Original Research Article

Identification and Characterization of Pathogenic Fungal Species Associated with Symptoms of Cassava Anthracnose in Côte d'Ivoire

ABSTRACT :

Cassava anthracnose is a plant disease that affectspathology of the aerial parts of cassava stems, petioles and fruits. The objective of this study waiis to analyze the diversity of symptoms of cassava anthracnose in Côte d'Ivoire and then to identify and characterize the associated pathogenic fungal species that are derived from them. Surveys were carried out throughout the country from July to November, in 2014, 2015, 2016 and 2017. Infected samples consisting of stems cut with a small number of superficial cankers (0.3%), deformed déformed stems ? (25.77%), lesioned ? and necrotic (65.18%) stems and petioles (65.18%). Also, withered and dried apical buds (8.76%) were collected. Fungal pathogens derived from the fungal alteration mycoflora ofinfected cassava aerial parts are of the genus *Colletotrichum gloeosporioides* (35.08%), the genus *Fusarium* sp (27.19%) and the genus *Botrytis* sp. (19.73%) and undetermined strains (17.98%). These different genera have revealedwere characterized a diversity by their morphological and microscopic characteristics. They applied a parasitic pressure of 80% and 100% for an average aggressiveness index of 3 and 4 respectively for the genus *Botrytis* sp and the genera *Colletotrichum gloeosporioides* and *Fusarium* sp. unclear The mycofloral of alteration of the aerial organs of cassava, linked to the symptoms of anthracnose, is composed of genera of great economic importance and great scientific interest.

Key words: cassava, anthracnose, mycoflora, Colletotrichum gloeosporioides, *Fusarium* sp*, Botrytis* sp.

Note that in the literature, the disease anthracnose on plants is attributed to species of Colletotrichum. The other fungi found may be secondary infections or the cause of similar diseases

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INTRODUCTION

Cassava is the second most important food crop in Côte d'Ivoire 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 Côte d'Ivoire ~~increased~~ decreased from 4,239,303 tonnes in 2014 to 3,674,818 and 3,210,614 tonnes 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 Côte d'Ivoire[3,4]. Notwithstanding abiotic factors and viral and bacterial phytopathological factors, cassava anthracnose disease causes enormous economic losses through the alteration of ~~cultured material~~ ? tuber quality? and yield losses related to young plant mortality [5,6]. It is a pathology of the aerial parts of cassava plants, especially the stem and leaf petioles [7]. Symptoms include cankers, deformities, 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 anthracnose symptoms. 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 Côte d'Ivoire Silué [10] have identified, on anthracnose symptomatic leaves ~~of anthracnose~~, *Colletotrichum gloeosporioides*, *Pestalotia heterocomis*, *Lasiodiplodia* theobromae and other

unidentified fungal strains. The openings ~~prior to the installation of~~that lead to cassava anthracnose could be secondary routes of infestation for some polyphytophagous fungal species. Knowledge on the mycoflora ~~of~~ associated with alteration of the aerial organs of cassava, in general, and that related to the symptoms of cassava anthracnose, particularly in Côte d'Ivoire, is not available.

The objective of this study is to analyse the diversity of symptoms of cassava anthracnose in Côte d'Ivoire and then to identify and characterise the pathogenic fungal species resulting from them.

2- MATERIAL AND METHODS

2-1- Plant material

Cassava is the main crop observed and evaluated. The various varieties in production in the seven agro-ecological zones (AEZ) of Côte d'Ivoire were examined in their development of anthracnose symptoms. These are precisely stems and petioles of infected cassava harvested from peasant plots and healthy cuttings used for assessments under semi-controlled conditions. Not clear what you mean.

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2-2- Cassava anthracnose disease symptomatic assessment and sampling

Surveys were carried out throughout the country from July to November, from 2014 to 2017. An average of three peasant plots, bordering the roads and 10 to 20 km apart, were subjected to health assessment through plant observations [11]. It was ~~discussed~~ essential to identify ~~the~~ different symptom~~atic types~~s of cassava anthracnose. The infected area of the plant, the morphology and coloration of the necrotic surface ~~allowed to appreciate the~~all contributed to symptomatic diversity. Samples were taken on the basis of the different symptomatic level of anthracnose defined by the IITA rating scale [12]. This scale is broken down as follows: 1 = Absence of symptoms; 2 =Few superficial 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 tasted ??), lesions on young stems and severe leaf axil necrosis; 5= Withering, strong defoliation and death of apical buds. I~~t collected~~ infected stems and petioles were collected for analysis.

