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Distribution of fungal diseases caused by *Lasiodiplodia* sp. in cashew and mango orchards in Côte d'Ivoire

Yaya KONE [1,*], Adjoa Marie Josephine KOUADIA [1], Akissi Sandrine YAO [1], Kan Ulrich Urbain KONAN [1,2], Eric-Olivier TIENEBO [1], Kouakou Theodore KOUADIO [1] and Kouabenan ABO [1]

[1] *Plant Biology and Health Research Unit, Joint Research and Innovation Unit in Agricultural Sciences and Transformation Processes (UMRI-SAPT). Félix Houphouët-Boigny National Polytechnic Institute (INP-HB), BP 1093 Yamoussoukro, Côte d'Ivoire.*
[2] *National Center for Agricultural Research (CNRA), Bimbresso Research Station, 08 BP 1536 Abidjan 01, Côte d'Ivoire.*

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Abstract

Objective: Fungal alterations constitute one of the major causes of losses in cashew nut and mango production in Côte d'Ivoire. The objective of this study is to contribute to improving the productivity of cashew and mango crops by controlling dieback diseases caused by *Lasiodiplodia* sp.

***Methodology and results*:** The study initially consisted in conducting a survey in the major production areas of these targeted crops in the country, in order to assess the incidence and severity of the disease. Then, the collected mango and cashew stem samples were isolated and the associated pathogens were identified. The symptoms of the disease are present in all the surveyed orchards. Agroecological zone 7 has the highest incidence rate values (82.06 ± 3.22) and average severity index (3.75 ± 0.03) and agroecological zone 2 presents the lowest values (78.00±0.05) and (3.46±0.26). Identification showed three morphotypes (whitish, grayish and blackish) of the fungus *Lasiodiplodia* sp., responsible for die-back diseases.

Conclusion and application of results: fungal diseases due to *Lasiodiplodia* sp. are widespread in all cashew and mango orchards surveyed in Côte d'Ivoire. These results are decision-making tools for the actors of these agricultural sectors, for a better management of this disease. However, characterization study could provide information on the morphological and genetic diversity of *Lasiodiplodia* sp. population in Côte d'Ivoire.

Keywords: Distribution; Fungal diseases; *Lasiodiplodia* sp; Côte d'Ivoire

1. Introduction

Cashew (*Anacardium occidentale* L.) and mango (*Mangifera indica* L.) represent the second and third most important cash crops respectively after cotton (*Gossypium hirsutum* L.) in northern Côte d'Ivoire [1]. From an economic point of view, these crops provide employment and also contribute to the development of the northern region [2]. Côte d'Ivoire is the third largest supplier of mango to the European market after Brazil and Peru and the world's leading producer of cashew nuts [3]. Apart from the economic importance of these main crops in the savannah zone of Côte d'Ivoire, it is important to note that they also play an essential role in social and environmental terms [4]. However, they are subject to several phytosanitary constraints that compromise yield in terms of quantity and quality [5]; [6]. Indeed, apart from pests, more than a dozen diseases have been described on these crops [7]. In Tanzania, four diseases including anthracnose (*Colletotrichum gloeosporioides*), powdery mildew (*Oidium anacardium*), leaf and nut rust

* Corresponding author: Yaya KONE

(*Cryptosporiopsis* sp.) and cashew wilt (*Laetiporus* sp., and *Ganoderma* sp.) have been responsible for significant damage [5]; [7]. Black spot disease caused by *Xanthomonas citri* pv *mangifera indica* L on mango in Burkina Faso [8], Alternaria leaf spot, the pathogen of which is *Altemaria altemata*, and scab due to *Elsinoe mangiferae* [9] have also been identified on mango. In Côte d'Ivoire, natural infections on plants and fruits have been associated with numerous fungi [1].

Among the pathogens responsible for these diseases, the fungus *Lasiodiplodia* sp. has been reported in approximately 70% of farms and has been linked to yield losses estimated at 80% to 100% in some countries [10]. It is a virulent, cosmopolitan, polyphagous, and opportunistic pathogen that attacks field crops and trees and causes different types of diseases responsible for declines and rots [11]; [12]. It has been widely observed in tropical and subtropical regions of the world [13] and has been identified on a very wide host range estimated at more than 500 species [14]; [15].

