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CASSAVA MOSAIC DISEASE INCIDENCE AND SEVERITY AND WHITEFLY VECTOR DISTRIBUTION IN GABON

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ABSTRACT

Cassava mosaic disease (CMD) is a major constraint to cassava (*Manihot esculenta* Crantz) production in sub-Saharan Africa. The objective of this study was to gain insights into the epidemiology of CMD in cassava production systems in Gabon. An epidemiological survey was conducted throughout Gabon from October 2020 to May 2021 to evaluate the epidemiology of cassava mosaic disease (CMD) in cassava farms. A phytosanitary diagnosis was conducted at each farm, using the harmonised and unified WAVE protocol to assess the incidence and severity of CMD and the abundance of its whitefly vector. All data collected were recorded using WAVE's mobile application and uploaded into the WAVE Cube data system. The results showed that CMD was present in all farms surveyed (n = 227; 100%), but with a variable incidence rate. At national level, the incidence of CMD was high, with an average of 64.29%. However, incidence differed among regions with 82.22 and 79.20% for Ogooue-Maritime and Nyanga; respectively, but only 40.42% for Estuaire. Woleu-Ntem had the highest mean severity (3.47) and Ogooue-Maritime the lowest (2.64). Overall, incidence and severity differed significantly among regions ($P< 0.05$). The average abundance was 2.58 whiteflies per plant (w/p), varying between 1.06 (Nyanga) and 5.25 w/p (Estuaire). The use of infected cuttings was the main mode of CMD spread (62.67%). These results highlight the need to identify the viruses responsible for the observed cases of CMD, to sanitise cultivated plant material, and to implement a CMD control strategy in Gabon.

Key Words: *Bemisia tabaci*, epidemiology, *Manihot esculenta*

RESUME

La maladie de la mosaïque du manioc (CMD) est une contrainte majeure à la production de manioc (*Manihot esculenta* Crantz) en Afrique subsaharienne. L'objectif de cette étude était de mieux comprendre l'épidémiologie de la CMD dans les systèmes de production de manioc au Gabon. Une enquête épidémiologique a été menée dans tout le Gabon d'Octobre 2020 à Mai 2021 afin de mieux connaitre l'épidémiologie de la mosaïque du manioc. Un diagnostic phytosanitaire a été fait dans chaque champ prospecté. L'incidence, la sévérité de la mosaïque et l'abondance des mouches blanches vectrices ont été estimées en utilisant le protocole harmonisé et unifié du programme WAVE. Toutes les données de terrain ont été saisies grâce à l'application mobile de WAVE puis stockées dans le gestionnaire de données de WAVE, le Cube. Les résultats de cette étude ont montré que la mosaïque du manioc était présente dans toutes les zones prospectées (n = 227; 100%), avec une incidence variable. Au niveau national, l'incidence de la CMD était élevée, avec une moyenne de 64,29% pour une sévérité moyenne de 3,16. Cette incidence varie entre les provinces avec respectivement 82,22% et 79,20% pour l'Ogooué-Maritime et la Nyanga, mais seulement, 40,42% pour l'Estuaire. La région du Woleu-Ntem a présenté la plus grande sévérité moyenne (3,47) et l'Ogooué-Maritime, la plus faible moyenne (2,64). L'incidence et la sévérité ont différé significativement entre les régions (P< 0,05). L'abondance moyenne des mouches blanches était de 2,58 mouches/plante (m/p). Cette abondance a oscillé entre 1,06 m/p (Nyanga) et 5,25 m/p (Estuaire). L'utilisation des boutures de manioc infectées est le principal mode de propagation de la CMD (62,67%). Ces résultats préliminaires mettent en exergue la nécessité impérieuse d'identifier les virus responsables des cas de CMD observés, d'assainir le matériel végétal cultivé et de mettre en place une stratégie de contrôle de la CMD au Gabon.

Mots Clés: *Bemisia tabaci,* épidémiologie, *Manihot esculenta*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the world's fourth most important food crop after maize, wheat and rice (FAO, 2021). Cassava is a high-energy food (64 - 87% dry matter) (Diallo *et al.*, 2013), with calories ranging from 125 to 140 kcal per 100 g of fresh peeled cassava (Macrae *et al.*, 1993).

In Gabon, cassava is the second most important food crop with annual production of 337,209 metric tonnes (FAO, 2021). It is consumed by more than 80% of the population in various forms at a rate of 159 kg per^{-1} *capita* per year (FIDA, 2008). Thus, it is considered one of the strategic crops for the revival of the agricultural sector in Gabon, as it provides income for numerous small-scale producers and ensures their food security (FAO, 2012). However, current production levels are too low (annual deficit stands at 81,000 tonnes) (FIDA, 2008) to cater for the food needs of the Gabonese population. This low cassava yield is linked to pests and disease attacks, including cassava mosaic disease (CMD) (FAO, 2012). This viral disease is widespread in cultivated areas of cassava in Africa (Adjata *et al.*, 2008) and can reduce yields by 40 - 70% (Legg and Fauquet, 2004). Studies have shown the extensive presence of CMD in Cameroon, the Republic of Congo and Gabon (Legg *et al.*, 2004; Ntawuruhunga *et al.*, 2007).

Several cassava mosaic geminiviruses (family *Geminiviridae*; genus *Begomovirus*) can cause CMD (Adjata *et al.*, 2008). These viruses are transmitted by carrier insects, mainly the whiteflies (*Bemisia tabaci*, Hemiptera: Aleyrodidae) in the genus *Bemisia* (Tocko-Marabena *et al.*, 2017). These widespread, polyphagous insects proliferate at the beginning of the rainy season in cassava-growing areas, where they feed on the phloem of plants. In Africa, CMD can be attributed to nine virus species, namely African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus, East African cassava mosaic Zanzibar virus, East African cassava mosaic Malawi virus, East African cassava mosaic Kenya virus, South African cassava mosaic virus (Bisimwa Basengere, 2012), African cassava mosaic Burkina Faso virus and Cassava mosaic Madagascar virus (Tiendrebeogo *et al.*, 2012; Harimalala *et al.*,2013). This disease is generally recognised by different symptoms such as yellow or light green discolouration of the leaves with or without distortion, shrinking of the leaf surface, thinning of the veins and stunting of the plants (Sseruwagi *et al.*, 2004; Zinga, 2012).

In Gabon, previous studies on cassava virus species have indicated the presence of ACMV, EACMV and EACMV-Uganda (EACMV-Ug) in some villages in Estuaire, Woleu-Ntem, Ogooue-Ivindo, Ngounie, Moyen-Ogooue and Nyanga regions (Legg *et al.*, 2004; Delêtre *et al.*, 2021). CMD can cause yield losses of up to 90% and socio-economic disasters; the severe 1990 epidemic in East Africa caused an annual economic loss of US$1.9 - 2.7 billion, famine and a high death toll (Legg *et al.*, 2006). This study is aimed at gaining an insights in the epidemiology of CMD in cassava production systems in Gabon.

