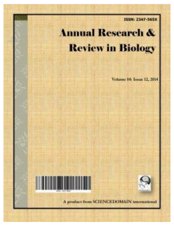

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Identification and Characterizations of Pathogenic Fungal Species Associated with Symptoms of Cassava Anthracnose in Ivory Coast

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Authors' contributions

This work was carried out in collaboration between all authors. Author EKJN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author KKG has helped to identify fungal isolates and corrected first draft. Author THMAC has helped to collect samples. Author AK read and corrected the first draft. Author KD is the scientific director of project. He corrected the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Cassava anthracnose is a plant disease that affects cassava stems, petioles and fruits. The aim of this study was to analyze the diversity of cassava anthracnose symptoms in Ivory Coast and then to identify and characterize the associated fungal genera. Surveys were carried out in all agricultural zones of the country from July to November, in 2014, 2015, 2016 and 2017. Infected samples

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consisting of stems cut with a small number of superficial cankers (0.3%), distorted stems (25.77%), and necrotic stems and petioles (65.18%) were collected. Also, withered and dried apical buds (8.76%) were harvested. Fungal pathogens derived from samples were *Colletotrichum gloeosporioides* (35.08%), *Fusarium* sp. (27.19%) and *Botrytis* sp. (19.73%) genera and undetermined strains (17.98%). Genera were characterized by morphological and microscopic characteristics. Parasitic pressure increased to 80 and 100% respectively for *Botrytis* sp. genus and *Colletotrichum gloeosporioides* and *Fusarium* sp genera. Fungal genera have caused lesions on stem and petioles in green house with diameters sizes 46, 71 and 72 mm respectively for genera *Botrytis* sp, *Fusarium* sp and *Colletotrichum gloeosporioides*. Aggressiveness index of *Botrytis* sp. genus was 3 and 4 respectively for *Colletotrichum gloeosporioides* and *Fusarium* sp. genera. The mycoflora of cassava aerial organs alteration, linked to the symptoms of anthracnose, is composed of genera of great economic importance and scientific interest.

Keywords: Cassava; anthracnose; mycoflora; Colletotrichum gloeosporioides; Fusarium sp; Botrytis sp.

1. INTRODUCTION

Cassava is the second most important food crop in Ivory Coast due to its tuber yield. Its production contributes to the reduction of the food deficit through the multiplicity of products derived from its artisanal and industrial processing while supporting economic activity for poverty reduction [1]. However, fresh cassava tuber yields in Ivory Coast decreased from 4,239,303 tons in 2014 to 3,674,818 and 3,210,614 tons respectively in 2015 and 2016 [2]. Cassava anthracnose, which is the most damaging fungal disease of cassava in the tropical zone, has reached worrying levels of incidence and severity in Ivory Coast [3,4]. Notwithstanding abiotic factors, viral and bacterial phytopathological factors, cassava anthracnose disease causes enormous economic losses through alteration of cuttings quality and yield losses related to young plant mortality [5,6]. It is a disease of the aerial parts of cassava plants, especially the stem and leaf petioles [7]. Symptoms include cankers, distortions, numerous lesions and severe necrosis on stems, petioles and leaf axils. Petiole wilt followed by severe defoliation leading to drying of the buds with stem exudate has also been described [4]. The establishment of anthracnose is promoted by injuries or tissue weakening that will constitute the entry points for the infectious propagules of *Colletotrichum gloeosporioides* Penz *manihotis* [8]. In addition, many other pathogenic fungal species or saprophytes have often been associated with cassava disease. Nyaka [9] have identified in Cameroon, on cassava root diseases, various fungal strains including *Colletotrichum* sp., *Fusarium* sp., *Pestalotia* sp., *Geotrichum* sp., *Sphaerostilberepens*, *Trichoderma viride* and *Botryodiplodia theobromae*. Also, in Ivory Coast Silué [10] have identified, on anthracnose symptomatic of Cashew (*Anacardium occidentale* L.), *Colletotrichum gloeosporioides*, *Pestalotia heterocomis*, *Lasiodiplodia theobromae* and other unidentified fungal strains. The openings that lead to cassava anthracnose could be secondary routes of infestation for polyphytophagous fungal genera. Knowledge concerning mycoflora associated with cassava aerial organs alteration, generally, and that related to the symptoms of cassava anthracnose disease, particularly in Côte d'Ivoire, is not available.

