the environment. This concerns mainly altitude which was measured with GPS, soil texture and vegetation. The overall parameters are summarized in Table 1.

Epidemiological data were collected by noting the presence or absence of diseases and Citrus trees decline. CTD may start with leaves discoloration, but this symptom could also be associated with diseases or deficiencies. In the case of this study, a tree was considered declining if the rate of leaf drop or dryness of branches was observed, whatever the season. CTD was considered present as at least 10 to 25% of leaves drop and/or dry stems was noticed.

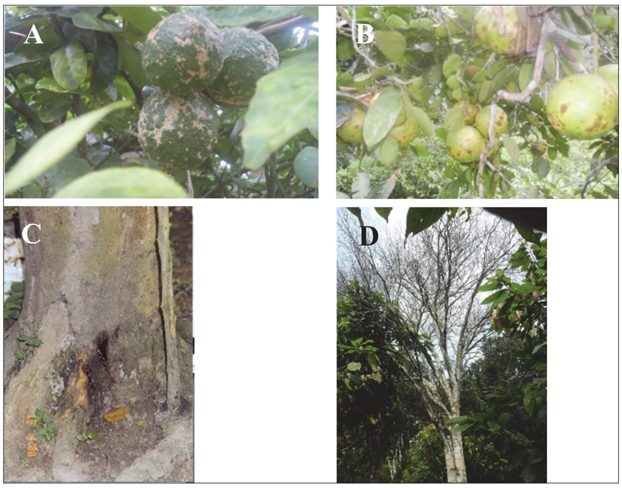

FIGURE 2. Symptoms of Scab on lemon fruits (A), *Pseudocercospora* on grapefruits (B), *Phytophthora* foot rot disease on orange tree (C), and declining citrus tree in a cocoa agro forest of Cameroon (D).

On one hand, the presence or absence of CTD was noticed (presence = 1, absence = 0), and on the other hand the presence or absence of spots due to *Pseudocercospora* leaf and fruit spot disease (PLFSD) on leaves (PLSD) and on fruits (PFSD), scabies due to Citrus scab on leaves and fruits, and rot on tree's foot due to *Phytophthora* foot rot disease (Figure 2). Citrus species, site parameters including altitude, temperature, rainfall, relative humidity and soil texture were noticed. The year of observation was also considered.

Prevalence, which is the percentage of trees affected by one of these diseases, was calculated by site for each disease. Since PLFSD and Citrus scab attack leaves and fruits, prevalence for those two diseases was calculated on leaves and on fruits.

Statistical analysis

A descriptive analysis of data was done through univariate and multivariate analysis for their exploration and to know the distribution, tendencies and dispersion of different variables used. Cross tabulations and graphical representations were done to illustrate the variation of diseases or CTD according to years, species or sites. The analysis of variance was performed to compare the different sites where epidemiological data were collected. Comparison was also done among the three years during which data were collected. Besides the treatments, means were compared using the Student-Newman-Keuls test at the 5% level of probability.

Quantitative (disease prevalence in %, calculated by site and by year for each disease) and qualitative (Citrus species, observation year and sites parameters, *i.e.*, altitude, temperature, rainfall, soil type and vegetation) data were used in the different analyses.

To determine the relationship between quantitative variables, a correlation test was done, using the Pearson's method.

An evaluation (using the two categories of data) was done, to select the main factors involved in CTD development. This was done through a regression analysis using GLM (General Linear Model) procedure of the SAS (Statistical Analysis System) software, version 9.3.

Results and discussion

Diseases prevalence

Citrus Tree Decline prevalence

Citrus Tree Decline was found in the 39 sites, during the three years of observation. CTD was also found on all the Citrus species of the study sites. This shows the importance of the impact of CTD in the Citrus production basins of Cameroon's humid zones.

The prevalence varied with Citrus species. Prevalence was higher on orange and grapefruit trees though not significantly different, whereas the difference between these two species and lemon trees was significant at 5% level. CTD mean prevalence varied from 12% to 30% (Figure 3).

Concerning the observation period, the analysis of variance showed less significant differences in CTD prevalence during the 3 years at 5% level of significance. CTD prevalence was slightly higher in year 1 than in year 2 and year 3.