However, there is little information on the different species, strains or types of this fungus on mango and cashew nut crops in Côte d'Ivoire. The objective of this study is to contribute to better management of the disease caused by *Lasiodiplodia* sp., by understanding its distribution on mango and cashew nut crops in Côte d'Ivoire.

2. Material

2.1. Plant material

The plant material consists of explant samples of symptomatic cashew and mango plants from the different mango and cashew producing regions of Côte d'Ivoire.

2.2. Technical equipment:

The laboratory studies required various standard equipment and consumables commonly used in plant pathology. These included a laminar flow hood, an autoclave, incubators, a Bunsen burner, glassware, a 90 mm Petri dish, and culture media.

2.3. Methods

Four major steps were followed during this study. A survey was conducted in different localities to assess the incidence and severity of dry-down disease. Then, symptomatic samples were collected from the sites. Subsequently, pathogens associated with the symptoms were isolated and identified. Finally, a distribution map of the disease was established.

2.3.1. Study site

The surveys were carried out in the main production areas of mango and cashew crops. These areas are spread across 15 regions, themselves located in 5 agroecological zones of Côte d'Ivoire [16]. A total of 146 locations were surveyed.

Table 1 Characteristics of the 7 agroecological zones (ZEA) in Côte d'Ivoire [30]

ZAE	Features	Altitude (m)	Rainfall (mm)	Annual average temperature in °C (Standard deviation)
I	Dense South Rainforest area	0-200	1400 - 2500 (bimodal)	29 (5.6)
II	Dense West Rainforest area	~1000 (Daloa)	1300-1750	23.5 (13.4)
III	Semi-western mountain forest area	> 1000 (Man)	1300-2300	24.5 (7.7)
IV	Dense semi-deciduous Rainforest area	0-200	1300-1750	23.5 (13.4)
V	Transitional Forest area	300-600	1300-1750 (unimodal)	23.5 (13.4)
VI	Tropical humid savannah area	300-500	1150-1350 (unimodal)	26.7 (1.1)
VII	Tropical dry savannah zone	300-500	1150-1350	26.7 (1.1)

2.4. Prospecting and description of symptoms

2.4.1. Determination of incidence and severity in cashew and mango orchards

Disease incidence and severity were assessed based on symptoms visible in the field. Incidence is the number of new cases of a disease or diseased individuals present at a given time in a plant population. It was assessed using the method of [17]. In each orchard, 5 rows of trees were randomly selected. Then, 4 to 21 trees per row were selected; a total of 21 to 50 trees per orchard. Incidence was determined using the following formula [18]:

$$\boldsymbol{Pi = \frac{ni}{N} X100}$$

With ni: symptomatic trees and N: the total number of trees.

Severity is the level of seriousness or degree of disease infection on a plant in a diseased plant population. It was assessed using a rating scale by [19] (Table 2). Severity was calculated using the formula by [20]:

$$\boldsymbol{ISM = \frac{\Sigma(Ni \times I)}{Nt}}$$

ISM: Average Disease Severity Index; I: Disease Severity Index (I ranging from 0 to 3); Ni: Number of trees with index I and Nt: Total number of trees examined

Table 2 Severity Rating Scale

Severity	Description of observed symptoms (OS)
0	Healthy tree
1	Presence of gum exudations on the main trunk or on the primary and secondary branches (SO 1)
2	Visible cracks, exposure of internal tissues, on the main trunk or on primary and secondary branches (SO 2)
3	Presence of well-defined canker and dieback of branches (SO 3)

2.4.2. Sample collection

Sample collection was carried out during a field survey in the major cashew and mango production areas, particularly in five (5) agro-ecological zones (Table 3). During the survey, mango and cashew trees of various varieties were examined in several locations across the country. Fragments (samples) were taken from symptomatic trees. All fragments from a diseased tree were grouped into one sample, and the number of samples per site varied depending on the number of diseased individuals or the diversity of symptoms present at the site. The samples thus collected were stored in plastic bags and transported to the Phytopathology and Plant Biology Laboratory of INP-HB in Yamoussoukro.

