Comparison of the prevalence of *Cocoa Swollen shoot virus* and the prevalence of *Phytophthora sp* in Petit-Bondoukou, South-West of Côte d'Ivoire

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Abstract— Background: Swollen shoot and Black pod disease are two major diseases that cause significant damage in Nawa region. In order to compare the prevalence of these two diseases, a study was conducted in Soubré area in the south-west of Côte d'Ivoire, at the site of Petit-Bondoukou. Methods: The observation system consists of a sentinel site of approximately 10 km X 10 km. Each sentinel site is made up of 16 clusters of 2.5 km X 2.5 km containing about 10 plots which constitute the different observation points. In this study, observations were made in 4 plots of the site. The variables that were measured during data collection were the total number of pods on each tree, the total number of pods with black pod disease symptoms and the total number of diseased trees in the swollen shoot outbreaks. The prevalence of each pathogen was deduced from the measured variables and then related using the Bravais-Pearson correlation test. Beforehand, a molecular analysis was carried out to identify the different species of Phytophthora. Results: The results of the molecular analysis showed that the only species identified at this site was Phytophthora palmivora. The descriptive analysis showed that the prevalence of CSSV was higher than that of Black pod disease with 50% and 32% respectively. Statistical analysis showed that there is a positive and significant correlation between the prevalence of swollen shoot and black pod disease. Conclusion: This study shows that most of the test trees at the site of Petit-Bondoukou show both Swollen shoot and Black pod disease symptoms and invites growers contribute to reducing the pressure of both diseases by leading regular activities plot maintenance.

Keywords— Cocoa trees, CSSV, black pod disease, Prevalence, Côte d'Ivoire.

I. INTRODUCTON

The cocoa tree (*Theobroma cocoa L.*) is a tropical perennial crop of Malvaceae family (Motamayor *et al.*, 2002) formerly classified in the Sterculiaceae family (Metcalfe and Chalk, 1950). The cocoa tree is native to South America and its origin is in the Amazon basin (Motamayor *et al.*, 2002). Cocoa cultivation has been introduced in various West African countries such as Ghana, Cameroon, Nigeria, Togo and Côte d'Ivoire where large plantations have been established (Janny *et al.*, 2003). The extension of cocoa orchards in Côte d'Ivoire is linked to land availability and an attractive and remunerative pricing policy. This is the reason why Côte d'Ivoire has been the world's leading cocoa producer since 1977 (Tano, 2012). This crop is therefore of great importance in the Ivorian economy where it accounts for 40% of export earnings and contributes up to 15% of gross domestic product (GDP) (Tano, 2012; Serges, 2014). However, these important socio-economic achievements related to cocoa production in Côte d'Ivoire should not make us lose sight of the many constraints related to diseases, pests and market price fluctuations (Freud *et al.*, 2000). The consequences of these constraints are lower production and

increased poverty in rural areas. In Côte d'Ivoire, black pod disease and swollen shoot are the two main diseases that pose a major threat to cocoa production (Kouakou *et al.,* 2011).

Black pod disease is caused by *Phytophthora spp*., with 44% crop losses (CNRA, 2017). In areas most favourable to black pod disease, losses can reach 80% of the crop (Koua et al., 2018). In addition, the reappearance of the swollen shoot virus disease in 2003 in the Ivorian orchard (Kébé and N'guessan, 2003) after 60 years, amplifies the threat to cocoa production in Côte d'Ivoire. Swollen shoot is one of the most significant viral diseases in the world (Dzahini-Obiatey *et al.,* 2010). The Cocoa Swollen shoot Virus (CSSV) responsible for this disease belongs to the genre Badnavirus and is transmitted by mealybugs of the Pseudococcidae family (N'guessan *at al.,* 2016). Swollen shoot disease has not progressed since its discovery in 1946 (Alibert, 1946), and it was only in 2003 that new outbreaks were discovered in the central-western part of the country, notably in Sinfra, Issia and Bouaflé (Kébé and N'guessan, 2003). Today, the swollen shoot species discovered in the new outbreaks are gradually spreading in the Ivorian cocoa plots to the point of posing a real threat to the major cocoa-producing basins such as the Soubré region in the south-west of Côte d'Ivoire. In addition to the damage caused by the swollen shoot disease (Fig.1), there is also the damage caused by black pod disease (Fig.2), thus increasing the risk of a drop in cocoa production. The various known pathogens that commonly cause black pod disease are represented by *Phytophthora palmivora* and *Phytophthora megakarya* (Kébé *et al.*, 2009).