MATERIALS AND METHODS

An epidemiological survey on CMD was part of an African regional programme known as the "Central and West African Virus Epidemiology (WAVE) for food security". The survey was conducted within the WAVE programme during October 2020 to May 2021. Within this framework, data collection and storage were carried out using the WAVE harmonised and standardised tools, including CMD data management through an innovative and comprehensive cloud-based data storage system (WAVE Cube).Data were recorded using a tablet with the survey software (iForm Zerion version 9.1.6) developed by the University of Cambridge, UK's Epidemiological Modelling Group.

This study was carried out using a harmonised field sampling protocol adopted by the WAVE programme, which includes Gabon, and based on methods previously described in Sseruwagi *et al.* (2004). In all, 227 cassava farms in accessible areas of the nine regions of Gabon were surveyed (Fig. 1) and their geographical coordinates (latitude, longitude and altitude) were taken with GPSMAP 64s device. In each region, the farms surveyed were selected on the basis of their proximity to villages, accessibility by road and cassava cultivation intensity. These cassava farms, growing plants that were 3–6 months old, were located approximately 10 Km apart in order to capture potential virus diversity for future work. In each farm, five epidemiological parameters were recorded, namely CMD symptoms, CMD incidence, CMD severity, abundance of whiteflies and mode of disease spread.

Plant material collection. In each cassava farm surveyed, diseased and healthy leaf samples were taken from three different plants. The collected samples were representative of all levels of disease severity within a field. Each leaf sample was coded with the first three letters of the region and district; and the first four letters of the village or town/neighbourhood where the sample was collected. The sample was numbered 001 NGO-DOO-MOUT. All collected samples were packed in duplicate A5 envelopes and placed in the herbarium press for preservation. The herbarium press was assembled by carefully gluing the sampled cassava leaves to corrugated boards.

Abundance of whiteflies. To assess the presence of whiteflies, we focused on the first five apical leaves of the 30 cassava plants selected in a similar 'X' diagonal manner as stated below for CMD data collection. We carefully turned each of these leaves to make

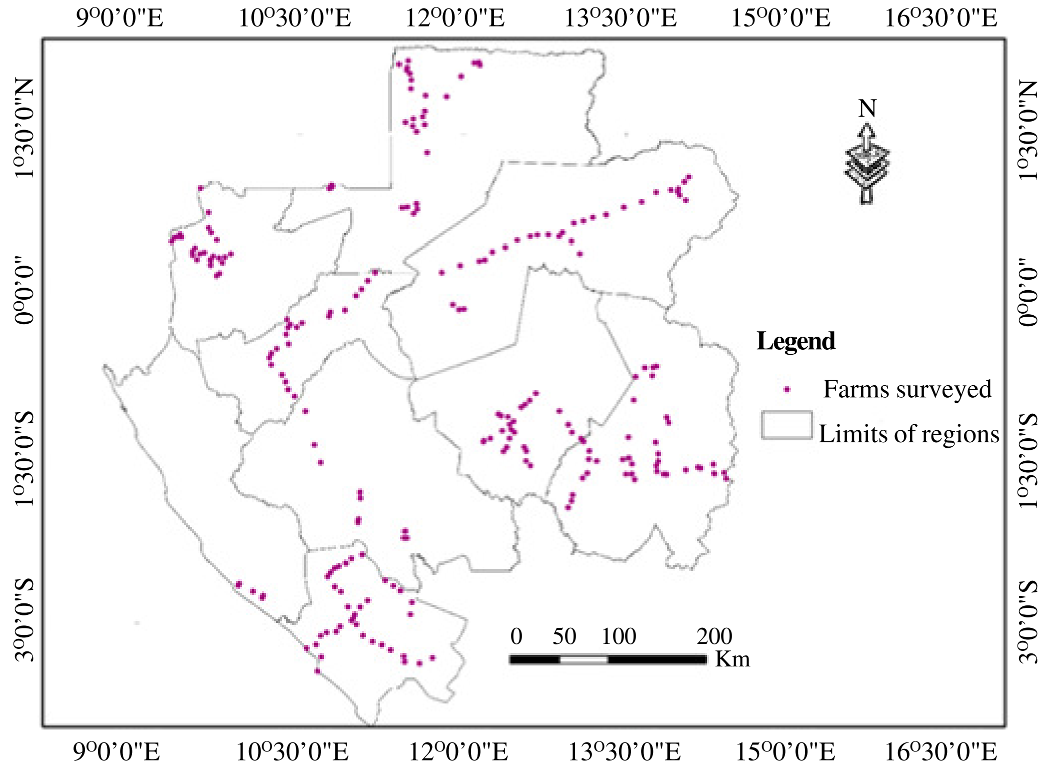

Figure 1. Surveyed areas

the back visible, and then counted all adult white flies present. This information was recorded in the WAVE mobile survey application (built in the iForm 9.12.7 software by the University of Cambridge, UK), using an electronic recording device. The number of whiteflies per plant was estimated using the method of Bah *et al.* (2011) (Table 1). Whitefly specimens were collected from the selected cassava plants using a fly aspirator (Tocko-Marabena *et al.*, 2017). The whitefly samples were placed in 1.5 ml Eppendorf tubes containing 70% ethanol for specimen preservation, pending molecular analysis. To calculate the average w/p, we divided the total number of whiteflies counted in the field by the total number of plants sampled.

Data recording. All field survey data were recorded using a tablet and the WAVE mobile survey application (built in the iForm software, as described above). This survey application consists of a questionnaire with open-ended questions, multiple-choice questions and automatically generated supplementary questions. Data were uploaded from the tablet into iForm's cloud-based database and then integrated into the WAVE Cube –a novel, multi-dimensional database that was developed to hold centrally all the field survey data collected in the different countries of the WAVE network.

TABLE 1. Assessment scale of whitefly abundance according to Bha *et al.* (2011)

Scale	Abundance assessment
1 - 50 whiteflies/plant	Low
51 - 100 whiteflies/plant	Medium
>100 whiteflies/plant	High

The information collected *via* the iForm application (and accessed through the WAVE Cube) is shown in Table 2.

Recording disease: symptomatic and asymptomatic plants. Images of the characteristic symptoms of CMD were obtained using a Canon EOS 250 D digital camera. Following this, CMD symptoms were observed on 30 randomly selected cassava plants along two 'X' diagonals. The symptoms recorded included leaf shape (filiform, distorted and mosaic pattern) and leaf colouration.

Determination of CMD incidence. To assess CMD incidence, 30 plants were assessed randomly along two diagonal transects across each cassava farm and we counted the apparently healthy and diseased cassava plants among the 30 selected plants. All data were collected in the tablet via the iForm 9.12.7 application. Subsequently, the CMD incidence (percentage of symptomatic cassava plants among the 30 plants observed), was calculated using the formula provided in Sseruwagi *et al.* (2004) below:

$$\text{Mean incidence (\%)} = \frac{\sum \text{Infected plants}}{\sum \text{Plants}} \times 100$$

Incidence is used to estimate the evolution or spread of the disease. The CMD incidence rates and levels are defined in Table 3.