2-3- Isolation, identification and characterization of fungal strains

Isolations and purifications took place in the laboratory according to the methodology of Fokunang and Dixon [13]. After 72 hours of incubation, all fungal colonies that emerged from explants were transplanted until a pure isolate was obtained. Media ? The cultural characteristics that contributed to ~~the appreciation of~~ the diversity of the isolates were the appearance and colouring of the aerial thallus. Morphological aspects were based on the general shape of the spores and mycelium. Strain 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 conidial dimensions. The evaluation of mycelial growth was done daily, based on the measurement, along two orthogonal axes, ~~of mycelial growth,~~ from a mycelial disc inoculated on a PDA medium (20%) [17]. The measurement of spores focused on measuring the length along the longitudinal axis and the width along the vertical axis of the spores. A drop of a spore suspension was mounted between the slide and the ~~lamella~~ cover slip and measurements were made under an optical microscope at 40X magnification ~~G×40~~ 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 (Potato Dextrose Agar 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 the ~~stand~~ species ? and proportion of fungal contaminants ~~hosted by~~arising from ? the ~~treated~~ 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 of Spurr and Welty [18]:

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

Contaminant Eff= Total contaminant ~~size~~ s in the collection; NE= Total number of samples treated.

2-5- Characterization of the pathogenic potential of strains and Koch's postulate

This characterization consisted in evaluating the aggressiveness of the strains 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 pot culture, a vigorous plant was artificially injured with a cold

sterile needle. The injuries were ~~sustained in~~applied to the knotted area, in the axil and on the petioles of the leaves~~.~~, giving three sites for each plant ?~~There were 3 of them, depending on the above-mentioned sites~~. Inoculations were performed by placing a mycelial disc a few millimeters in size, taken from a 14-day-old culture medium, on the wounds. Three morphotypes ? of each strain were used for pathogenicity assessment. The evaluations took place 30 days after inoculations. The diameter of the necroses was calculated according to the formula (1) used by Kouamé [20]:

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

The aggressiveness of isolates was assessed using a rating scale used by Wokocha [19] with a change in intervals. The aggressivity index (AI) was calculated according to the following formula (2):

$$Aggressivity\ index\ (AI) = \frac{\sum Ri}{N} \quad (2)$$

R = number of lesions for showing ? each degree of infection; i = degree of infection ; N = total number of lesions.

Degree 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. Did the pathogens produce spores and were lésions from different species identical, different etc.

2-6- Statistical analysis

~~The s~~Statistical analyses were carried out using Statistica version 7.1 software. Morpho-metric data of the strains 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 the Duncan test at the 5% threshold.

3- RESULTS

3-1- Proportion of infection stage and fungal genera hosted

In the infected plots visited, symptoms observed on the collected samples consisted of a small number of superficial cankers (0.3%) located on the added parts ?? of the 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 injured petioles covered with necrotic lesion (65.18%). Finally, discoloured and dried apical buds (8.76%) were collected. The fungal species hosted by these symptoms consist of strains 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. was hosted only by stages 3, 4 and 5. *Botrytis* sp. isolates 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 alteration of the aerial organs of cassava, depending on the samples treated. They are ~~illustrated~~ shown in figures 1-3 under different phenotypes depending on the appearance of the aerial thallus and the shape of the conidia. The genus *Colletotrichum gloeosporioides* presented several morphotypes with cylindrical and fusiform conidia (Figure 1). The genus *Fusarium* sp. also presented several morphotypes producing fusiform macro-conidia and cylindrical micro-conidia (Figure 2). Morphotypes of the genus *Botrytis* sp. had rounded conidia (Figure 3). Mean radial mycelial growth was higher between 72 and 120 hours. Three morphotypes of each genus were used for morphological and microscopic characterization. The average lengths of the cylindrical conidia of ?? 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 had average lengths between 6.63 and 7.40 µm and average widths between 6.67 and 7.94 µm.

infection stage of the samples	Proportion by stage of infection (%)			
	Botrytis sp.	Colletotrichum	Fusarium sp.	Non identifiées

Table I: Fungal genera associated with cassava anthracnose disease stages

		gloeosporioides		
Stage 2	0	1	0	48
Stage 3	65	45	48	27
Stage 4	35	53	51	15
Stage 5	0	1	1	10
Proportion by genus (%)	19,73 c	35,08 a	27,19 b	17,98 d

The letters (a, b, c and d) refer to the different classes of the statistical averages with a significant difference according to Duncan's test at the 5% threshold. ???