Table 3 Collection areas for symptomatic samples of mango and cashew fruit trees in Côte d'Ivoire

ZAE	Régions	Sub-Prefectures	Localities	Crops
Zone 7	Tchologo	2	3	Cashew and Mango
	Kabadougou	9	35	Cashew and Mango
	Folon	2	4	Cashew
	Poro	6	11	Cashew and Mango
	Bagoue	5	10	Cashew and Mango
Zone 5	Hambol	3	14	Cashew and Mango
	Worodougou	6	17	Cashew and Mango
	Bere	6	17	Cashew
	Gbeke	4	10	Cashew and Mango
Zone 4	Belier	2	4	Cashew and Mango
	Gontougo	3	5	Cashew
	Yamoussoukro	2	2	Cashew
Zone 3	Haut Sassandra	5	10	Cashew
	Marahoue	3	6	Cashew
Zone 2	Guemon	1	3	Cashew
Total	**15**	**59**	**146**	**02**

ZAE : agroecological zone

2.4.3. Laboratory isolation of the pathogen

This step took place in the laboratory and consisted of isolating the fungi present in the samples. The method used is based on that of [21] and [22]. To do this, one-centimeter-long pieces of mango or cashew stems were superficially disinfected with a 7 °C bleach (NaClO) solution diluted to 50% with sterile distilled water for one minute. These fragments were then rinsed three times successively for the same duration with sterilized distilled water. Then, they were placed on Potato Dextrose Agar (PDA) artificial culture medium. The Petri dishes containing the culture substrate and having received the apparently sterilized pieces of tissue were incubated at 30 °C with alternating light and darkness for three days. After these three days of incubation, the dishes were examined and all the fungi that had developed on the medium around the wood tissues were subcultured onto PDA medium. The inseminated media were also incubated under the same conditions as before for another 7 days.

2.4.4. Identification of isolates

The last step consists of identification of the isolated pathogens. The last step consists of identification of the isolated pathogens. A macroscopic observation (color and appearance) of the colonies and microscopic (morphology and characteristics) of the conidia was carried out. The identification was done by following the determination keys of [23] and [24]

2.4.5. Statistical analyses

XLSTAT 2021 software was used for statistical analyses. The Shapiro-Wilk normality test was performed to verify the normal distribution of variables at the 5% significance level. In case of normality of the variables, analysis of variance was used to compare the incidence and severity of the disease at the 5% significance level. In case of significant difference, the Turkey test was used to form homogeneous groups.

3. Results

3.1. Symptoms of *Lasiodiplodia* sp in the studied localities

Figures 1 and 2 show typical symptoms corresponding to infections due to *Lasiodiplodia* sp., which were observed in all the surveyed plots in the different localities studied. Indeed, the symptoms begin at the tips in the form of dry rot. The disease subsequently affects the branches and vegetative buds, causing hypertrophy and gum exudation on the main trunk or on the primary and secondary branches (SO 1), followed by intense defoliation and progressive death from the tip to the base called drying or dieback (SO 3). On the primary and secondary branches and the main trunk, the infection begins from the external surface of the wood, and from natural cracks in the bark (SO 2). The presence of well-defined canker and dieback of branches (SO 3) is the most common symptom (92.08%) followed by the presence of rubber exudations on the main trunk or on the primary and secondary branches SO 1 (45.42%). Finally, visible cracks or exposure of internal tissues, on the main trunk or on the primary and secondary branches (SO 2) are less common (22.38%) in our surveyed orchards.

Figure 1 Some symptoms of drying observed on cashew trees

Legend: SO 2: Visible cracks, exposure of internal tissues, on the main trunk or on the primary and secondary branches; SO 3: Presence of well-defined canker and dieback of branches

Figure 2 Some symptoms of drying observed on mango trees

3.2. Disease incidence and severity

The analysis of variance shows only a significant difference (p=0.0001) at the level of the average severity indices between the two crops (Table 4). Cashew cultivation presents the highest values (3.67±0.61) at the level of average severity indices. The incidence of the dry-down disease for mango and cashew were 81.03% and 79.69% respectively.

Table 4 Incidence rate and average severity of dry-out disease on cashew and mango crops in Côte d'Ivoire

Crops	**Incidence (%)**	**Severity Index (ISM)**
Mango (*Mangifera indica*)	81.03±0.93 a	3.59±0.29 b
Cashew (*Anacardium occidentale*)	79.69±0.44 a	3.67±0.61 a
F	1,6515	16,9576
Pr > F	0.2004	< 0.0001

In the same column, the mean values followed by the same letter are not statistically different according to the Turkey test at the 5% significance level.

The analysis of variance shows a significant difference between the regions only in cashew cultivation (p=0.0001) unlike mango (p=0.3362) (Table 5). In fact, Agro-ecological Zone 7 presents the highest incidence rate values (82.06±3.22) and average severity index (3.75±0.03) and Agro-ecological Zone 2 presents the lowest values (78.00±0.05) (Table 5).