Estimating CMD severity. To estimate CMD severity, we observed and assessed the number of diseased cassava leaves on the main stem. This allowed us to determine a severity rating for each observed plant using the scale defined by the International Institute of Tropical Agriculture (IITA) (Table 4). As a result, a severity field average was calculated for the diseased plants only (Sseruwagi *et al.*, 2004).

The severity represents the degree to which a plant is infected by CMD. Mean severity was calculated as follows:

$$\text{Mean severity} = \frac{\sum_2^5 \text{Plants severity score}}{\sum \text{Infected plants}}$$

Infection sources. The types of cassava mosaic infection observed on each plant were categorised on the basis of the location of symptoms on the apical and basal leaves. Thus, if plants showed CMD symptoms only on the basal leaves or on all leaves, the infection was deemed to be related to the use of contaminated cuttings. However, if the cassava showed symptoms on the apical leaves only, the infection was attributed to the action

TABLE 2. Data recorded on the tablet during the surveys

Parameters	Details recorded
Surveyor details	Full name (given name, surname)
Cassava farm characteristics	Sequence number from the first farm, age of plantation, area, cropping system (monoculture or mixed cropping)
Environmental conditions	Presence/absence of rain, sunshine
Cassava farm location	Region, county, district, village, altitude, distance travelled from the last farm surveyed, GPS coordinates
Cultivar type	The three dominant cultivars, the number of varieties
Vector data	Total number of whiteflies on each of the first five leaves of the plant
Epidemiology (CMD)	Location and description of symptoms, incidence, severity
Coding convention	Code used for leaf and whitefly samples

TABLE 3. CMD incidence assessment scale

CMD rate (%)	Incidence level
0 - 25	Low
25 - 50	Medium
50 - 75	High
75 - 100	Very high

TABLE 4. CMD severity assessment (source: IITA, 1990)

Severity values	Symptoms
1	No symptoms (healthy)
2	Slight chlorosis, slight distortion at the base of most leaves, while the remaining parts of the leaves and leaflets appear green
3	Pronounced mosaic patterns on most leaves, shrinkage and distortion of the lower third of the leaflets
4	Severe mosaic distortion of two-thirds of most leaves and general reduction in leaf size and stunting of plants
5	Very severe mosaic patterns on all leaves, distortion, deformation and severe reduction of leaves, accompanied by severe stunting of plants

of whitefly vectors. This distinction on the source of infection was recorded on the field tablet. This information is helpful when developing CMD management methods to cope with infected cuttings and/or whitefly control. The rate of infection by cuttings (RIC) was calculated from the following formula:

$$\text{RIC (\%)} = \frac{\text{Number of plants infected by cuttings}}{\text{Total number of plants observed}} \times 100$$

The rate of infection by whiteflies (RIW) was calculated using the following formula:

$$\text{RIW (\%)} = \frac{\text{Number of plants infected by whiteflies}}{\text{Total number of plants observed}} \times 100$$

Data processing and analysis. Field data were collected in a tablet *via* the iForm 9.12.7 application. All data from Gabon were uploaded from the tablet into iForm's cloud-based database and then integrated into, and managed in, the WAVE Cube data system. Data pertinent to this study were selected and visualised as histograms. Additionally, all of these data were analysed using R version 3.6.1. Pearson's correlation analyses between some parameters and Wilcoxon tests were used to compare means at $P < 0.05$. Using the WAVE Cube coordinates, maps were produced using the Microsoft Power BI tool and geographic information software ArcGIS 10.1.

Ethical considerations. This epidemiological survey of CMD was complied with ethical considerations. Each of our field trips was conditional on obtaining permission from the WAVE-Gabon Country Director. In each province and department, the WAVE-Gabon field team met with local officials of the Ministry of Agriculture, as well as with administrative and civil officials, to present the purpose of the mission and the WAVE programme, as well as the members of the mission. On these occasions, the Regional Governors and District Prefects signed and stamped the mission orders to attest to their authorisation to work in their locality. Before any sampling was carried out in the cassava fields, the owner or their representative was interviewed by the mission leader in order to present the objective of the work and the methods used for this purpose. After their verbal consent, the observations and epidemiological evaluations were made in the field concerned.

RESULTS

Incidence of CMD in Gabon. All the cassava fields surveyed (100%) in the study fields were infected with CMD. The incidence of CMD was high, with a national average of 64.29%. However, the incidence varied among regions (provinces) (Fig. 2). In Ogooue-Maritime and Nyanga regions, CMD incidence was very high, with values of 82.22% and 79.20%, respectively (Fig. 2).

Although incidence was also high in Woleu-Ntem, Ogooue-Ivindo, Haut-Ogooue, Moyen-Ogooue, Ngounie and Ogooue-Lolo regions, it was less than in Ogooue-Maritime and Nyanga, with values in the range of 55.38–73.78%. Estuaire region, however, had an average incidence level of 40.42% (Fig. 2). There were significant differences in mean incidence among the regions (P< 0.05).

Severity of CMD in Gabon. CMD severity was high with an average of 3.16. Across the regions, the severity values did not vary much, ranging from 2.64 to 3.47 (Fig. 2). However, the highly infected regions of Ogooue-Maritime and Nyanga had moderate severity levels, with values of 2.64 and 2.79, respectively. In contrast, the other regions had higher levels of disease severity (Fig. 2). There were significant differences in mean severity among the regions (P<0.05).