CTD prevalence was variable in the 39 sites. The mean prevalence of CTD was almost 25% (Table 2). However, differences were noticed among sites, meaning that CTD varies with environmental factors. Those differences were more

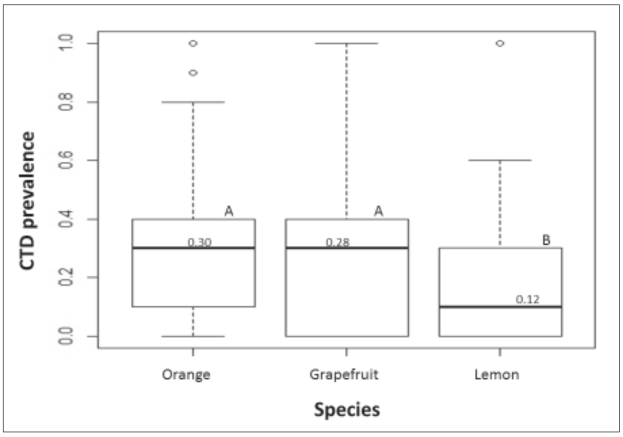

FIGURE 3. CTD prevalence on lemon, orange and grapefruit trees in Citrus production basins of Cameroon.

significant with 4 sites. The sites Mbangassina and Nkolakok, belonging respectively to Mbangassina and Okola-Evodoula Citrus production basins in the monomodal humid zone, presented the highest prevalence. On the other hand, Limbé and Edea from Idenau and Edea Citrus production basins in the bimodal humid zone presented the lowest prevalence.

Associated diseases prevalence

Pseudocercospora leaf and fruit spot disease, Citrus scab, and *Phytophthora* foot rot disease were found in the 39 sites (Table 3). This result was expected, as the weather conditions of the studied zones are favourable to the development of pathogenic agents responsible of the diseases. *Pseudocercospora* leaf and fruit spot disease incidence increases with drop in climatic conditions (Kuate *et al.*, 2002). Humidity is also one of the principal factors of spore production and infection in the case of scab disease (Dewdney and Timmer, 2012) and *Phytophthora* spp. produces large number of motile zoospores that can swim in water for short distances under high moisture and temperature conditions (Futch and Graham, 2012).

For each disease, the prevalence differed with sites. PLFSD and *Phytophthora* foot rot disease presented the highest prevalence in many sites. In many cases, prevalence of the two diseases was higher than 50%. The mean prevalence of scab was situated around 20% in the majority of sites.

The correlation between *Pseudocercospora* leaf spot disease and *Pseudocercospora* fruit spot disease was positive and significant, indicating that the disease varies proportionally on leaves and on fruits. Conversely, scab on leaves and scab on fruits were not correlated (Table 4). This could be explained by the fact that fruits are more infected than leaves. Leaves are immune to scab infection in a few days, while fruits remain susceptible up to two months (Agostini *et al.*, 2003).

The prevalence of the diseases also varied with Citrus species (Figure 4). PLFSD and *Phytophthora* root rot disease prevalence were higher on grapefruit and orange trees, whereas scab prevalence was higher on lemon trees. This confirms the results found in previous studies. Differences noticed were due to the high susceptibility of orange and grapefruit trees against PLFSD (Seif and Hillocks, 1999; Bella Manga *et al.*, 1999; Kuate *et al.*, 2006b). Scab disease is generally a severe problem for many tangerines, lemons and limes (Nelson, 2008). Concerning *Phytophthora* foot rot disease, evaluation of Citrus susceptibility has indicated a

TABLE 2. Variation of CTD prevalence on Citrus in the different sites of Cameroon's Citrus production basins.