The aim of this study was to analyse the diversity of cassava anthracnose symptoms in Ivory Coast and then to identify and characterize associated pathogenic fungal genera.

2. MATERIALS AND METHODS

2.1 Plant Materials

Cassava is the main crop observed and evaluated. The various varieties produced in the seven agro-ecological zones (AEZ) of Ivory Coast were examined in their development of anthracnose symptoms. Specifically, some stems and petioles infected and healthy cuttings were harvested from farmer plots for screening.

2.2 Cassava Anthracnose Disease Symptomatic Assessment and Sampling

Surveys were conducted in all the agricultural areas of the country from July to November, from 2014 to 2017. An average of three farmer plots, bordering the roads and 10 to 20 km apart, were subjected to health assessment through plant observations [11]. It was essential to identify different cassava anthracnose symptoms. The infected area of the plant, morphology and coloration of necrotic surface have been considered for symptomatic diversity. Samples were taken on the basis of the different symptomatic level of cassava anthracnose disease defined by the IITA rating scale [12]. This scale is defined as follows: 1 = No symptom; 2 =Shallow cankers on woody stems appearing towards the end of the season; 3= Many deep cankers on stems that have become woody and deformed; 4= Many oval lesions on green stems (herbaceous, not woody), lesions on young stems and severe leaf axil necrosis; 5= Withering, strong defoliation and death of part of or whole apical buds. Infected stems and petioles were collected for analysis.

2.3 Isolation, Identification and Characterizations of Fungi Associated with Cassava Anthracnose Disease Symptoms

Isolations and purifications took place in the laboratory according to the methodology of Fokunang and Dixon [13]. Samples were cleaned with 70% alcohol and five explants were taken from front of necrosis. They were disinfected in 10% sodium hypochlorite for 3 minutes. Explants were also washed 3 times for 3 minutes with sterile distilled water. They were dried and seeded on PDA (Potato Dextrose Agar) medium (20%). After 72 hours of incubation, fungal colonies emerged were transplanted on new PDA medium until a pure isolate was obtained. The cultural characteristics that contributed to the diversity of the isolates were the appearance and colouring of the aerial thallus. Morphological aspects were based on the general shape of the conidia and mycelium. Genera identifications, based on these characters, were made using the keys of Webster and Weber [14], Barnet and Hunter [15] and Malloch [16]. Morpho-metric assessments focused on radial mycelial growth and conidia size. Mycelial growth evaluation was done daily, based on the measurement, along two orthogonal axes, from a mycelial disc inoculated on a PDA medium (20%) [17]. The measurement of conidia size focused on measuring length along the longitudinal axis and width along the vertical axis of conidia. A drop of a conidia suspension was mounted between the slide and the cover slip and measurements were made under an optical microscope at 40X magnification to 20 µm.

2.4 Assessment of the Fungal Parasitic Pressure of Cassava Anthracnose Symptoms

A total of 5 explants were inoculated on PDA medium (20%), for each sample treated. Four of the explants were taken at the growth front and the last one was taken in the center of the symptom initiation zone. Parasite pressure was assessed by determining population and proportion of fungal contaminants arising from infected samples. The fungal population was assessed through the diversity of emerging strains on all treated samples. The Proportion of Contaminant (Pc) was calculated according to the formula (1) of Spurr and Welty [18]:

$$\text{Contaminant proportion (Cp)} = \frac{\text{Contaminant Eff}}{5 \times \text{NE}} \times 100 \tag{1}$$

Contaminant Eff= Total contaminants in the collection; NE= Total number of samples treated.