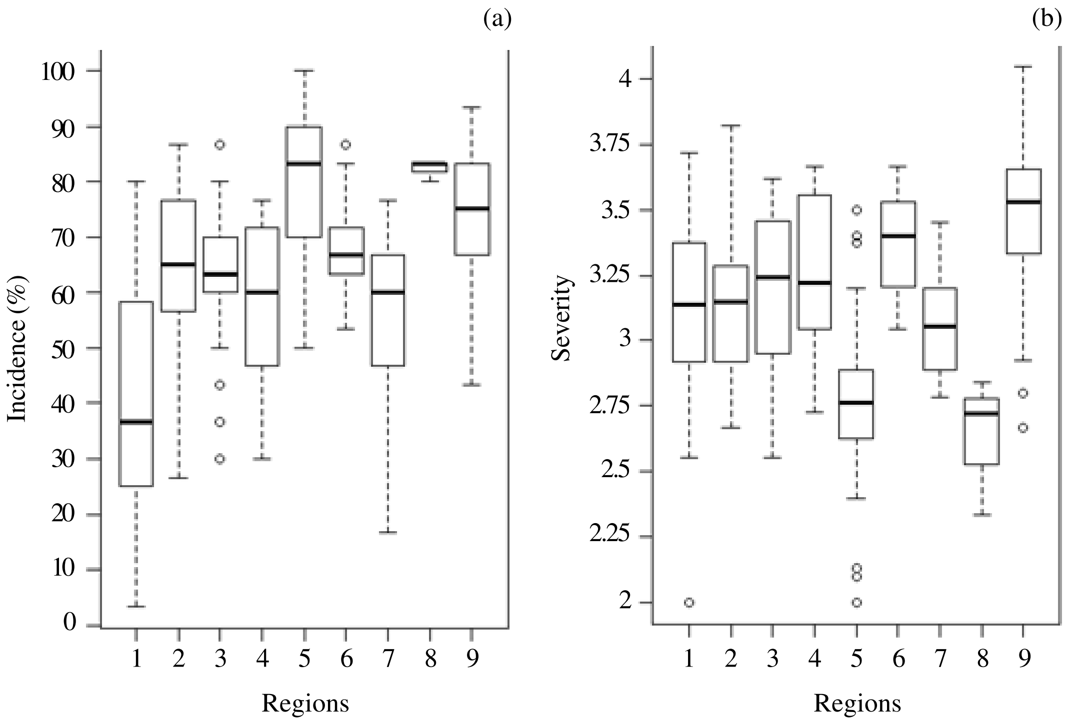

Figure 2. CMD (a) incidence and (b) severity in all regions in Gabon. Regions: 1 = Estuaire, 2 = Haut-Ogooue, 3 = Moyen-Ogooue, 4 = Ngounie, 5 = Nyanga, 6 = Ogooue-Ivindo, 7 = Ogooue-Lolo, 8 = Ogooue-Maritime, 9 = Woleu-Ntem

Abundance of whiteflies. The average number of whiteflies per cassava plant at national level was relatively low, with a value of 2.58 w/p (Fig. 3). At regional level, Estuaire (5.25 w/p), Ogooue-Lolo (3.96 w/p), Ogooue-Maritime (3.94 w/p) and Ogooue-Ivindo (3.29 w/p) had the highest numbers; and Ngounie (1.65 w/p), Haut-Ogooue (1.61 w/p), Woleu-Ntem (1.51 w/p) and Nyanga (1.06 w/p) had very low whitefly numbers. There were no significant differences in mean numbers of whiteflies among regions (P >0.05).

Sources of CMD infection. Using the data on whitefly symptomatology and abundance (Table 5), we identified the origins of infection. The CMD infection in cassava farms was due to both the use of contaminated cassava cuttings and to the action of whiteflies. However, contamination by infected cuttings was higher (62.67%) than for the whitefly vector (2.06%).

CMD severity, CMD incidence and number of whiteflies. Pearson's correlation analysis showed a weak but significant positive correlation between severity and altitude (r = 0.381, Table 6), i.e. higher altitude was associated with more severe CMD. No other significant correlations were found among parameters at P>0.05. CMD incidence and altitude (r = 0.112) were positively correlated;

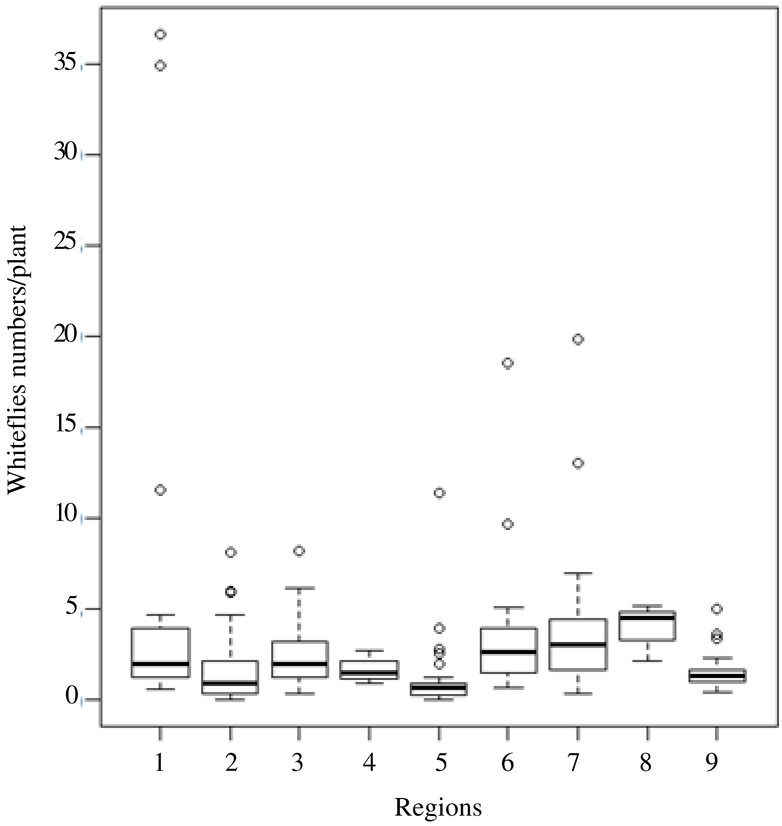

Figure 3. Mean numbers of whiteflies per plant in Gabon regions. Regions: 1 = Estuaire, 2 = Haut-Ogooue, 3 = Moyen-Ogooue, 4 = Ngounie, 5 = Nyanga, 6 = Ogooue-Ivindo, 7 = Ogooue-Lolo, 8 = Ogooue-Maritime, 9 = Woleu-Ntem

TABLE 5. Sources of CMD infection in Gabon

Region	Infection source (%)	
	Cutting	Whitefly
Estuaire	39.58	0.83
Haut-Ogooue	61.46	2.96
Moyen-Ogooue	62.73	0.91
Ngounie	57.61	0.60
Nyanga	76.77	2.54
Ogooue-Ivindo	68.17	1.18
Ogooue-Lolo	51.54	4.74
Ogooue-Maritime	82.22	0.00
Woleu-Ntem	72.33	1.56
Overall	62.67	2.06

TABLE 6. Results of correlation analysis: CMD severity and CMD incidence against each other and against other parameters across all fields surveyed

Parameter 1	Parameter 2	Pearson's r	P-value
CMD severity	Altitude	0.381	0.012
CMD incidence	Altitude	0.112	0.091
CMD severity	CMD incidence	-0.329	0.387
CMD severity	Number of whitefly	-0.179	0.646
CMD incidence	Number of whitefly	-0.515	0.156

however, negative correlations were found among CMD severity, CMD incidence and number of whiteflies, i.e. these parameters moved in opposite directions (Table 6).

Infection types and CMD incidence according to regions. There was a weak correlation between infection due to whiteflies and CMD incidence (r = 0.085, Table 7). However, there was a strong correlation between infection due to cuttings and CMD incidence (r = 0.705).