Production basins	Sites	General CTD prevalence mean ± SD*
Boumnyebel	Makaï	0.19±0.33 ^{BAC}
	Minse, Maloung	0.14±0.12 ^{BAC}
	Nkon Madog	0.33±0.29 ^{BAC}
Mbangassina	Mbangassina	0.49±0.30 ^{A}
	Goura	0.28±0.28 ^{BAC}
	Etam Nyat	0.26±0.36 ^{BAC}
Bokito	Kedia	0.29±0.42 ^{BAC}
	Begni	0.33±0.40 ^{BAC}
	Ombessa	0.24±0.19 ^{BAC}
Obala	Nkolmelen	0.28±0.23 ^{BAC}
	Nkoltomo, Efok	0.22±0.24 ^{BAC}
	Batschenga	0.22±0.11 ^{BAC}
Okola-Evodoula	Nkol akok	0.46±0.11 ^{BA}
	Nguibassal	0.24±0.21 ^{BAC}
	Ekekam	0.33±0.11 ^{BAC}
Pouma	Seppè, Makondo	0.13±0.09 ^{BC}
	Nkonga	0.23±0.14 ^{BAC}
	Tjiedikoï	0.29±0.06 ^{BAC}
Njombé	Njongo	0.15±0.14 ^{BAC}
	Loum chantier	0.11±0.31 ^{BC}
	Nlohé	0.18±0.25 ^{BAC}
Edéa	Edéa	0.04±0.15 ^{C}
	Kopongo	0.13±0.17 ^{BC}
	Beon	0.35±0.21 ^{BAC}
Kumba	Kumba	0.39±0.21 ^{BAC}
	Ekiliwindi	0.15±0.17 ^{BAC}
	Mabonji-Tantcha	0.17±0.43 ^{BAC}
Muyuka	Bombé	0.22±0.15 ^{BAC}
	Banga	0.12±0.26 ^{BC}
	Yoké-Malendé	0.13±0.18 ^{BC}
Ekona	Mautu	0.37±0.43 ^{BAC}
	Ekona town	0.11±0.15 ^{BC}
	Buéa	0.22±0.26 ^{BAC}
Idénau	Idénau	0.20±0.18 ^{BAC}
	Bakingili	0.22±0.23 ^{BAC}
	Limbé	0.09±0.10 ^{C}
Jakiri	Jakiri	0.12±0.12 ^{BC}
	Kifue	0.18±0.25 ^{BAC}
	Waïnamah	0.28±0.30 ^{BAC}

± = Standard deviation. Numbers followed by the same letters are not significantly different at $\alpha = 5\%$.

wide range of rootstocks that are tolerant or less susceptible to *Phytophthora* diseases of Citrus including 'Volkamer' lemon, most rough lemon selections, sour orange, 'Troyer' and 'Carrizo' citrange (Graham and Timmer, 2014). Trees grafted with those rootstocks better resist to *Phytophthora* spp. However, the majority of Citrus trees planted in those zones are not grafted (Ndo, 2007; Ndo *et al.*, 2010), therefore without a tolerant rootstock, orange and grapefruit trees are seriously attacked by *Phytophthora* spp.

In general, for the studied fungal diseases, since, small scale Citrus producers in humid zones of Cameroon use no chemical or inappropriate treatments (without respecting

TABLE 3. Mean prevalence and standard deviations of Citrus diseases in different sites of Cameroon's Citrus production basins.