Table 5 Incidence rates and average severities of cashew and mango wilt diseases according to agroecological zones of Côte d'Ivoire

	Incidence (%)		**Severity Index (ISM)**	
ZAE	**Mango**	**Cashew**	**Mango**	**Cashew**
Zone 2	-	78.00±5.32ab	-	3.46±0.26b
Zone 3	-	76.12±1.33b	-	3.56±0.06b
Zone 4	78±0.10a	75.75±1.88b	3.46±0.15a	3.52±0.09b
Zone 5	81.33±2.30a	78.57±0.71ab	3.61±0.08a	3.64±0.03b
Zone 7	81.1±3.44a	82.06±0.66a	3.60±0.02a	3.75±0.03a
Averages	80.14±1.19	78.10±1.98	3.55±0.08	3.59±0.09
F	11.55	8.58	0.4200	3,4428
Pr<F	0.080	< 0.0001	0.660	0.010

In the same column, the mean values followed by the same letter are not statistically different according to the Turkey test at the 5% probability threshold, ISM: Severity Index, ZAE: Agroecological Zone.

3.3. Identification of *Lasiodiplodia* sp.

Macroscopic observation: It was carried out on the third and fourteenth day after subculturing and revealed the presence of different morphotypes (3) within the isolates of *Lasiodiplodia* sp. studied. The different morphotypes observed are illustrated in Figures 5. Indeed, on the third day, the isolates were characterized by a whitish, sparse or cottony mycelium on the front face of the Petri dish. This mycelial facies gradually evolved to fill the Petri dish. From the fifth day, the initial coloration gradually changed from whitish (A) to grayish (B) or blackish (C) with a cottony appearance and a downy structure (Figure 3).

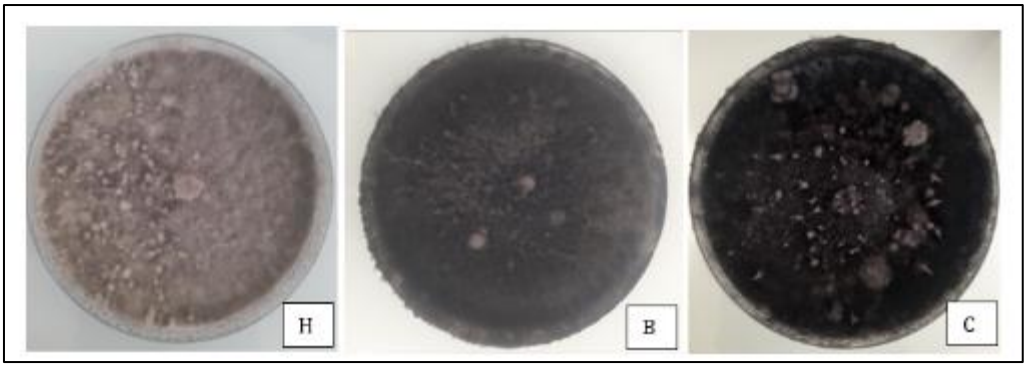

Figure 3 Different morphotypes (3) within the isolates of *Lasiodiplodia* sp. studied

Microscopic observation: Figure 4 presents the microscopic characteristics of the *Lasiodiplodia* sp isolates studied. Microscopically, the conidia appeared highly variable from one isolate to another, with ovoid, oblong, and ellipsoid conidia presenting two overall colors, including brown and hyaline. These conidia sometimes presented irregular longitudinal striations and rings at the septa.