Based on agro-ecological zones. Figure 4 shows mean severity and incidence for CMD between different agro-ecological zones in Gabon. Mean incidence was high in savannah (70%) and relatively low in forest (68%). However, values of severity between these two agro-ecological zones contrasted with the results for incidence. Indeed, severity was higher in the forest (3.23) than in savannah (3.00).

Wilcoxon test results showed no significant differences in incidence among agro-ecological zones (P = 0.1213). Nevertheless, there were significant differences in severity among agro-ecological zones ($P < 0.05$). The abundance of whiteflies was higher in forest areas and very low in savannah areas (Fig. 5).

Based on cropping type. There were no significant differences in CMD incidence and severity according to cropping type (Fig. 6).

TABLE 7. Correlation analysis of CMD incidence with no. of whitefly and no. of cuttings

Parameter 1	Parameter 2	Pearson's r	P-value
CMD incidence	Number of whitefly	0.085	0.205
CMD incidence	Number of cuttings	0.705	<0.001

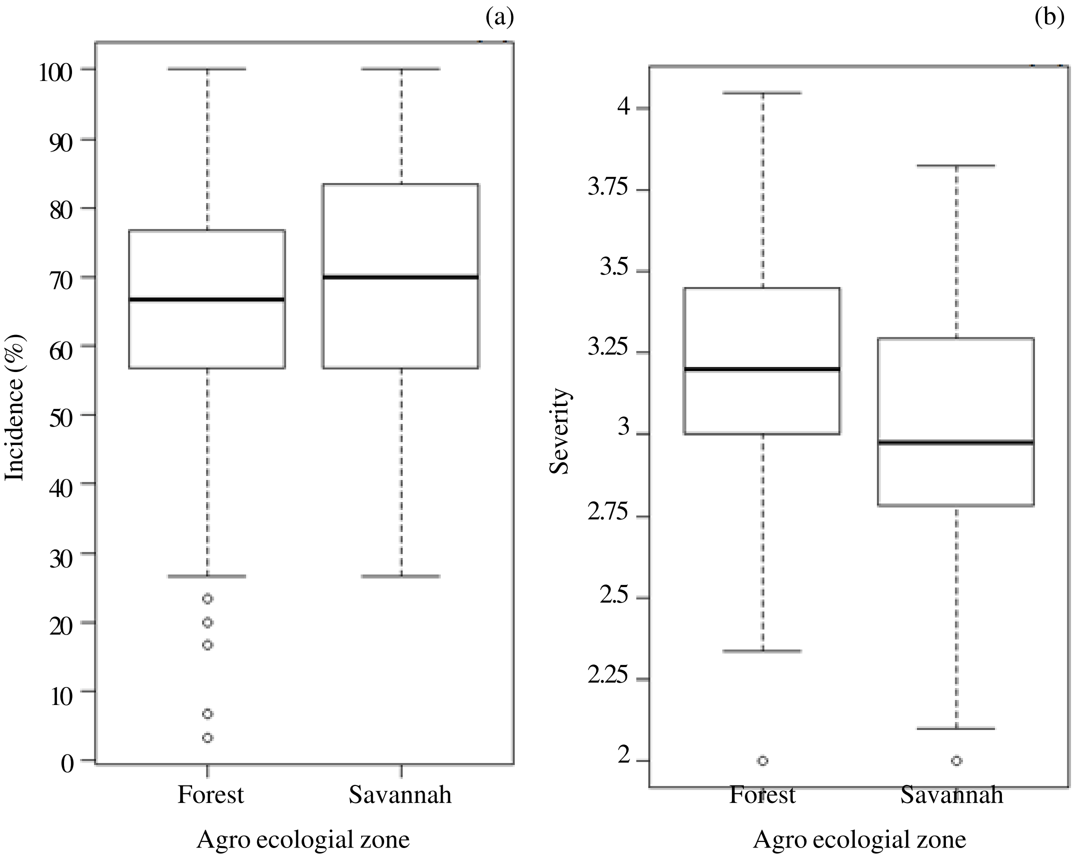

Figure 4. CMD (a) incidence and (b) severity in agro-ecological zones in Gabon.

However, the mean values of incidence (70%) and severity (3.2) were higher in cassava monoculture than in fields where intercrops were present.

The abundance of whiteflies differed depending on cropping type, namely high for mixed crops (intercrop) and low for monoculture (Fig. 7).

DISCUSSION

Symptoms associated with CMD in Gabon. This study revealed that all the farms surveyed were infected with CMD and that the cassava plants observed had diseased leaves that presented discolouration (100%) and deformation (96.47%) (severity range of 2-

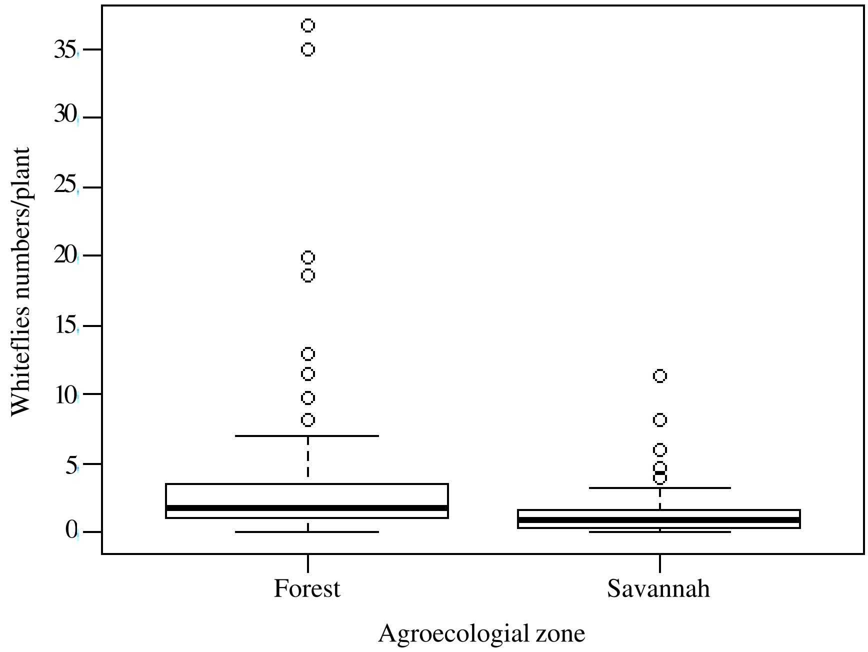

Figure 5. Abundance of whiteflies in agro-ecological zones in Gabon.