Sites	PFSD*	PLSD*	*Phytophthora* foot rot	Scab on leaves	Scab on fruits
Makaï	0.16±0.33	**0.35±0.42**	0.14±0.19	0.01±0.02	0.03±0.05
Minse, Maloung	0.14±0.18	**0.37±0.33**	0.24±0.18	0.04±0.09	0.07±0.10
Nkon Madog	0.23±0.22	**0.45±0.29**	0.27±0.18	0.11±0.14	0.15±0.21
Mbangassina	**0.30±0.35**	**0.34±0.36**	**0.50±0.33**	0.24±0.36	0.10±0.13
Goura	0.28±0.29	0.26±0.29	**0.38±0.34**	0.17±0.22	0.17±0.18
Etam Nyat	**0.36±0.35**	**0.54±0.33**	0.19±0.17	0.12±0.18	0.23±0.32
Kedia	**0.42±0.30**	**0.37±0.26**	**0.41±0.22**	0.16±0.25	0.10±0.09
Begni	**0.40±0.32**	**0.59±0.33**	**0.34±0.31**	0.16±0.22	0.16±0.18
Ombessa	**0.36±0.37**	**0.44±0.31**	0.23±0.24	0.19±0.21	0.09±0.11
Nkolmelen	**0.43±0.41**	**0.56±0.40**	0.24±0.31	0.23±0.34	0.15±0.22
Nkoltomo, Efok	0.28±0.40	**0.37±0.36**	0.20±0.15	0.17±0.24	0.20±0.20
Batschenga	**0.41±0.32**	**0.47±0.33**	0.15±0.13	0.25±0.33	0.28±0.31
Nkol akok	0.15±0.21	0.23±0.27	**0.30±0.20**	0.17±0.21	0.17±0.27
Nguibassal	0.27±0.22	0.20±0.14	**0.49±0.34**	0.13±0.20	0.23±0.23
Ekekam	0.29±0.29	**0.39±0.33**	**0.37±0.17**	0.11±0.12	0.24±0.22
Seppè, Makondo	0.10±0.15	0.28±0.26	**0.35±0.30**	0.08±0.12	0.18±0.15
Nkonga	0.16±0.17	0.22±0.24	**0.38±0.32**	0.06±0.12	0.12±0.11
Tjiedikoï	0.19±0.20	**0.46±0.40**	**0.44±0.46**	0.18±0.19	0.15±0.17
Njongo	0.12±0.13	0.16±0.15	**0.40±0.23**	0.22±0.16	0.15±0.12
Loum chantier	0.13±0.13	0.28±0.37	0.24±0.17	0.17±0.15	0.17±0.19
Nlohé	0.25±0.18	**0.43±0.32**	**0.33±0.19**	**0.41±0.33**	0.21±0.18
Edéa	0.00±0.00	0.09±0.13	0.02±0.06	0.23±0.31	0.09±0.23
Kopongo	0.04±0.07	0.13±0.13	**0.40±0.26**	0.14±0.15	0.10±0.08
Beon	0.09±0.15	0.14±0.16	0.23±0.15	0.12±0.17	0.15±0.14
Kumba	**0.59±0.45**	**0.58±0.40**	0.14±0.13	0.22±0.29	**0.30±0.41**
Ekiliwindi	**0.42±0.39**	**0.49±0.44**	**0.31±0.41**	0.05±0.11	0.11±0.19
Mabonji-Tantcha	**0.41±0.33**	**0.43±0.36**	0.04±0.08	0.26±0.37	0.36±0.35
Bombé	0.12±0.11	**0.31±0.33**	0.21±0.17	0.29±0.31	0.22±0.23
Banga	0.21±0.30	**0.32±0.39**	**0.51±0.49**	0.08±0.13	0.20±0.36
Yoké-Malendé	0.17±0.23	**0.44±0.30**	**0.50±0.49**	**0.31±0.39**	**0.32±0.47**
Mautu	0.08±0.20	**0.36±0.44**	**0.48±0.38**	0.20±0.32	0.12±0.14
Ekona town	0.05±0.09	0.11±0.09	0.25±0.26	0.13±0.09	0.19±0.21
Buéa	0.06±0.09	0.03±0.09	**0.61±0.25**	0.18±0.25	**0.30±0.34**
Idénau	0.02±0.05	0.24±0.29	**0.38±0.10**	0.16±0.24	**0.36±0.35**
Bakingili	0.24±0.31	0.32±0.29	0.12±0.13	0.12±0.16	0.23±0.23
Limbé	0.07±0.10	0.40±0.36	0.12±0.12	**0.32±0.38**	0.09±0.12
Jakiri	0.03±0.06	0.01±0.04	0.16±0.15	0.18±0.25	0.25±0.24
Kifue	0.10±0.26	0.12±0.26	0.19±0.24	**0.30±0.28**	0.11±0.13
Waïnamah	0.13±0.26	0.06±0.17	0.08±0.14	**0.32±0.40**	0.18±0.22

*PFSD = *Pseudocercospora* fruits spot disease; PLSD = *Pseudocercospora* leaves spot disease; ± = Standard deviation.

TABLE 4. Pearson's correlation coefficients between Citrus diseases prevalences in Cameroon.

	Pearson correlations				
	PFSD	*Phytophthora* foot rot	PLSD	Scab on fruits	Scab on leaves
PFSD	–	-0.0916 ^{ns}	0.7682***	0.1574 ^{ns}	0.0033 ^{ns}
Phytophthora foot rot	–	–	-0.0544 ^{ns}	0.0472 ^{ns}	-0.1674 ^{ns}
PLSD	–	–	–	0.0469 ^{ns}	0.0069 ^{ns}
Scab on fruits	–	–	–	–	0.2580 ^{ns}
Scab on leaves	–	–	–	–	–