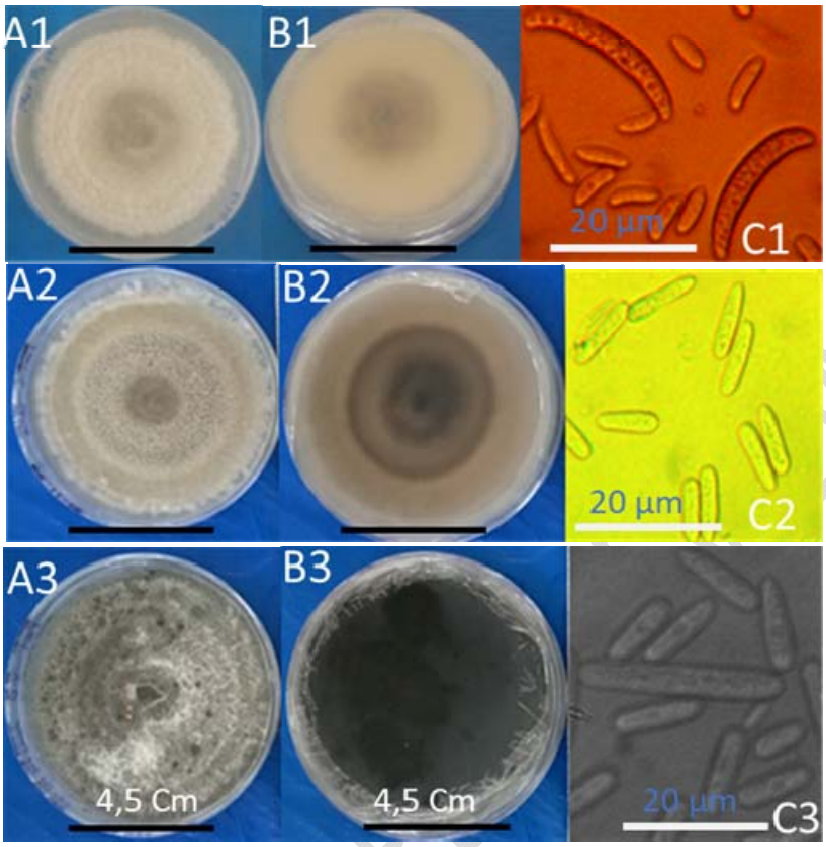

Figure I: Morpho-cultural and microscopic characteristics of isolates of the genus *Colletotrichum gloeosporioides*

(A1 and B1: cottony phenotype with 10.5 mm/d growth; C1: conidia of 27.37 × 7.01 µm)

(A2 and B2: cottony phenotype with 8.42 mm/d growth; C2: conidia of 28.9 × 7.5 µm)

(A3 and B3: cottony phenotype with 15.1 mm/d growth; C3: conidia of 29.9 ×6.9 µm)