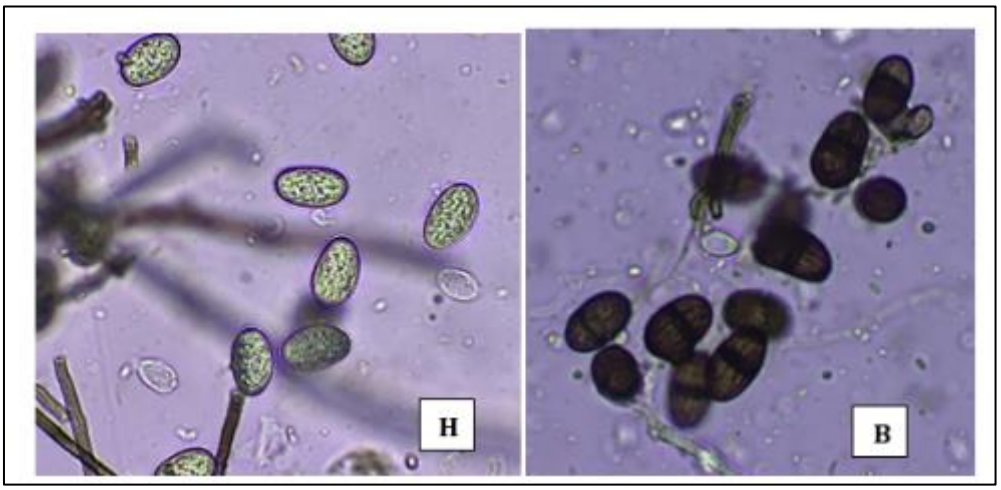

Figure 4 Different conidia within isolates of Lasiodiplodia sp., studied Immature conidia of *Lasiodiplodia* sp.; B-Mature conidia of *Lasiodiplodia* sp

3.4. Disease distribution

Figure 5 shows the distribution map of the wilting disease caused by *Lasiodiplodia* sp. in cashew and mango orchards in Côte d'Ivoire. Symptoms of the disease are present in all orchards (100%) prospected (Figure 3) with a cumulative incidence rate of 80%. The average severity index is 3.59±0.09, i.e. category 3.

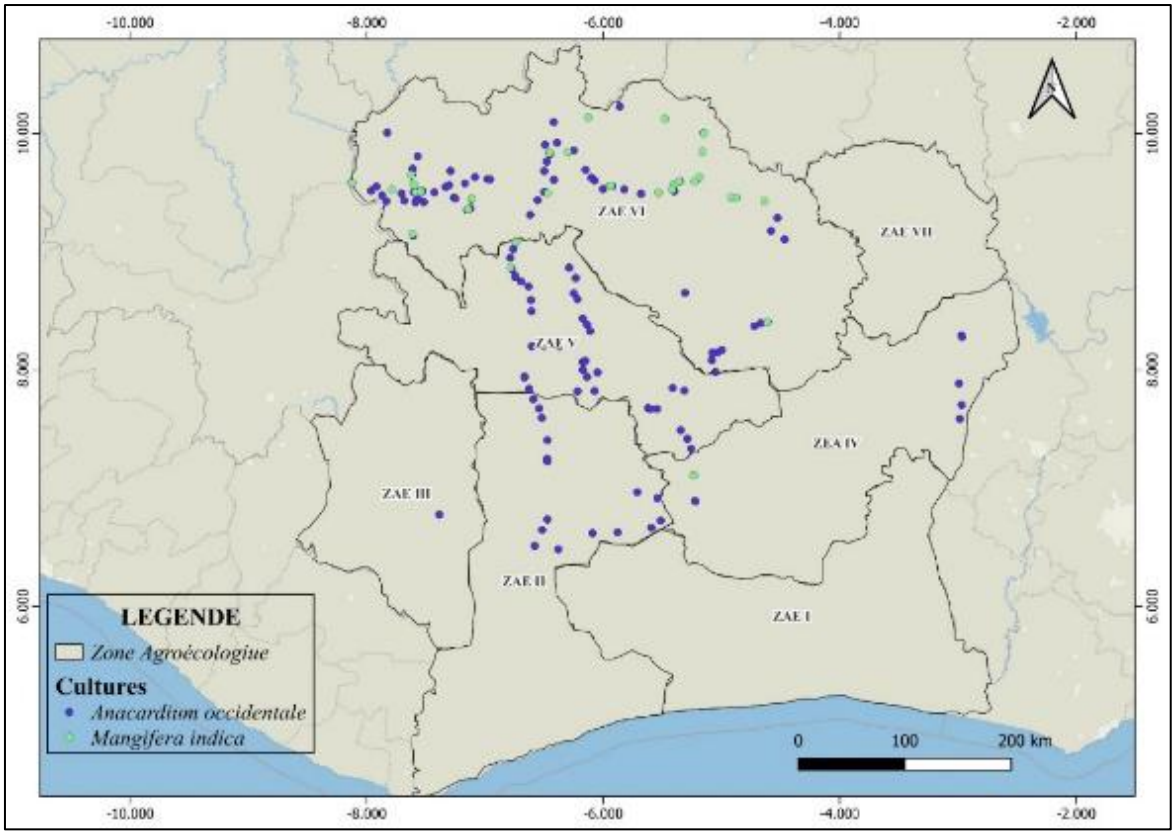

Figure 5 Distribution map of die-back disease due to Lasiodiplodia sp. in cashew and mango orchards in Côte d'Ivoire

4. Discussion

Several symptoms such as drying of the lower end of the stem, the presence of gum exudations, cracks and exposure of internal tissues, on the main trunk or on the primary and secondary branches were observed. These different symptoms could be associated with the fungus *Lasiodiplodia* sp. [25] isolated fungi from mango trees that were affected by regressive death.