*** = highly significant, ns = non-significant at $\alpha = 5\%$.

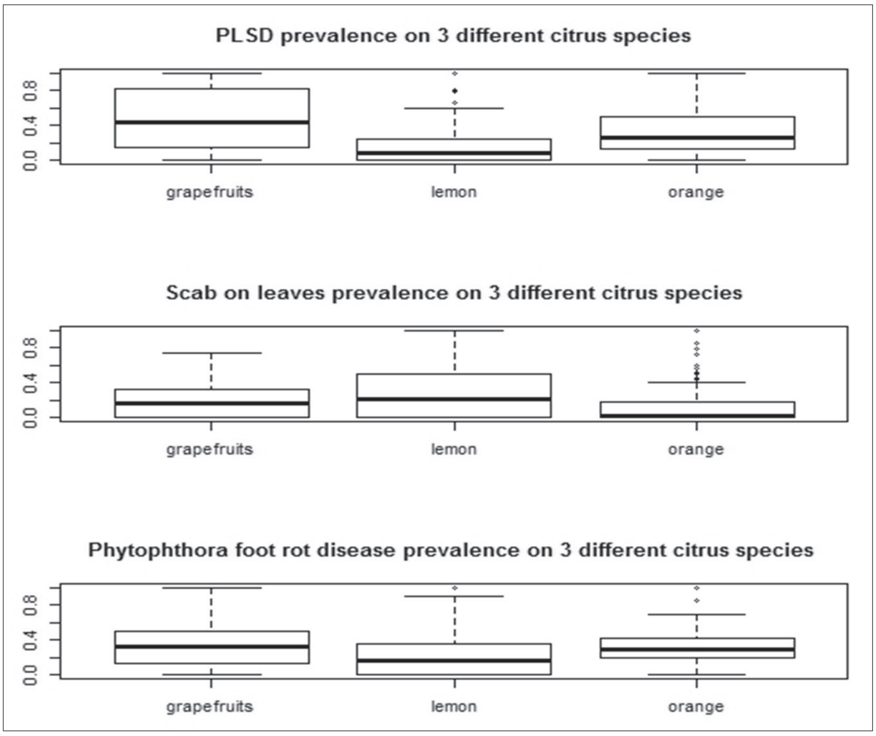

Figure 4. *Pseudocercospora* leaf spot disease (PLSD), Scab on leaves and *Phytophthora* foot rot disease prevalence on grapefruit, lemon and orange trees.

indications and doses) (Seif and Hillocks, 1999; Ndo *et al*., 2010), the difference in disease prevalence between sites or production basins is mostly explained by the susceptibility of tree species used by producers, and ecological conditions.

Factors associated with CTD prevalence on Citrus

The regression analysis showed that Citrus species, vegetation, annual rainfall, *Pseudocercospora* fruit spot disease, *Phytophthora* foot rot disease and scab on leaves were the main variables associated with the prevalence of Citrus tree decline in Cameroon's humid zones (Table 5). The year of observation, soil type, and scab on fruits also had a less significant effect on CTD. Those variables therefore are correlated with the prevalence of CTD. However, correlation does not imply causation.

Pseudocercospora leaf and fruit disease tends to cause leaf drop fairly quickly on new flushes (Kuate, 1998; Kuate *et al*., 2002), so many symptomatic leaves may have already abscised by the time the survey was undertaken, which can explain why the effect of *Pseudocercospora* leaf spot disease is not significant on CTD. Severe attacks of PLSFD can cause leaf drop in such a manner that the presence of this disease on some declining trees may be unnoticed.

As far as scab is concerned, it is known that Citrus scab is a foliar fungal disease that causes damage on leaves and fruits but very rarely enough tree damage to cause leaf drop (Ki Woo Kim *et al*., 2004). Even highly infected leaves or fruits, where visible tissue is difficult to see, do not tend to fall off the tree. So organs infected with scab were still present on trees in decline. Scab cannot therefore be considered as one of the main causes of CTD.

For *Phytophthora* foot rot disease, symptoms are sometimes difficult to detect. The detection of symptoms is easier when disease is at a stage of high severity (Graham *et al*., 2000; Drenth *et al*., 2006). Hence, sometimes the effect of *Phytophthora* foot rot disease on CTD may be neglected or attributed to other factors. Anyway, this study clearly shows the significant effects of this disease on CTD.