2.5 Characterization of the Pathogenic Potential of Fungal Genera and Koch's Postulate

This characterizations consisted in evaluating the aggressiveness of three genera on the original host and satisfying Koch's postulate [19]. The local variety, sensitive to anthracnose, Yacé was used for the pathogenicity test. After 6 weeks of culture in green house, a vigorous plant was artificially injured with a cold sterile needle. The injuries were applied to the knotted area, in the axil and on the petioles of the leaves, of each plant. Inoculations were performed by placing a small mycelial disc, taken from a 14-day-old culture medium, on the injuries. Three morphotypes of each genus were used for pathogenicity assessment. The evaluations took place 30 days after inoculations. The diameter of the necrosis was calculated according to the formula (2) used by Kouamé [19]:

$$\textit{Diameter of lesions (LD)} = \frac{\textit{Length of lesions} + \textit{Width of lesions}}{2} \tag{2}$$

The aggressiveness was assessed using a scoring scale used by Wokocha [20] with a change in intervals. Aggressiveness index (AI) was calculated according to following formula (3):

$$\textit{Aggressivity index (AI)} = \frac{\sum R_i}{N} \tag{3}$$

R = Number of infected points with the same size; i = level of infection ; N = total number of lesions.
Levels of infection: 1 = lesion < 1 mm ; 2 = lesion from 1 to 3 mm ; 3= lesion from 4 to 6 mm ;
4 = lesion from 7 to 10 mm; 5 = lesion > 10 mm.

2.6 Statistical Analysis

Statistical analyses were carried out using Statistica version 7.1 software. Morpho-metric data of isolates and their aggressiveness on the host plant were subjected to the ANOVA analysis of variance (one factor). The significantly different averages were classified according to Duncan's grouping test at 5% threshold.

3. RESULTS

3.1 Symptomatic Diversity of Cassava Anthracnose Infection Stages and Mycoflora Hosted

In the infected plots visited, symptoms observed on the collected samples are shown in (Fig. 1). They consisted of a small number of superficial cankers (0.3%) located on added stems. Also, deformations (25.77%) of stems due to bulges and distortions were collected on stems and petioles. As a result, we collected green stems and petioles injured covered with necrotic lesion (65.18%). Finally, dieback and dried apical buds (8.76%) were collected. The fungal population hosted consist of genera of *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botrytis* sp. and unidentified strains. Isolates of *Colletotrichum gloeosporioides* and unidentified strains were isolated from all symptomatic stages while *Fusarium* sp. genus was hosted only by stages 3, 4 and 5. *Botrytis* sp. genus were found in stages 3 and 4 mycoflora (Table 1).

3.2 Phenotypic Diversity of Mycoflora of Cassava Anthracnose Symptoms

Three fungal genera were distinguished in the mycoflora of cassava anthracnose symptoms, depending on the samples treated. They are shown in Figs. (2-4) under different phenotypes depending on the appearance of the aerial thallus and the shape of the conidia. *Colletotrichum gloeosporioides* genus presented several morphotypes with cylindrical and fusiform conidia (Fig. 2). *Fusarium* sp. genus also presented several morphotypes producing fusiform macro-conidia and cylindrical micro-conidia (Fig. 3). Morphotypes of *Botrytis* sp. genus had rounded conidia (Fig. 4). Mean radial mycelial growth was higher between 72 and 120 hours. The average lengths of the cylindrical conidia of *Colletotrichum gloeosporioides* were between 27.37 and 29.82 for average widths between 6.67 and 7.53 µm. The mean lengths of the fusiform conidia were between 40.97 and 55.40 µm for mean widths between 4.45 and 7.13 µm. The rounded conidia of *Botrytis* sp. genus had average lengths between 6.63 and 7.40 µm and average widths between 6.67 and 7.94 µm.

3.3 Parasitic Activity of Mycoflora Morphotypes of Casava Anthracnose Disease Symptoms

Three genera have induced lesions on the internodes of the stems, on the petioles and in the axil of the leaves. Average diameter of lesions and mean aggressiveness index of genera were revealed with significant difference (P = 0.01). Parasitic pressure was very high for all three genera. *Colletotrichum gloeosporioides* and *Fusarium* sp. genus were the most abundant fungal population and were found with total parasitic pressure on the seeded explants. *Botrytis* sp. genus could be revealed by four out