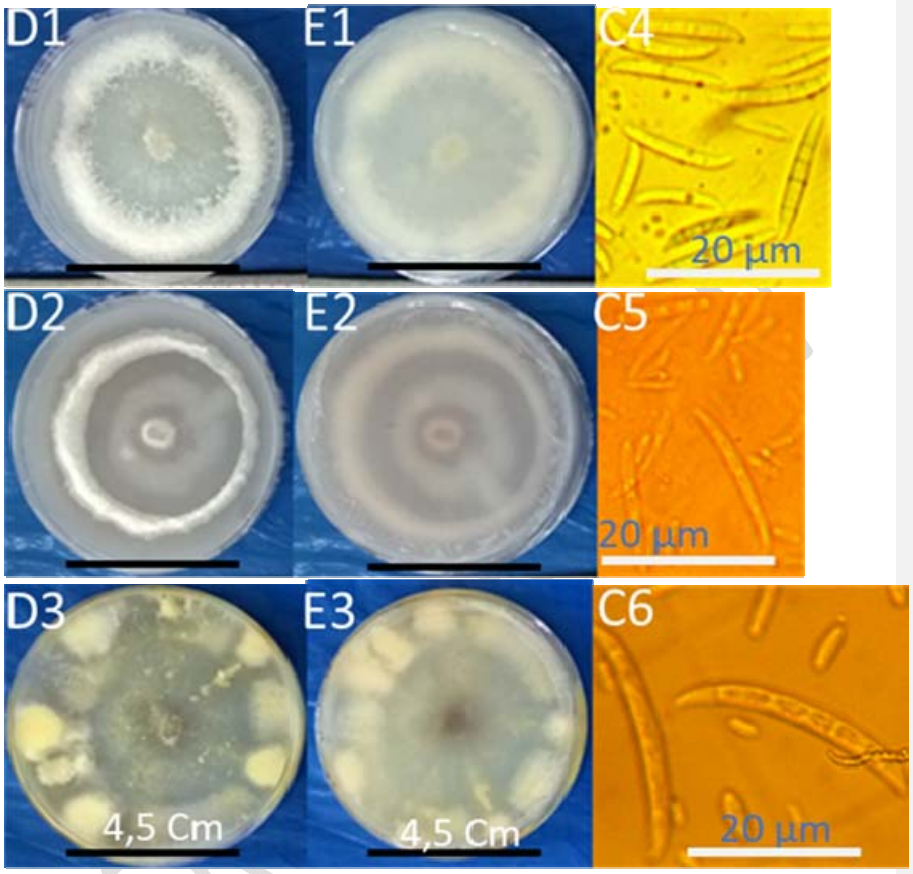

Figure 2: Morpho-cultural and microscopic characteristics of isolates of the genus *Fusarium* sp.

(D1 and E1 : cottony phenotype with 15.2 mm/d growth; C1 : conidia of 55.4 ×4.4 µm)

(D2 and E2 : cottony phenotype with 17 mm/d growth; C2 : conidia of 53.2 × 7 µm)

(D3 and E3: cottony phenotype with 15.1 mm/d growth; C3: conidia of 40.9 ×7.1 µm)

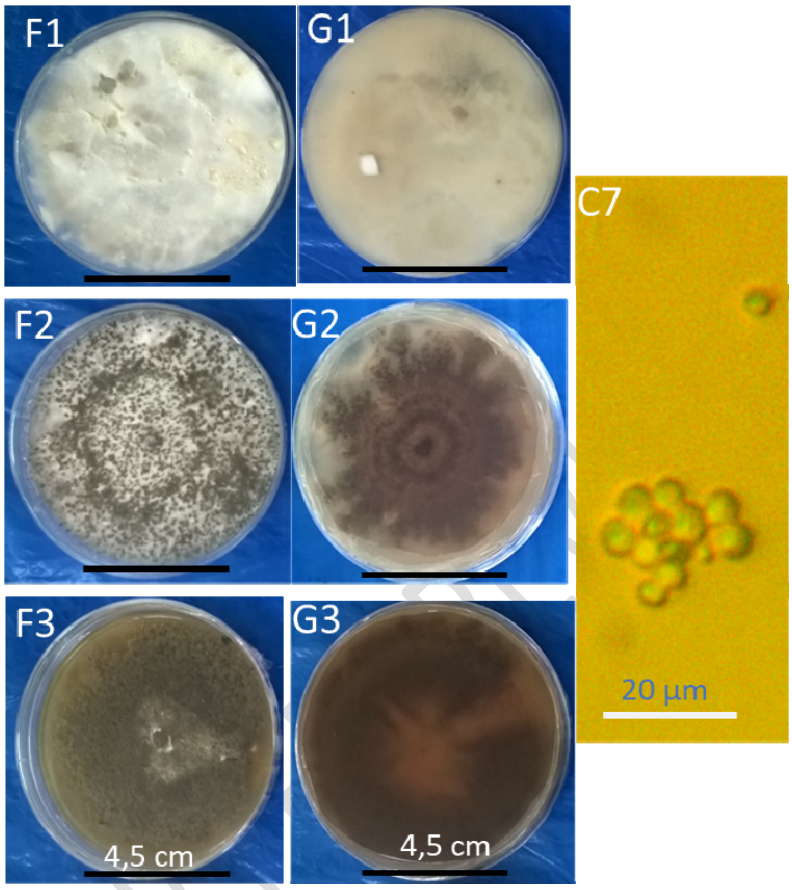

Figure 3: Morpho-cultural and microscopic characteristics of isolates of the genus *Botrytis* sp.