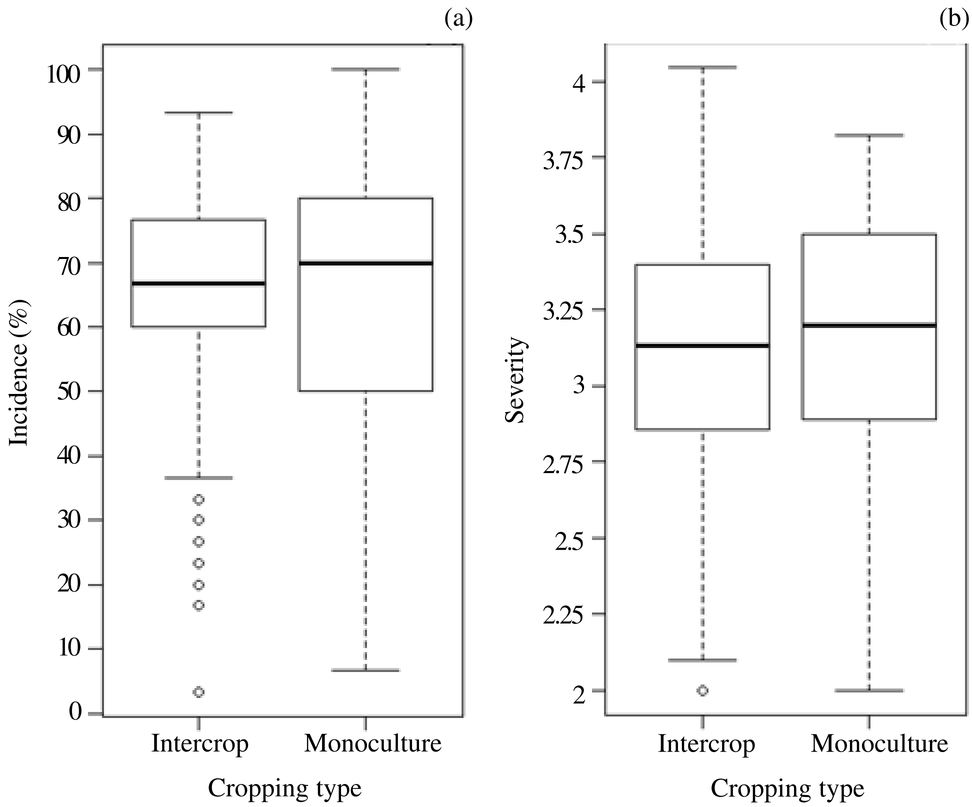

Figure 6. CMD (a) incidence and (b) severity according to cropping type in Gabon.

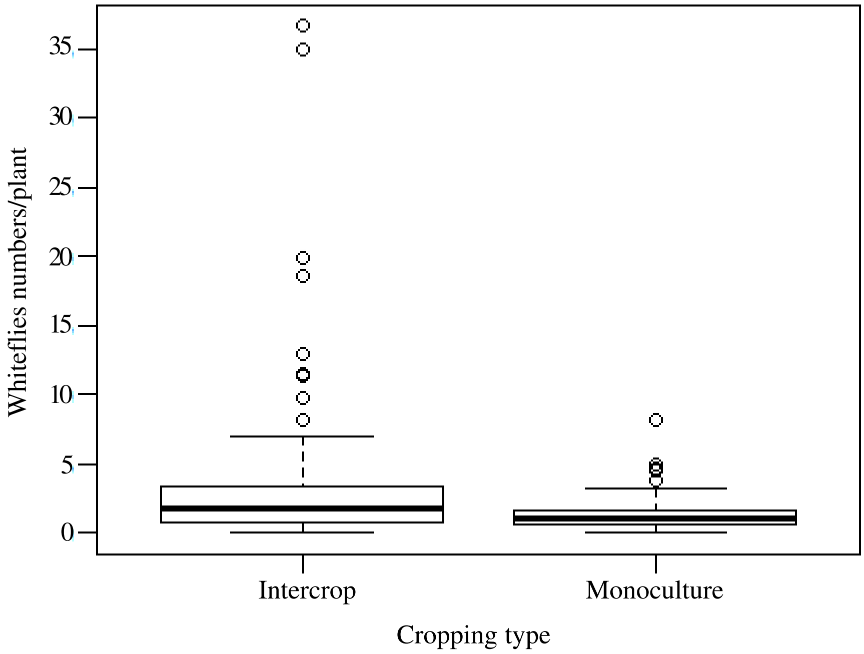

Figure 7. Abundance of whiteflies according to cropping type in Gabon.

4). Thus CMD is present and widespread in Gabon. The disease is similarly reported to be widely spread in the Congo Republic (Central Africa), Nigeria, Burkina Faso, Côte d'Ivoire (West Africa), Tanzania (East Africa) and South Africa (Eni *et al*., 2021; Okelola *et al*., 2021).

The high incidence observed during this survey suggests that infected planting materials were the cause of the spread of CMD in cassava farms. Indeed, farmers traditionally re-used cuttings from their own farms as planting materials and these cuttings were often diseased (Mivedor *et al*., 2020). Generally, cassava farmers did not recognise the symptoms of CMD as a sign of virus attack (unpublished data). They interpreted these symptoms as the effects of poor seasons or drought. This could be due to their lack of disease information. There is, therefore need for farmer training in simple disease detection to safeguard them from further inadvertent spread of the disease. This is attested by Chikoti (2011), who showed that CMD could go unnoticed by uninformed farmers in areas of Zambia. Furthermore, other conditions such as green mite, necrosis and cassava mealybug spots observed in several plantations visited could also explain this difficulty of farmers to identify CMD: these symptoms sometimes mask those of simple mosaic mottling, thus causing symptom clustering (Zinga *et al.*, 2013). This symptom clustering can create bias in the scoring of the incidence and severity of CMD during epidemiological observations (Zinga *et al.*, 2013).

Overall, the observations made in this survey should focus research attention on the production and distribution of healthy and quality planting material (cuttings from disease-resistant varieties), as CMD is a very serious threat to cassava crops.

Incidence and severity of CMD and abundance of associated whiteflies. The high CMD incidence and severity values obtained in the present study reflect that the disease is well spread and severe in Gabon. This high national CMD incidence could be explained by the recurring use of local, highly susceptible and unsanitised cassava planting materials. Similar observations were made by Delêtre *et al.* (2021) in Gabon in a study on the genetic diversity of cassava; whereby farmers grew a number of varieties traditionally passed from generation to generation to renew their fields, sometimes without considering their phytosanitary status. In contrast, the lower incidence (40.42%) recorded in the Estuaire region can be explained by the differences in agricultural practices in this locality. These differences can be attributed to the following factors: (i) most farmers in the region were previously civil servants and thus pay particular attention to maintenance of their plantations; (ii) the training provided within the framework of agricultural projects is concentrated in the peri-urban areas of the region; and (iii) expatriates living in the outlying areas of the capital often use improved plant materials (disease-tolerant varieties) brought from their country of origin (e.g. Ghana, Nigeria, Togo, Benin and Burkina Faso) for cultivation in Gabon. Nearly 88.88% of the regions surveyed had a CMD incidence of over 50%. Similarly high rates of disease occurrence were also observed in Nigeria (88.66%), Zambia (62%), DRC (80%), South Africa (63%), Côte d'Ivoire (52%) and Central African Republic (85%) (Mabasa, 2007; Ntawuruhunga *et al.*, 2007; Zinga *et al.*, 2013; Toualy *et al.*, 2014; Abubakar *et al.*, 2019).