Table 1. Fungal genera associated with cassava anthracnose disease stages

Infection stages of the samples	Fungal populations proportion (%)			
	***Botrytis* sp. genus**	***Colletotrichum gloeosporioides***	***Fusarium* sp. genus**	**Non identifiées**
Stage 2	0	1	0	48
Stage 3	65	45	48	27
Stage 4	35	53	51	15
Stage 5	0	1	1	10
Fungal population (%)	19,73 c	35,08 a	27,19 b	17,98 d

Letters (a, b, c and d) refer to different statistical averages classes of fungal population proportion with significant difference according to Duncan's grouping Test at 5% threshold.

of five explants and was lower than those of the other two genera in fungal population. The average diameters of necrosis caused by morphotypes of *Colletotrichum gloeosporioides* and *Fusarium* sp. were larger than those of morphotypes of *Botrytis* sp. genus. Morphotypes of *Colletotrichum gloeosporioides* and *Fusarium* sp. genera were more aggressive on stems and petioles than morphotypes of the genus *Botrytis* sp. (Table 2).

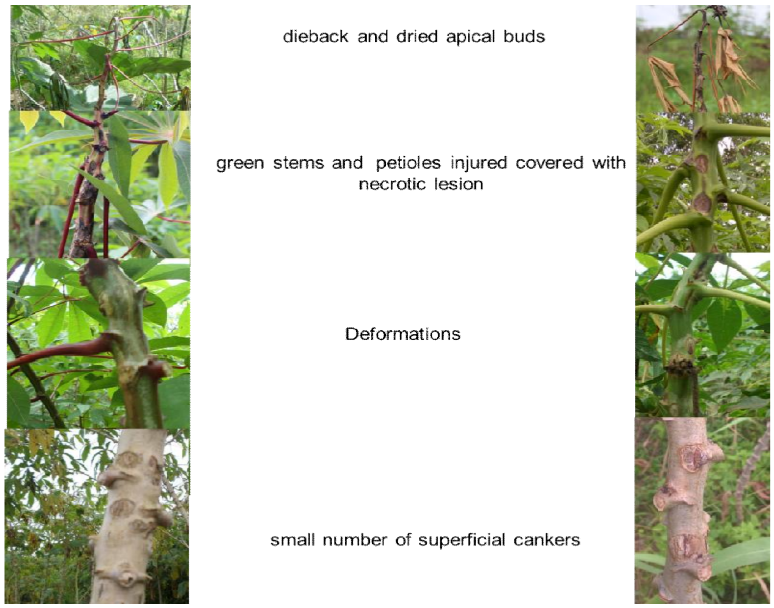

Fig. 1. Symptomatic diversity of cassava anthracnose disease in Ivory Coast

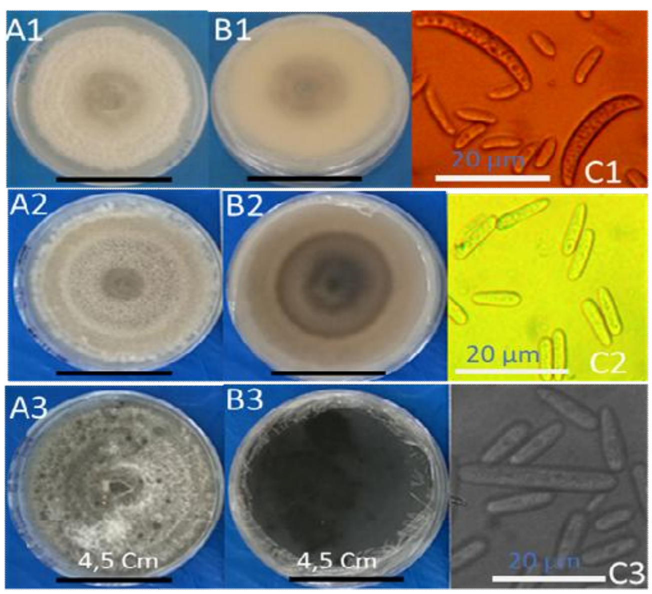

Fig. 2. Morpho-cultural and microscopic characteristics of isolates of the genus *Colletotrichum gloeosporioides*