In Côte d'Ivoire, *Phytophthora megakarya* which did not exist in cocoa plots is also beginning to spread significantly (Coulibaly *et al.*, 2013). This species is however the most aggressive (Brasier and Griffin, 1979) unlike *P. palmivora* which is the least aggressive and most widespread species. In addition, the prevalence of *P. megakarya* is poorly known in Côte d'Ivoire and an interaction between *P. megakarya* and *Cocoa Swollen shoot Virus* (CSSV) is suspected. This is the reason this study is being conducted.

The objective of this study is to compare the prevalence of swollen shoot virus and that of *Phytophthora sp* in order to assess the damage caused in plantations by each pathogen. Specifically, the aim is to first characterize the species responsible for black pod disease and then to study the relationship between the prevalence of CSSV and the prevalence of *Phytophthora sp*.

Fig. 1: The different indicators in the plot affected by swollen shoot disease.

(A): redness on young leaves of cocoa trees, (B): Outbreak of swollen shoot disease, (C): Cocoa tree stem swelling (Photo taken by Oro Franck)

Fig. 2 : Symptoms of black pod disease on immature cocoa pod (Photos taken by Oro Franck)

II.   MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Petit Bondoukou site (Fig.3) located 51 kilometers from Soubré town in Côte d'Ivoire. The Soubré region is currently the main cocoa production area in Côte d'Ivoire. This site is characterized by a humid tropical climate with an average rainfall of 1,485 mm per year, and an average temperature of 25.8°C per year. Vegetation and soil are characterized by dense, humid forests and a deep, permeable and well-drained soil that allows for anthropogenic activities related to agriculture (N'go *et al.*, 2012). This vegetation is now giving way to shreds of forest and huge plantations of traditional or industrial perennial crops (Le paysan, 2012).
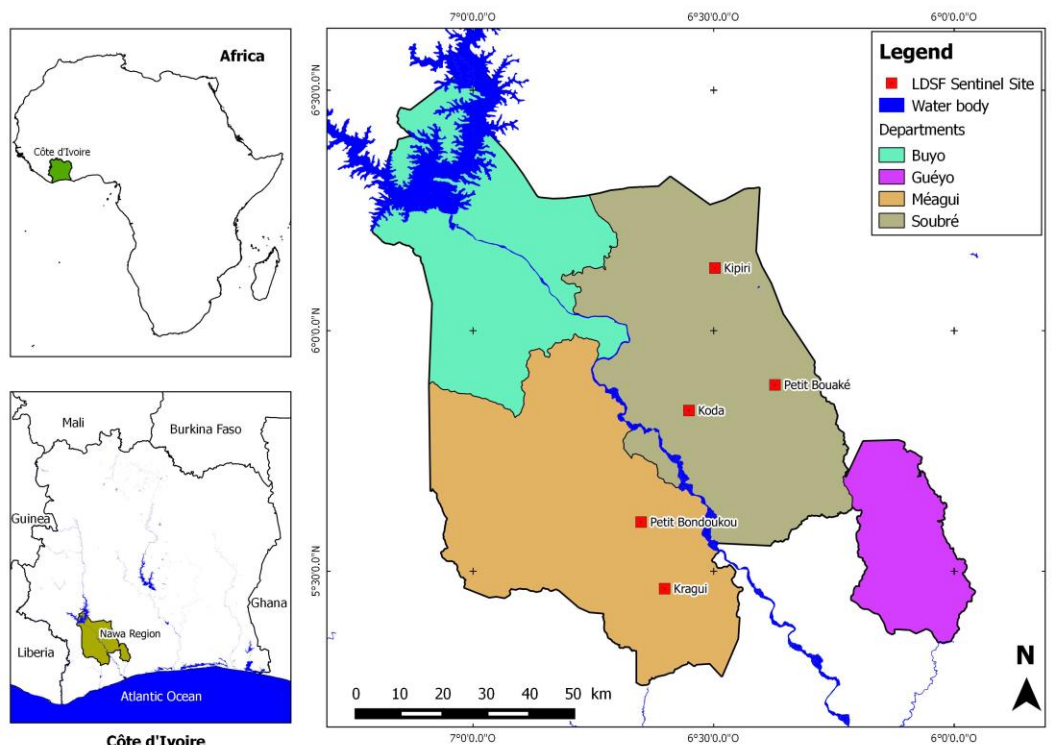

Fig. 3 : Map of the study area showing the site of Petit-Bondoukou (Diby et al., 2014).