In this study area where own-rooted Citrus trees are common, with a high rainfall, *Phytophthora* foot rot disease would be the leading cause of CTD. However, the result may not reflect the real level of *Phytophthora* foot rot disease on the field and thus it is a real contribution to the CTD prevalence. It is therefore necessary in further studies to use laboratory analysis to detect the presence of *Phytophthora* spp. when symptoms are not yet visible.

In Cameroon, rootstocks like *Citrus volkameriana* 'Volkamer' lemon perform well and are less susceptible to *Phytophthora* diseases and *Citrus tristeza* virus; farmers should therefore be encouraged to use plants grafted with such rootstocks which are already used by professionals in tree nurseries, extensionists and researchers to reduce at least the severity of these diseases.

Site parameters that are linked with CTD are mainly vegetation, annual rainfall, and soil texture. These are some of the factors also linked with the development of PLFSD and/or *Phytophthora* diseases (Ndo *et al*., 2010; Graham *et al*., 2014; Futch and Graham, 2012).

In fact, as far as vegetation is concerned, recent studies showed that in cocoa agroforests where Citrus are cultivated in majority in Cameroon, associated trees and their spatial distribution could regulate the dispersion of diseases such as PLFSD or *Phytophthora* foot rot disease (Akoutou *et al*., 2017; Ndo *et al*., 2019). Concerning soil texture, it is known that sandy-clayey soils are suitable for the development of Citrus (Ndo *et al*., 2010). Clayish soils are known to maintain and even prolong soil humidity during rainy seasons, thus favourable for *Phytophthora* diseases (Graham and Timmer, 1992). This confirms that CTD prevalence is associated with a combination of biotic and abiotic factors, which was our initial hypothesis.

Concerning Citrus species, the analysis of variance showed that CTD prevalence was higher on orange and grapefruit trees and less on lemon trees. The regression analysis was also done with CTD prevalence per species. Results showed that CTD prevalence for every species is

TABLE 5. Variables associated with CTD prevalence on Citrus in the different sites of Cameroon's Citrus production basins in the humid zones of Cameroon following a regression analysis.

Source	DF	Root mean square	F value	Pr > F
Species	2	0.40	11.53	<0.0001
Years	2	0.14	4.16	0.0166
Vegetation	2	0.29	8.56	0.0002
Soil type	8	0.09	2.69	0.0147
Altitude	1	0.07	2.09	0.1497
Meant	1	0.02	0.61	0.4354
Mean relative humidity	1	0.00	0.00	0.953
Mean annual rainfall	1	0.35	10.09	0.0016
PFSD	1	1.42	40.94	<0.0001
PLSD	1	0.13	3.86	0.0504
Phytophthora foot rot disease	1	1.29	37.02	<0.0001
Scab on leaves	1	0.43	12.49	0.0005
Scab on fruits	1	0.16	4.82	0.0290
Model's parameters				
R-square	0.372031			
CV	77.37516			
DF	21			
F value	8.41			
Pr > F	<0.0001			

also associated with the highlighted parameters at different levels. It is known that for each of the fungal diseases, there is a species-sensitivity scale. As far as PLFSD is concerned, the dissemination of less susceptible Citrus species has always been one of the major concerns of research (Bella Manga *et al.*, 1999; Kuate *et al.*, 2006b).

While fungal disease can contribute to Citrus tree decline, there are also other important agents like viruses, Citrus greening, and insects, nematodes that could also be contributing to the decline. It would be therefore important to confirm the role played by these agents in further studies, to safely conclude on main factors contributing to Citrus tree decline in Cameroon.

Conclusion

The aim of this study was to determine among many factors namely, Citrus species, observation period, altitude, temperature, rainfall, soil texture, vegetation, *Pseudocercospora* leaf and fruit disease (PLFSD), Citrus scab and *Phytophthora* foot rot disease, those that contribute the most to the development of Citrus tree decline (CTD), a serious constraint faced by Citrus producers in the humid zones of Cameroon.

Our results analysis showed that CTD is present in all the sites and its prevalence differs with sites and Citrus species. Citrus species and environmental factors (rainfall, soil texture, vegetation) were associated with the development of CTD. Although the three diseases are present in almost all sites, *Phytophthora* foot disease and PLFSD was found to be the leading cause of CTD. However, further, studies should consider all the likely causes of CTD, including pests, other Citrus diseases and other environmental factors, to develop a risk assessment model that takes into account all the important parameters.