(Morphotype AgYSt4: A1 and B1: cottony phenotype with 10.5 mm/day (growth); C1: conidia size 27.37 × 7.01 µm)
(Morphotype BotPet: A2 and B2: cottony phenotype with 8.42 mm/day (growth); C2: conidia size 28.9 × 7.5 µm)
(Morphotype PetVB: A3 and B3: cottony phenotype with 15.1 mm/day (growth); C3: conidia size 29.9 ×6.9 µm)

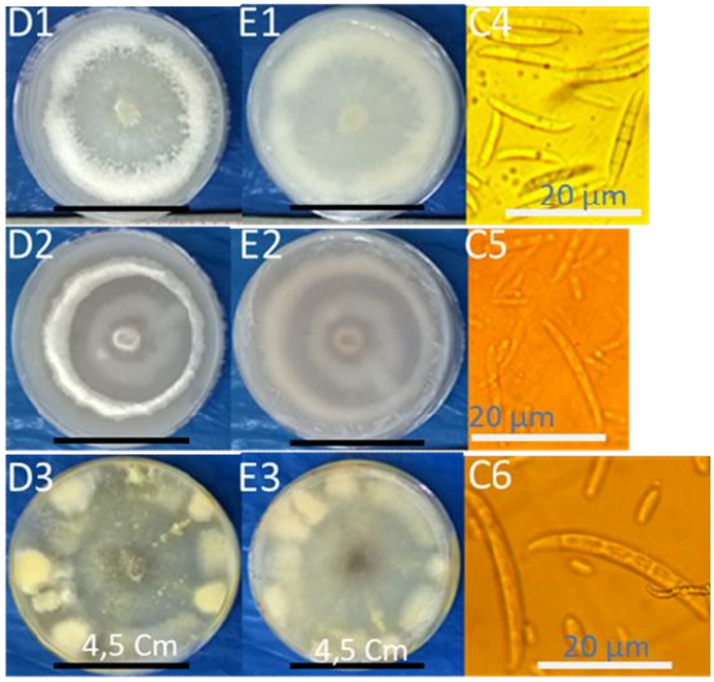

Fig. 3. Morpho-cultural and microscopic characteristics of isolates of the genus *Fusarium* sp.
(Morphotype SahYSt3: D1 and E1: cottony phenotype with 15.2 mm/day (growth); C4: conidia size 55.4 ×4.4 µm)
(Morphotype MbaASt1: D2 and E2: cottony phenotype with 17 mm/day (growth); C5: conidia size 53.2 × 7 µm)
(Morphotype DaoASt4: D3 and E3: cottony phenotype with 15.1 mm/day (growth); C6: conidia size 40.9 ×7.1 µm)

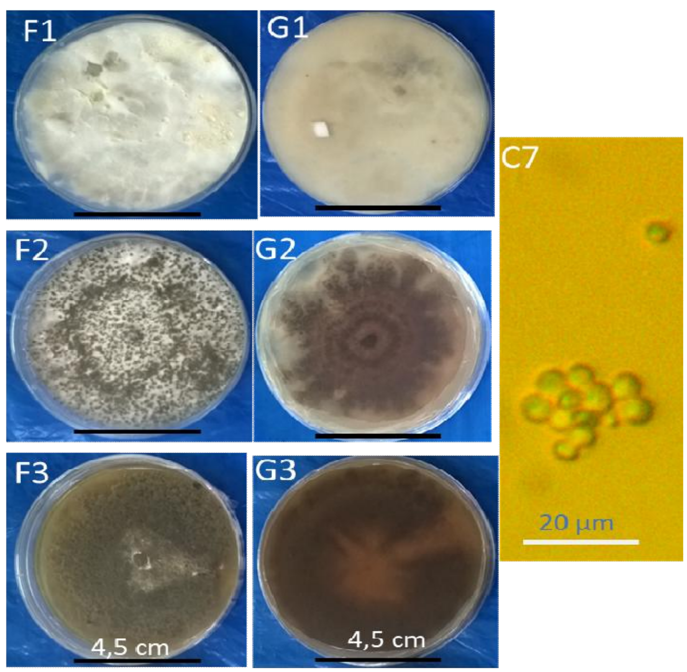

Fig. 4. Morpho-cultural and microscopic characteristics of isolates of the genus *Botrytis* sp.
(Morphotype BiaRSt3: F1 and G1: cottony phenotype with 17 mm/day (growth); C7: conidia size 6.6 ×7.9 µm)
(Morphotype MakASt4: F2 and G2: cottony phenotype with 17 mm/day (growth); conidia size 7.4 × 7 µm)
(Morphotype DivASt3: F3 and G3: cottony phenotype with 10.1 mm/day (growth); conidia size 6.7 ×6.6 µm)