2.2. Experimental device

The experimentation took place in peasant cocoa farms based on prospection surveys. These surveys collected data on swollen shoot and *Phytophthora sp*. These surveys were conducted according to the Land Degradation Surveillance Framework (LDSF) protocol (Diby *et al.,* 2014). The LDSF (Figure 4) is a protocol that was initially developed to monitor soils suitable for agriculture in West Africa. This protocol was adapted in this study for epidemiological investigations of swollen shoot disease and Black pod disease caused by *Phytophthora sp* (Diby *et al.,* 2014). The LDSF represents a sentinel site of 10 Km × 10 Km. The site is divided into 16 clusters of 2.5 Km × 2.5 Km (Fig.4). Each cluster has 10 observations points, called plots.
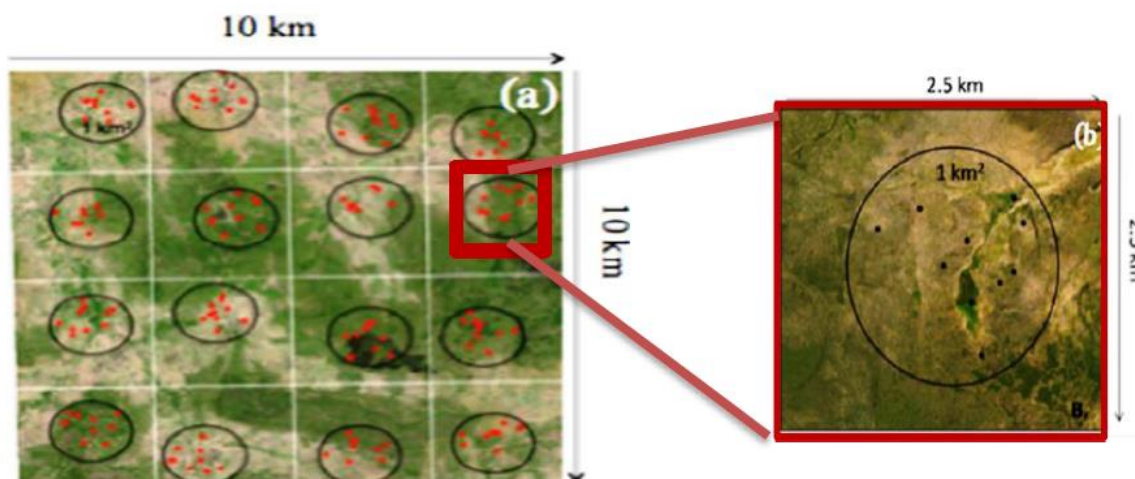

Fig.4 : Land Degradation Surveillance Framework (LDSF) Device (Diby et al., 2014)

2.3. Observation and data collection

The data collected are related to swollen shoot and Black pod disease. These data were collected in 4 plots of the Petit-Bondoukou site that were randomly selected. Observations were made on 10 cocoa trees in each plot, corresponding to 40 test trees.

II.3.1 Collection of CSSV data

The CSSV data collection was carried out at each observation plot, previously identified using the Garmin 64s GPS. Around the plot, an observation area with a radius of 50 m was delimited. In this observation area, infection outbreak were detected on the basis of several indicators including clearings (Fig.1B), leaf symptoms (Fig.1A) and stem symptoms (Fig.1C). Symptoms on young leaves are represented by redness along the veins and discoloration while stem symptoms are related to swellings on young stems. In each observation area several outbreaks were detected in case of presence of swollen shoot disease. Epidemiological data were collected from each outbreak. It is in these outbreaks that the number of healthy and diseased cocoa trees were counted. The areas of the outbreaks were measured using a Garmin 64 S GPS. A prepared survey form was then filled in to compile the physical data.