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References

Agostini, J.P., Bushong, P.M., and Timmer, L.W. (2003). Greenhouse evaluation of products that induce host resistance for control of scab, melanose, and Alternaria brown spot of Citrus. Plant Disease *87*(1), 69–74. https://doi.org/10.1094/PDIS.2003.87.1.69.

Akoutou Mvondo, E., Ndo, E.G.D., Ngo Bieng, M.A., Ambang, Z., Bella Manga, Cilas, C., Tsouga Manga, M.L., and Bidzanga Nomo, L. (2017). Assessment of the interaction between the spatial organization of citrus trees populations in cocoa agroforests and *Phytophthora* foot rot disease of citrus severity. Agroforestry Systems *93*(2), 493–502. https://doi.org/10.1007/s10457-017-0140-3.

Bella-Manga, Dubois, C., Kuate, J., Mimbimi-Ngbwa, M., and Rey, J.Y. (1999). Sensibilité à *Phaeoramularia angolensis* de divers agrumes cultivés en zone forestière humide du Cameroun. Fruits *54*, 167–176.

Cacciola, S.O., and di San Lio, G.M. (2008). Management of citrus diseases caused by *Phytophthora* spp. In Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria, A. Ciancio, and K.G. Mukerji, eds. (The Netherlands: Springer), https://doi.org/10.1007/978-1-4020-8571-0_4.

Dagnew, A., Belew, D., Admassu, B., and Yesuf, M. (2014). Citrus production, constraints and management practices in Ethiopia: The case of *Pseudocercospora* leaf and fruit spot disease. Sci. Technol. and Arts Res. J. *3*(2), 4–18. https://doi.org/10.4314/star.v3i2.2.

Dewdney, M.M., and Timmer, L.W. (2012). Florida Citrus Pest Management Guide: Citrus Scab. (Gainesville: University of Florida, Institute of Food and Agricultural Sciences), 146 pp. http://edis.ifas.ufl.edu.

Diallo, M.T.S. (2003). Vers une lutte contre la cercosporiose des agrumes en Guinée. Fruits *58*(6), 329–344. https://doi.org/10.1051/fruits:2003019.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., Irvine, G., and Irwin, J.A.G. (2006). Development of a DNA-based method for detection and identification of *Phytophthora* species. Australasian Plant Pathol. *35*, 147–159. https://doi.org/10.1071/AP06018.

Futch, S.H., and Graham, J.H. (2012). Field Diagnosis and Management of *Phytophthora* Diseases. (Gainesville: University of Florida, Institute of Food and Agricultural Sciences). http://edis.ifas.ufl.edu.

Gibbon, P. (2003). Value-chain governance, public regulation and entry barriers in the global fresh fruit and vegetable chain into the EU. Developm. Policy Rev. *21*, 615–625. https://doi.org/10.1111/j.1467-8659.2003.00227.x.

Graham, J.H., and Menge, J.A. (2000). *Phytophthora*-induced diseases. In Compendium of Citrus Diseases, L.W. Timmer, S.M. Garnsey, and J.H. Graham, eds. (St. Paul, MN: APS Press), p. 12–15.

Graham, J.H., and Timmer, L.W., (1992). *Phytophthora* diseases of citrus. Plant Diseases of Int. Importance *3*, 250–269.

Graham, J.H., and Timmer, L.W. (2014). Florida citrus pest management guide: *Phytophthora* foot rot and root rot. Rev. September 2013, University of Florida, IFAS Extension. http://edis.ifas.ufl.edu.

Ki Woo Kim, Jae-Wook Hyun, and Eun Woo Park (2004). Cytology of cork layer formation of citrus and limited growth of *Elsinoe fawcettii* in scab lesions. Eur. J. Plant Pathol. *110*, 129–138. https://doi.org/10.1023/B:EJPP.0000015330.21280.4c.

Kuate, J. (1998). Cercosporiose des agrumes causée par *Phaeoramularia angolensis*. Cahiers Agriculture *7*(2), 121–129.