(D1 and E1 : cottony phenotype with 17 mm/d growth; C1 : conidia of 6.6 ×7.9 µm)

(D2 and E2 : cottony phenotype with 17 mm/d growth; C2 : conidia of 7.4 × 7 µm)

(D3 and E3: cottony phenotype with 10.1 mm/d growth; C3: conidia of 6.7 ×6.6 µm)

3-3- Parasitic activity of mycoflora morphotypes of anthracnose symptoms

The three morphotypes of each genus induced lesions on the internodes of the stems, on the petioles and in the axil of the leaves. The average diameter of the lesions and the genus aggressiveness index were revealed with a significant difference between the genus (P = 0.01). Parasitic pressure was very high for all three genera. The genus *Colletotrichum gloeosporioides* and the genus *Fusarium* sp. were the most abundant fungal stands of symptoms and were found with total parasitic pressure on the seeded explants. The genus *Botrytis* sp. could be revealed by four out of five explants for a stand lower than those of the other two genera. The average diameters of necroses caused by morphotypes of the genera *Colletotrichum gloeosporioides* and Fusarium sp. were larger than those of morphotypes of the genus *Botrytis* sp.. Morphotypes of the genera *Colletotrichum gloeosporioides* and *Fusarium* sp. were more aggressive on stems and petioles than morphotypes of the genus *Botrytis* sp. (Table 2).

Table 2: Parasitic activity of fungal populations of mycoflora related to symptoms of cassava anthracnose disease

Stand of mycoflora	Parasitic pressure ??	Mean diameter of necrosis (mm)	Mean aggressiveness index
Botrytis sp.	0,82 b	46 b	3 b
Fusarium sp.	1 a	71 a	4 a
Colletotrichum gloeosporioides	1 a	72 a	4 a
Non identifiées	0,75 c	ND	ND

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

4-DISCUSSION

Cassava anthracnose is manifested by a small number of superficial cankers located on the exposed parts of the stems. It has also been observed ~~like~~ in the form of stem deformations due to swelling and distortions of petioles and green stems. Also, damaged and necrotic green stems and petioles as well as dried apical buds were observed. ~~Alteration m~~Mycoflora, ~~linked to~~associated with cassava anthracnose, include~~s~~ fungal morphotypes of the genera *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botrytis* sp. and unidentified species. Indeed, this diversity of fungal pathogenic strains linked, on the one hand, to cassava infections, was revealed by the results of Nyaka [9] after the identification of pathogenic fungi associated with cassava root rot in Cameroon. On the other hand, the diversity of fungal species linked to anthracose-like symptoms has been observed in Côte d'Ivoire by Silué[10] through the identification of the genera *Colletotrichum gloeosporioides*, *Pestalotia heterocomis*, *Lasiodiplodia theobromae* and unidentified fungal strains on cashew nuts (*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 [21] and Burgess [22] 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. The genera *Colletotrichum gloeosporioides* and *Fusarium* sp. were more often observed on samples from non-lignified parts and on petioles of plants with lesions and necrosis. The genus *Botrytis* sp. has been specifically encountered more on deformations. Indeed, these three genera are reputed to be very damaging on all aerial parts of monocotyledons and dicotyledons plants. The genus *Colletotrichum gloeosporioides* has been identified as an infectious agent of 703 genera and 167 plant families of monocotyledons and dicotyledons by cankers, lesions and necrosis [23]. The genus *Fusarium* sp. has been identified as infecting 150 plant species in several modes of infection [24], most commonly causing stalkrots. The genus *Botrytis* sp. has been identified as an infectious agent in the leaves, stems, flowers, fruits and seeds of 500 plant species like deformations and brown rot~~tenness~~ [25]. As a necrotroph, it often takes advantage of damage resulting from other pathogens to produce symtoms often call gray mold. 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 [26].

CONCLUSION

~~The symptoms~~Tissues sympomatic of cassava anthracnose, caused by *Colletotrichum gloeosporioides* Penz *manihotis*, host a diverse associated mycoflora of which the genera *Fusarium* sp. and Botrytis sp. are the most representative. They are a source of high parasitic pressure through the activity of different morphotypes, encountered at all stages of anthracnose infection. These three genres are of economic and scientific importance recognized by the scientific community. Their revelation in the mycoflora of alteration of the aerial organs of cassava poses 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.

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