2.3.2. Collection data of *Phytophthora sp*

The *Phytophthora sp* data were collected in the same area as the CSSV data. In contrast to the CSSV data, epidemiological data on black pod disease were collected within a radius of 300 meters around the same test plots (Fig.5). In the case of this study, surveys were only conducted in four (04) plots on the site of Petit-Bondoukou. For each test tree, the total number of pods was counted, as well as the number of pods affected by black pod disease. For the characterization of isolates of *Phytophthora* species, two samples of pods with symptoms were taken from each test tree. These sampled pods with symptoms were wrapped in newsprint to slow down the development of *Phytophthora sp*, and then labelled with an identifying code. These pod samples were cross-sectioned along the progressing front of the rotten spot, then a rot explant was taken from the cortex and from an eppendorf tube in the presence of a medium culture favourable to the development of *Phytophthora sp*. The different tubes were labelled with the codes corresponding to the identifier of sample pods.
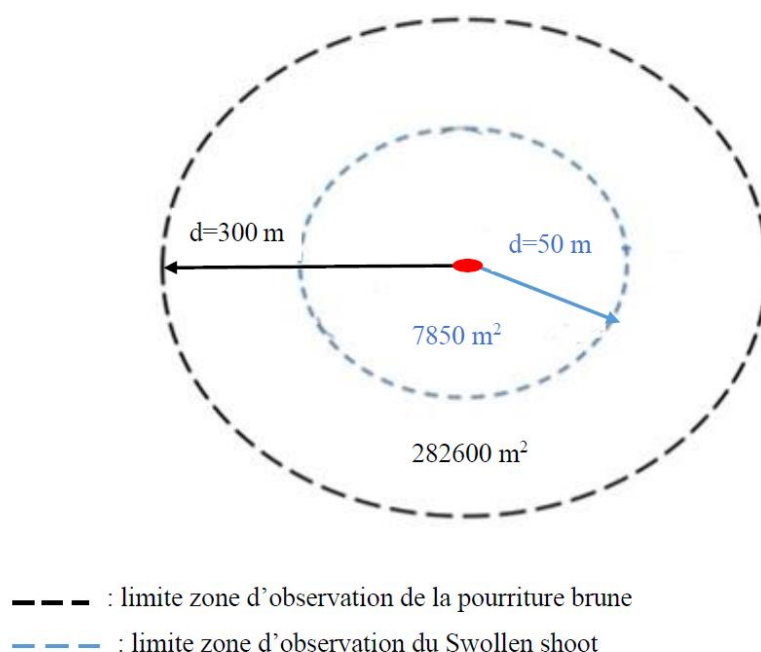
d=300 m   d=50 m

7850 m²

282600 m²

━ ━ ━ : limite zone d'observation de la pourriture brune

━ ━ ━ : limite zone d'observation du Swollen shoot

Fig.5 : Observation areas and data collection for CSSV and Phytophthora sp (Diby et al., 2014).

2.4. Data Analysis

Data analysis included molecular analysis to identify the different species of *Phytophthora sp* and statistical analysis to assess the relationship between the prevalence of CSSV and that of black pod disease.

2.4.1. Molecular analysis

Molecular analysis of black pod disease data was carried out at the CIRAD Joint Research Unit on Biology and Genetics of Plant-Parasite Interactions in Montpellier, France. This analysis allowed the identification of different *Phytophthora* species. In the laboratory, the sampled explants were grown in Petri dishes on the "V8" medium culture, which consists of 1/10 of a vegetable juice cocktail, agar at 15g / L and CaCO3 at 3g / L for 4 days in the dark, at 25°C. After 4 days, the agar explants containing *Phytophthora sp* mycelium culture were removed from the fungal growth front with a scalpel and then transplanted into the V8 medium culture under the same conditions described above for 7 days. The resulting mycelium is used for DNA extraction. In case the strains were contaminated with other fungi, several transplants on H2O-Agar culture medium at 15g/L were necessary to purify the strain. After purification, the strain is cultured again on "V8" medium at 25°C for 4 days and then 7 days in the dark to obtain a mycelium typical of *Phytophthora sp*. The mycelium obtained after purification is used for DNA extraction. The identification of the different species of *Phytophthora* was performed by

PCR (*Polymerase Chain Reaction*) with species-specific ITS primers (*P. megakarya* and *P. palmivora*).