Kuate, J., Fouré, E., Foko, J., Ducelier, D., and Tchio, F. (2002). La phaeoramulariose des agrumes au Cameroun due à *Phaeoramularia angolensis*: expression parasitaire à différentes altitudes. Fruits *54*(4), 207–218. https://doi.org/10.1051/fruits:2002018.

Kuate, J. (2003). La cercosporiose des agrumes au Cameroun provoquée par *Phaeoramularia angolensis*: Evaluation de la lutte chimique et de la sensibilité variétale au champ comme méthode de contrôle. Mémoire du Diplôme d'Etudes Approfondies (Gembloux, Belgique: Communauté Française de Belgique, Faculté des Sciences Agronomiques), 93 pp.

Kuate, J., Bella-Manga, Damesse, F., Kouodiekong, L., Ndindeng, S.A., David, O., and Parrot, L. (2006a). Enquête diagnostic sur les fruitiers dans les exploitations familiales agricoles en zone humide du Cameroun. Fruits *61*, 373–387. https://doi.org/10.1051/fruits:2006037.

Kuate, J., Foko, J., Ndindeng, S.A., Jazet-Dongmo, P.M., Fouré, E., and Damesse, F. (2006b). Effects of essential oils from citrus varieties on *in vitro* growth and sporulation of *Phaeoramularia angolensis* causing citrus leaf and spot disease. Eur. J. Plant Pathol. *114*, 151–161. https://doi.org/10.1007/s10658-005-2928-7.

Mariau, D. (1999). Les Maladies des Cultures Pérennes Tropicales (France : CIRAD ed.).

Ndo, E. (2007). Analyse du risque épidémiologique des populations d'agrumes vis à vis de la cercosporiose du scab et de la gommose dans les zones humides du Cameroun. M.Sc. Thesis (Université de Dschang, Faculté d'Agronomie et des Sciences Agricoles), 118 pp.

Ndo, E.G.D., Bella-Manga, F., Ndindeng, S.A., Ndoumbé-Nkeng, M., Fontem, A.D., and Cilas, C. (2010). Altitude, tree species and soil type are the main factors influencing the severity of *Phaeoramularia* leaf and fruit spot disease of citrus in humid zones of Cameroon. Eur. J. Plant Pathol. *128*, 385–397. https://doi.org/10.1007/s10658-010-9660-7.

Ndo, E.G.D., Akoutou Mvondo, E., Ambang, Z., Bella Manga, Cilas, C., Bidzanga Nomo, L., Gidoin, C., and Ngo Bieng, M.A. (2019). Spatial organisation influences Citrus *Pseudocercospora* leaf and fruit spot disease severity in cocoa-based agroforestry systems. Am. J. Plant Sci. *10*, 221–235. https://doi.org/10.4236/ajps.2019.101017.

Nelson, S. (2008). Citrus scab, Plant Disease (Mãnoa: University of Hawaï, Cooperative Extension Service).

Olife, I.C., Ibeagha, O.A., and Onwualu, A.P. (2015). Citrus fruits value chain development in Nigeria. J. Biol., Agric. and Healthcare *5*(4), 37–48.

Seif, A.A., and Hillocks, R.J. (1999). Reaction of some citrus cultivars to *Phaeoramularia* fruit and leaf spot in Kenya. Fruits *54*(3), 23–29.

Suhr, K.I., and Nielsen, P.V. (2003). Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J. Appl. Microbiol. *94*, 1–11. https://doi.org/10.1046/j.1365-2672.2003.01896.x.

Temple, L. (2001). Quantification des productions et des échanges de fruits et légumes au Cameroun. Cahiers Agriculture *10*, 87–94.

Timmer, L.W., Priest, M., Broadbent, P., and Tan, M.K. (1996). Morphological and pathological characterization of species of *Elsinoë* causing scab diseases of citrus. Phytopathology *86*(10), 1032–1038. https://doi.org/10.1094/Phyto-86-1032.

Tyson, J.L., and Fullerton, R.A. (2001). Disease notes or new records. First report of benomyl resistance in *Elsinoe fawcettii* in New Zealand citrus orchards. Australasian Plant Pathol. *30*, 69. https://doi.org/10.1071/AP01059.

Yesuf, M. (2007). Distribution and management of *Phaeoramularia* leaf and fruit disease of citrus in Ethiopia. Fruits *66*(2), 99–106. https://doi.org/10.1051/fruits:2007003.

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