Table 2. Parasitic activity of fungal populations of mycoflora associated with cassava anthracnose disease symptoms

Fungal populations	Parasitic pressure of genera (%)	Mean diameter of necrosis (mm)	Mean aggressiveness index
Botrytis sp.	82 b	46±0,07 b	3± 0,00 b
Fusarium sp.	100 a	71± 0,24 a	4±0,75 a
Colletotrichum gloeosporioides	100 a	72± 0,22 a	4±0,80 a
Non identifiées	75 c	ND	ND

ND: Not determined; letters (a, b and c) refer to the different statistical averages classes with a significant difference according to Duncan's grouping Test at 5% threshold.

4. DISCUSSION

Cassava anthracnose disease is manifested by a small number of superficial cankers located on added stems. It has been observed deformations due to swelling and distortions of petioles and stems. Also, damaged and necrotic green stems and petioles as well as dried apical buds and dieback were observed. Similar symptomatic observations have also been reported in assessments of cassava anthracnose in Cote d'Ivoire [4], Congo [21,22] and Nigeria [7,11]. Mycoflora, associated with cassava anthracnose symptoms, include fungal morphotypes of genera *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botrytis* sp. and unidentified species. Indeed, this diversity of fungal pathogenic genus, linked to cassava infections, on one side, was revealed by the results of Nyaka [9] after identification of pathogenic fungi associated with cassava root rot in Cameroon. On other side, diversity of fungal genera associated with anthracose symptoms has been observed in Ivory Coast. Silué [10] identified *Colletotrichum gloeosporioides*, *Pestalotia heterocomis*, *Lasiodiplodia theobromae* genera and unidentified fungal strains on symptomatic cashew (*Anacardium occidentale* L.). The identifications were based on morphological and microscopic characteristics. Our morpho-metric results, both macroscopic and microscopic, are consistent with those of Fokunang [8], Ferrada [23] and Burgess [24] respectively for the genera *Colletotrichum gloeosporioides*, *Botrytis sp*. and *Fusarium* sp.. In addition, the proportion and parasitic fungal pressure were significant for all genera encountered. *Colletotrichum gloeosporioides* and *Fusarium* sp. genera were more observed on samples from non-lignified parts and on petioles of plants with lesions and necrosis. Indeed, the genus *colletotrichum gloeosporioides* has been identified as infectious agent causing cankers, lesions, necrosis and dieback on all the aerial organs of cassava affected by anthracnose in Ivory Coast [4], Congo [22] and Nigeria [19]. Also, *Fusarium* sp. genus has been identified as infecting 150 plant species in several modes of infection [25], most commonly causing stalkrots. The genus *Botrytis* has been more encountered on stems deformations and necrosis. This genus would cluster infectious agent from leaves, stems, flowers, fruits and seeds of 500 plant species causing distortions, cankers and rotting. As a necrotroph, it often takes advantage of damage resulting from other pathogens to produce symtoms often call gray mold [26,27]. These three genera *Botrytis* sp., *Fusarium* sp. and *Colletotrichum* sp. occupy respectively the 2nd, 4th and 8th ranks, in terms of fungal pathogens of economic and scientific importance [28].

5. CONCLUSION

Tissues symptomatic of cassava anthracnose disease host a diverse associated mycoflora of which *Colletotrichum gloeosporioides* species and the genera *Fusarium* and *Botrytis* are the most representative fungal population. They are a source of high parasitic pressure through the activity of different morphotypes, encountered at all stages of anthracnose infection. These three genera have economic and scientific importance recognized by the scientific community. Their occurrence in the mycoflora of cassava anthracnose disease symptoms reveals a major phytosanitary problem that opens up multiple fields of study including control approaches, such as the use of bio-pesticides, and molecular analysis of morphotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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