The visible cracks and exposures of internal tissues, which were observed, could be caused by the wind or the producers themselves during the maintenance of the plots [26]. As for the presence of gum exudations, it could be due to a defense mechanism put in place to regenerate its tissues damaged by producers, animals and insects [9].

Isolated fungi are certainly present due to the actions of wind, rain or the producers' work tools, thus causing an epidemic. Indeed, according to some authors, the penetration of the fungus into plant tissues can occur through injuries or not [27]. Regarding the presence of well-defined canker and branch dieback, the ends of the plants being fragile with poorly developed tissues could facilitate infection by the fungus. This could also be explained by the fact that some fungi present on the surface of fruits or explants can be in the form of spores that can germinate [1]. According to [28], pathogens on the surface of plants that are able to penetrate plants through lenticels, shocks or injuries at the upper end of the plant. The same observation was made by [29] in a study on the evolution of wasting disease caused by *Lasiodiplodia* sp.

Of all these symptoms, stem drying and the presence of rubber exudations are those that are present in all cashew and mango production areas in Côte d'Ivoire. Therefore, they could be considered as the main symptoms of *Lasiodiplodia* sp diseases in cashew and mango orchards, given their incidence and severity. [19] corroborate this result through a study carried out in Mexico. However, according to this author, like [7], environmental conditions are determining factors in the etiology of this disease and it is advisable to maintain a state of vigilance in a context of climate change. Indeed, in agro-ecological zones 7 and 5, rainfall is between 1150 and 1350 mm and is low with high temperature (26.7±1.1 C) compared to other agro-ecological zones [30]. This could explain the variation in distribution and severity from one locality or agro-ecological zone to another. The high severity in these areas could be caused by the extreme climatic conditions of these areas where the temperature is extremely high with low rainfall. [31] corroborates this result. The

severity of the disease may be caused by environmental pressures, especially in semi-arid regions, where climatic conditions are favorable and uniform, such as temperature; low relative humidity and low rainfall [32]; [22]. These factors, combined with biological pressure from pathogens and pests expanding their geographical boundaries, promote the development of diseases related to *Lasiodiplodia* sp. [34]. Macroscopic and microscopic characteristics confirm the presence of *Lasiodiplodia* sp. [35]. The different morphotypes observed are whitish, grayish, and blackish. These results are similar to previous studies by [36] who showed that mycelial colonies of *Lasiodiplodia* sp. isolates evolve after subculturing. These observed microscopic characteristics have been reported by several authors [37]. According to [38], conidia of *Lasiodiplodia* sp. are initially hyaline, unicellular, ellipsoid to oblong, thick-walled, and granular in content. Upon maturity, they become bicellular, dark brown, and have pigmented longitudinal bands. [39] reported that the main features that distinguish *Lasiodiplodia* sp. from other closely related fungal genera are the presence of pycnidial paraphyses and longitudinal striations on mature conidia [36].

The presence of this pathogen in cashew and mango orchards in Côte d'Ivoire could lead to a drop in yields of these crops in Côte d'Ivoire. This same observation was made by [7] in Benin in relation to the yield of cashew cultivation.

5. Conclusion

This study determined the distribution of wilting due to *Lasiodiplodia* sp. in cashew and mango orchards in Côte d'Ivoire. Various symptoms related to wilting were identified in the orchards. The incidence rate and severity of the disease varied from one crop to another and from one agro-ecological zone to another. The presence of the genus *Lasiodiplodia* sp. was found to be highly responsible for fungal diseases in the orchards surveyed. It is therefore fair to deduce that reducing the impact of wilting of mango and cashew trees will require the control of *Lasiodiplodia* sp. However, additional studies, including morphological, microscopic and molecular characterization are necessary to establish a more precise link between this pathogenic fungus and mango and cashew plant diseases.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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