The average severity of CMD symptoms ranged from moderate to high (2.64 - 3.47). The severe symptoms could be explained by erosible co-infection of viruses carried by different whitefly populations. These results agree with those of studies conducted in Zambia that indicated severe CMD symptoms in plants infected with ACMV and EACMV (Chikoti *et al.*, 2013). Additionally, Toualy *et al.* (2014) also reported viral co-infections in Côte d'Ivoire. This result could be linked to the presence of virulent viral strains in the surveyed agricultural areas, such as the EACMV-Ug strain first identified by Legg *et al.* (2004) in south-eastern Gabon. The surveys conducted by Delêtre *et al.* in 2004, 2007, 2014b and 2015 in 11 villages in Gabon highlighted the existence of EACMV-Ug strains in the north, west and centre of Gabon (Delêtre *et al.*, 2021). The spread of these viral strains throughout the country could explain the severe nature of the disease observed throughout Gabon.

The inventory of whiteflies showed an average of 2.58 whiteflies/plant (w/p) and whiteflies were involved in infection in 2.06% of cases. The whitefly numbers varied between 1.06 w/p (Nyanga) and 5.25 w/p (Estuaire). Although the climatic and environmental conditions in Gabon favour development of whiteflies, the low density of whiteflies observed per plant could be explained by the differences in collection times, which varied according to field conditions, and by the fact that the cassava farms surveyed were in association with several crops, namely maize, groundnuts and plantain. Fondong (1999) showed that plots where cassava was cultivated in association with maize and beans had low populations of whiteflies per plant. Under those conditions, crop association decreases the number of whiteflies per plant, as associated crops such as maize can be trap crops (Metty, 2010). However, the whitefly numbers in our study were lower than those obtained by Fondong (1999) in Cameroon (32.3 w/p) and Tajebe *et al.* (2015) in Tanzania (76.7 w/p). With respect to these statistics, other studies in East Africa have shown that whitefly populations are high in areas growing whitefly-susceptible cassava varieties and in areas that have high-fecundity whitefly biotypes (Dinsdale *et al.*, 2010; Omongo *et al.*, 2012; Boykin *et al.*, 2018; Mugerwa *et al.*, 2019).

Sources of cassava mosaic infection in Gabon. The epidemiological survey analysis showed that infection through contaminated cuttings was the major source of CMD infection (62.67%) in Gabon. This result could be linked to the use and transfer of infected cuttings by farmers because these would favour the development and spread of CMD throughout the country. In fact, in the surveyed areas, farmers were unable to select healthy cuttings due to factors such as ignorance of the disease, their low economic status, strong human–wildlife conflicts and absence of quality seeds in the country. As a result, farmers were forced to regularly recycle infected cuttings from old fields when planting new cassava fields. These agricultural behaviours are believed to favour the spread of CMD, and thus increase its severity (Toualy *et al.*, 2014). Studies in the Congo Republic, the Central Africa Republic and South Africa have long reported that infected plant materials could be the primary means of spreading viral diseases (Fargette *et al.*, 1985; Mabasa, 2007; Ntawuruhunga *et al.*, 2007; Zinga *et al.*, 2013).

Since some virulent mosaic virus species have been reported in several African countries, and with the movement of cassava cuttings across borders, it is important to consider cross-border joint actions to find a strategy to deal with the problem of cassava virus infections in Gabon. The use of improved local varieties that are tolerant to CMD could prove an advantageous strategy for managing CMD.

CONCLUSION

Cassava Mosaic Disease (CMD) is endemic in Gabon and widespread in the cassava-producing areas. It is spread mainly through the use of infected cuttings. Therefore, there is an urgent need to put in place a CMD control strategy and to focus on awareness-raising and training of producers on this disease. Research and plant breeding activities should be continued in order to select local varieties that are tolerant to CMD infections. Additionally, work should be undertaken to identify the whitefly species within the *B. tabaci* complex that were collected.

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REFERENCES

Abubakar, M., Mohammed, I.U., Anas, H. and Mohammed, M.T. 2019. Severity of cassava mosaic disease in north east Nigeria. *Journal of Innovative Research in Life Sciences* 1:1-8.

Adjata, K.D., Muller, E., Peterschmitt, M., Aziadekey, M. and Gumedzoe, M.Y.D. 2008. Incidence of cassava viral diseases and first identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in cassava (*Manihot esculenta* Crantz) fields in Togo. *American Journal of Plant Physiology* 3(2):73-80. https://doi.org/10.3923/ajpp.2008.73.80

Bah, E.S., Bamkefa, B.A., Winter, S. and Dixon, A.G. 2011. Distribution and current status of cassava mosaic disease and begomoviruses in Guinea. *African Journal of Root Tuber Crops* 9:17-23.

Bisimwa Basengere, E. 2012. Epidémiologie, diversité génétique, distribution et contrôle des virus de la mosaïque africaine du manioc et de son vecteur (*Bemisia tabaci*) dans la région du Sud-Kivu en République Démocratique du Congo. Thèse de Doctorat, Université Catholique de Louvain, Belgique.

Boykin, L.M., Kinene, T., Wainaina, J.M., Savill, A., Seal, S., Mugerwa, H., Macfadyen, S., Tay, W.T., De Barro, P. and Kubatko, L. 2018. Review and guide to a future naming system of African *Bemisia tabaci* species. *Systematic Entomology* 43:427-433.

Chikoti, P.C. 2011. Development of cassava (*Manihot esculenta* Crantz) cultivars for resistance to cassava mosaic disease in Zambia. Ph.D. Thesis. University of KwaZulu-Natal, South Africa. 168pp.

Chikoti, P.C., Ndunguru, J., Melis, R., Tairo, F., Shanahan, P. and Sseruwagi, P. 2013. Cassava mosaic disease and associated viruses in Zambia: occurrence and distribution. *International Journal of Pest Management* 59:63-72.

Delêtre, M., Lett, J.-M., Sulpice, R. and Spillane, C. 2021. Kinship networks of seed exchange shape spatial patterns of plant virus diversity. *Nature Communications* 12:1-10.

Diallo, Y., Gueye, M.T., Sakho, M., Gbaguigi Darboux, P., Kane, A., Barthelemy, J.-P. and Lognay, G. 2013. Importance nutritionnelle du manioc et perspectives pour l'alimentation de base au Sénégal (synthèse bibliographique). Biotechnologie, *Agronomie, Société et Environnement* 17(4):634-643.

Dinsdale, A., Cook, L., Riginos, C., Buckley, Y.M. and De Barro, P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Annals of the Entomological Society of America* 103:196-208.