2.4.2. Determination of the prevalence of the two plant pathogens

The prevalence of swollen shoot disease is the ratio of the number of diseased cocoa trees counted to the total number of cocoa trees sampled in the observation area. This prevalence is represented by equation 1. The prevalence of black pod disease is obtained from the ratio of the number of rotten pods to the total number of pods on the selected trees in the plot according to equation 2. In addition, the prevalence of black pod disease for each species of *Phytophthora sp* was also determined from the ratio of the number of pods infected by each species to the total number of pods sampled at the site. This prevalence was obtained according to equation 3.

$$\text{P CSSV (\%)} = \frac{\text{Number of diseased trees sampled}}{\text{Total number of trees sampled from pl}} \times 100 \quad \textbf{(1)}$$

$$\text{P Phyto (\%)} = \frac{\text{Number of rotten pods}}{\text{Total number of pods on the plot}} \times 10 \quad \textbf{(2)}$$

$$\text{P Phyto sp (\%)} = \frac{\text{Number of pods infected by species}}{\text{Total number of pods sampled on site}} \times 100 \quad \textbf{(3)}$$

P CSSV (%): Prevalence of Swollen shoot disease

P Phyto (%): Prevalence of black pod disease

P Phyto sp (%): Prevalence of each *Phytophthora* species

2.4.3. Statistical analysis

Statistical analysis was applied on the prevalence of swollen shoot disease and the prevalence of black pod disease. This analysis first provided a description of these two quantitative variables. Second, it evaluated the relationship between the two variables using the Bravais-Pearson correlation test in SPSS Statistics 20 software.

III. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1. Different species identified

Molecular analysis revealed a diversity of species of the genre *Phytophthora*, of which only one was identified as *Phytophthora palmivora*. The results of molecular analysis showed that the other *Phytophthora* species are more dominant than *Phytophthora palmivora* at 93% compared to 8% of the pods sampled (Table I). This indicates a prevalence of 23% for other *Phytophthora* species compared to nearly 2% for *Phytophthora palmivora* (Table II). The species *Phytophthora megakarya* was not identified at the Petit-Bondoukou site.

3.1.2. Description of prevalences of Swollen shoot disease and black pod disease

The result of the descriptive analysis (Table III) shows that the prevalence of swollen shoot disease at the site of Petit-Bondoukou is between 0% and 100%, for an average of 50% ± 32%. The prevalence values of Swollen shoot disease rather scattered around the average show that the prevalence of swollen shoot disease differs strongly from one plot to another at the site of Petit-Bondoukou. In addition, more than half of the plots are affected by swollen shoot disease (Table IV). This shows that there are more diseased trees (53%) than healthy trees (48%).

Black pod disease also affects this site with a prevalence of between 21% and 39%. The average prevalence of black pod disease over the whole site is 32 ± 7%. The prevalence of black pod disease less dispersed around the average explains why the Black pod disease differs little or not at all from one plot to another on the site of Petit-Bondoukou.

Table I: Number of trees infected by the different species of Phytophthora identified at the site of Petit-Bondoukou

Phytophthora species	Number of infected trees	Percentage
P. palmivora	3	7,5
P. megakarya	0	0
Other species	37	92,5
Total	40	100

Table II : Prevalence of each species of Phytophthora identified at the site of Petit-Bondoukou

	Number of infected pods	Prevalence (%)
Phytophthora megakarya	0	**0,00**
Phytophthora palmivora	17	**1,62**
Other species of *Phytophthora*	237	**22,59**
Total number of infected pods	254	**24,21**
Total sampled pods		
	1049	

Table III : Results of the descriptive analysis of the prevalence of Swollen shoot and Black pod disease

	N	Minimum	Maximum	Average	Standard Deviation
Prevalence CSSV (%)	40	00	100	50	35,81
Prevalence phyto (%)	40	21,36	38,74	31,82	6,98

Table IV : CSSV status of sampled trees

Status of trees	Number	Percentage
Diseased	21	52,5
Healthy	19	47,5
Total	40	100

3.1.3. Relationship between the prevalence of swollen shoot and black pod disease

The result of the Bravais-Pearson correlation test (Table V) showed that there is a positive and significant correlation (r=0.814; p = 0.000) between the prevalence of CSSV and the prevalence of *Phytophthora sp.* This shows that the development of one of these diseases favours the development of the other.

Table V: Result of the correlation test between the prevalence of CSSV and the prevalence of Phytophthora sp.

Correlation		Prevalence CSSV (%)	Prevalence *Phytophthora*
Prevalence CSSV (%)	Correlation of Pearson	1	**0,814**[**]
	Sig. (bilateral)		**0,000**
	N	40	40

** The correlation is significant at the 0.01 level (bilateral).

3.2. DISCUSSION

The result of the descriptive analysis showed that swollen shoot disease is very present in Petit-Bondoukou with a proportion of 50%. This result is in line with the results of the work of Diby *et al* (2014) which showed that the Petit-Bondoukou site is a sentinel site with a high presence of swollen shoot disease with a prevalence rate of 63.4%.The results of molecular analysis also showed that the *Phytophthora* species identified at the study site are *Phytophthora palmivora* and *other Phytophthora* species that were not the subject of this study. The species *Phytophthora megakarya* has not been identified at the site of Petit-Bondoukou. This result is in agreement with the work of Babacauh (1980) which showed that the only *Phytophthora* species responsible for black pod disease were *Phytophthora palmivora* and *Phytophthora citrophthora*. From the above, damage related to other species not identified in this study could be linked to *Phytophthora citrophthora*. Indeed, the absence of *Phytophthora megakarya* at the site of Petit-Bondoukou could be explained by the sampling period which took place towards the end of August. At this period, opportunistic pathogens take over the development of *Phytophthora palmivora* and *P. megakarya* (Kébé *et al.,* 2001; Koné, 1999). Statistical analysis has shown that the presence of CSSV is significantly related to the presence of black pod disease, i.e. the presence of one of these diseases favours the occurrence and/or development of the other. This result shows that both diseases are increasing strongly at the site of Petit-Bondoukou. This means that most of the test trees show both signs of swollen shoot and black pod disease.

Indeed, observations were made on early-infected trees still showing a high level of leaf cover. This high rate of leaf cover could be at the origin of a humid microclimate (Yao et al., 2014; Koua et al., 2018) which could favour the dispersal of the *Phytophthora* pathogen, as well as the development of scale insects that are vectors of swollen shoot disease. This is in line with the studies carried out by Bigger (1972) and Nguyen-ban (1984) which reveal that these climatic conditions favour the outbreak of mealybugs in cocoa trees. These arguments are supported by Brasier and Hansen (1992) who showed that rainfall favours the establishment and development of phycomycetes diseases such as black pod disease due to *Phytophthora sp*. As for mealy bugs of the family Pseudococcidae, the vector species of the swollen shoot virus, have a development cycle which varies according to climatic conditions including temperature, humidity and according to the host plant (Kenza, 2017). According to Goldasteh *et al* (2009) the optimal temperature for development of scales to adulthood is between 15° and 32°C. These development conditions correspond to the climatic parameters of the Soubré region where swollen shoot disease occurs.

IV.   CONCLUSION

Swollen shoot and black pod diseases are two major cocoa diseases that cause significant losses in the region of Soubré. At the end of this study, it appears that the site of Petit-Bondoukou is a sentinel site with a high presence of swollen shoot disease. Molecular analysis showed that the only species identified on this site is *Phytophthora palmivora* although there is a strong propensity for other unidentified species responsible for black pod disease. This study also showed that there is a strong positive correlation between the prevalence of swollen shoot and the prevalence of Black pod disease. This shows that most of the test trees at the site of Petit-Bondoukou show both Swollen shoot and Black pod disease symptoms. To limit the spread of these two diseases, firstly, regular plot maintenance should be carried out by regular weeding and regular pruning of the plots to adjust the light and thus break the cycle of *Phytophthora sp*. Secondly, to limit the spread of Swollen shoot, growers should be made aware of the recognition of symptoms so that they can take action by destroying the first trees bearing the symptoms of the disease. This combined action could greatly contribute to reducing the pressure of both diseases.

V.   CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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