Eni, A.O., Efekemo, O.P., Onile-ere, O.A. and Pita, J.S. 2021. South West and North Central Nigeria: Assessment of cassava mosaic disease and field status of African cassava mosaic virus and East African cassava mosaic virus. *Annals of Applied Biology* 178:466-479.

FAO. 2012. Appui à la production et protection integrée du manioc au Gabon (projet TCP/ GAB/3203(D)), Rapport terminal du projet (Libreville). Libreville, Gabon. 37pp.

FAO. 2021. Crop production data 2019, FAOSTAT [WWW Document]. Rome Italy. URL http://www.fao.org/faostat/fr/ #data/QCL (accessed 7.30.21).

Fargette, D., Fauquet, C. and Thouvenel, J.-C. 1985. Field studies on the spread of African cassava mosaic. *Annals of Applied Biology* 106:285-294.

FIDA. 2008. Etude des potentialités de commercialisation des produits derivés du manioc sur les marchés CEMAC. Rapport d'activités du projet Initiative Regionale pour la Production et la Commercialisation du Manioc. Initiative Régionale Transformation et Commercialisation du Manioc (IRPCM), Cameroun.

Fondong, V.N. 1999. Viruses associated with the cassava mosaic disease in Cameroon: Spread and molecular characterisation. Ph.D. Thesis. University of the Witwatersrand, Johannesburg, South Africa. 173pp.

Harimalala, M., De Bruyn, A., Hoareau, M., Andrianjaka, A., Ranomenjanahary, S., Reynaud, B., Lefeuvre, P. and Lett, J.-M. 2013. Molecular characterization of a new alphasatellite associated with a cassava mosaic geminivirus in Madagascar. *Archives of Virology* 158:1829-1832.

International Institute of Tropical Agriculture (IITA). 1990. Cassava in tropical Africa: A reference manual. IITA, Ibadan, Nigeria. 184pp.

Legg, J.P. and Fauquet, C.M. 2004. Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* 56:585-599.

Legg, J.P., Ndjelassili, F. and Okao-Okuja, G. 2004. First report of cassava mosaic disease and cassava mosaic geminiviruses in Gabon. *Plant Pathology* 53(2):232. doi: 10.1111/j.0032-0862.2004.00972.x

Legg, J.P., Owor, B., Sseruwagi, P. and Ndunguru, J. 2006. Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of a regional pandemic. *Advances in Virus Research* 67:355-418.

Mabasa, K.G. 2007. Epidemiology of cassava mosaic disease and molecular characterization of cassava mosaic viruses and their associated whitefly (*Bemisia tabaci*) vector in South Africa. Master of Science. University of the Witwatersrand, Johannesburg, South Africa. 114pp.

Macrae, R., Robinson, R.I.C. and Sadler, M.J. 1993. Encyclopaedia of food science, food technology and nutrition. Vol. 1. New York, USA: Academic Press.

Metty, T. 2010. Évaluation de l'impact d'une bordure de maïs sur les populations d'Helicoverpa zea et de *Bemisia tabaci* sur une culture de tomate. Mémoire de Master en Sciences Technologiques Biodiversité tropicale. Écosystèmes naturels et exploités, Université des Antilles et de la Guyane, Pôle de Recherche Agro-environnementale de la Martinique, Pointe-à-Pitre, France. 51pp.

Mivedor, A.S., Dansou-Kodjo, K.A., Adjata D.K. and Pita J.S. 2020. Identification and incidence of cassava mosaic begomoviruses in Togo. *Asian Journal of Plant Pathology* 14:11-20.

Mugerwa, H., Rey, M.E.C., Tairo, F., Ndunguru, J. and Sseruwagi, P. 2019. Two sub-Saharan Africa 1 populations of *Bemisia tabaci* exhibit distinct biological differences in fecundity and survivorship on cassava. *Crop Protection* 117:7-14.

Ntawuruhunga, P., Okao-Okuja, G., Bembe, A., Obambi, M., Mvila, J.A. and Legg, J.P. 2007. Incidence and severity of cassava mosaic disease in the Republic of Congo. *African Crop Science Journal* 15:1-9. doi:10.4314/acsj.v15i1.54405

Okelola, T.A., Asala, S.W., Oyerinde, A.A. and Folarin, O.O. 2021. Incidence and distribution of African Cassava Mosaic Virus in the Guinea Savannah Vegetation zone of Nigeria. *Journal of Plant Science* 9:1-8.

Omongo, C.A., Kawuki, R., Bellotti, A.C., Alicai, T., Baguma, Y., Maruthi, M.N., Bua, A. and Colvin, J. 2012. African cassava whitefly, *Bemisia tabaci*, resistance in African and South American cassava genotypes. *Journal of Integrative Agriculture* 11:327-336.

Sseruwagi, P., Sserubombwe, W.S., Legg, J.P., Ndunguru, J. and Thresh, J.M. 2004. Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. *Virus Research* 100:129-142.

Tajebe, L.S., Boni, S.B., Guastella, D., Cavalieri, V., Lund, O.S., Rugumamu, C.P., Rapisarda, C. and Legg, J.P. 2015. Abundance, diversity and geographic distribution of cassava mosaic disease pandemic-associated *Bemisia tabaci* in Tanzania. *Journal of Applied Entomology* 139:627-637.

Tiendrebeogo, F., Lefeuvre, P., Hoareau, M., Harimalala, M.A., De Bruyn, A., Villemot, J., Traore, V.S., Konaté, G., Barro, N. and Reynaud, B. 2012. Evolution of African cassava mosaic virus by recombination between bipartite and monopartite begomoviruses. *Virology Journal* 9:67-74.

Tocko-Marabena, B.K., Silla, S., Simiand, C., Zinga, I., Legg, J., Reynaud, B. and Delatte, H. 2017. Genetic diversity of *Bemisia tabaci* species colonizing cassava in Central African Republic characterized by analysis of cytochrome c oxidase subunit I. *PLoS One* 12:e0182749.

Toualy, M.N., Akinbade, S., Koutoua, S., Diallo, H. and Kumar, P.L. 2014. Incidence and distribution of cassava mosaic begomoviruses in Cote d'Ivoire.

International Journal of Agronomy and Agricultural Research 4:131-139.

Zinga, I. 2012. Epidémiologie de la maladie de la mosaïque du manioc en République Centrafricaine, résistance variétale et assainissement par thermothérapie. Thèse de Doctorat, Université de la Réunion, Ecole Doctorale Sciences, Technologies et Santé ED N° 542, Saint-Denis, Ile de la Réunion, France. 170pp.

Zinga, I., Chiroleu, F., Legg, J., Lefeuvre, P., Komba, E.K., Semballa, S., Yandia, S.P., Mandakombo, N.B., Reynaud, B. and Lett, J.-M. 2013. Epidemiological assessment of cassava mosaic disease in Central African Republic reveals the importance of mixed viral infection and poor health of plant cuttings. *Crop Protection* 44:6-12.