International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 44 No. 3 Jan. 2025, pp. 674-685

http://www.ijias.issr-journals.org/

Molecular identification of the causal agents of mango anthracnose disease in North Côte d'Ivoire

Kouadjo Claude Ghislaine[1], Doga Dabe[1-2], Loukou Charles[3], Koffi Ghislain[1], and Coulibaly Sidonie[1]

[1]Centre National de Recherche Agronomique (CNRA), Laboraroire Central de Biotechnologies, Côte d'Ivoire

[2]Centre National de Recherche Agronomique (CNRA), Station de recherche de LATAHA, Côte d'Ivoire

[3]Université Nangui Abrogoua, Côte d'Ivoire

ABSTRACT: Anthracnose disease of mango contributes to a huge loss of mango fruits in côte d'Ivoire. This disease is the main pre- and post-harvest fungal disease infecting mango trees worldwide, and represents the 2nd major constraint to mango production and export in Côte d'Ivoire. However, information on the causal agent of this disease in Côte d'Ivoire remains scarse but presumed to be *Colletotrichum gloeosporioides* as reported in early studies that were based on morphological characteristics. Since emerging information evidenced on one hand a possible intraspecific diversity within *Colletotrichum gloeosporioides* and on the other the existence of other emerging anthracnose causing agents, it was important to thoroughly identify these in North Côte d'Ivoire one of the main mango growing region. 41 fungal isolates were collected from diseased mango fruits in North Côte d'Ivoire, of which forty were morphologically identified as *Colletotrichum gloeosporioides* and one as *Fusarium sp*. Further molecular studies using ITS identified *Colletotrichum gloeosporioides* exhibiting an intraspecific diversity and *Fusarium concentricum* as the causal agents of anthracnose disease in mango in North Côte d'Ivoire.

KEYWORDS: Colletotrichum, *Mangifera indica L*., species identification, distribution, Côte d'Ivoire.

1 INTRODUCTION

Anthracnose is a prominent fungal disease affecting many fruit crops globally, with mangoes (*Mangifera indica*) being particularly impacted due to its high qualitative export value (Iqbal et al 2022). Caused primarily by the pathogen *Colletotrichum gloesporioides,* anthracnose manifests through dark, sunken lesions on ripe and unripe fruit, often leading to significant post-harvest losses (Freeman et al., 1998; Dinh 2002; Tarnowski and Ploetz 2008; Jayasinghe and Fernando, 2009). In orchards, the pathogen attacks all parts of the mango tree. Symptoms on fruits usually appear only after harvest. In tropical regions, the disease does not only compromises the quality and marketability of mangoes but can also negatively impact the livelihoods of farmers dependent on this vital cash crop (Tarekegn et al 2022).

Mango is the second fruit exported by Côte d'Ivoire, after banana and before pineapple. Along with these other fruits, it contributes 4% of the national GDP and 10% of Côte d'Ivoire's agricultural GDP (La côte d'Ivoire agricole 2022). Indeed, Côte d'Ivoire is one of West Africa's leading producers of mangoes, with a mango production estimated at 150,000 tonnes per year in a large orchard of traditional mango trees and some modern plantations (MEMINADER 2022). However, the prevalence of anthracnose poses a serious challenge to the mango industry, raising concerns about sustainability and food security. Furthermore, during the 2008 and 2009 campaigns, there was a significant decline in mango exports (Fruitop 2008). This decrease in production was attributed to competition from other markets and, notably, the quality of the mangoes, which was adversely affected by round spots associated with fungal diseases, especially anthracnose, as observed in exported fruits in 2007 (Guerbaud, 2008). Additionally, Hala and Coulibaly's (2006) indicated a resurgence of certain diseases in mango orchards in northern Côte d'Ivoire, with anthracnose identified as the primary fungal disease affecting mangoes there. In regions with

high rainfall and humidity, the incidence of this disease can reach up to 100% (Arauz, 2000). The country's unique climatic conditions and extensive mango cultivation practices create an environment conducive to the proliferation of *C. gloesporioides (Fruitop 2017;* Peralta-Ruiz et al 2023*).* Understanding that the dynamics of this disease within the local ecological context is essential for developing effective management strategies. It is then important to collect information on the genetic diversity of the causal agent. Indeed, few studies have been conducted on the *Colletotrichum spp.* communities of fungi responsible for mango anthracnose in Côte d'Ivoire. The identification of the causal agent and the evaluation of their genetic diversity should allow a better management of the disease notably the development of resistant mango varieties and integrated disease management practices. Through this comprehensive study, we seek to contribute valuable knowledge that should enhance our understanding of mango anthracnose dynamics in Côte d'Ivoire, ultimately supporting the sustainability of mango production in the region.The objective of this study was 1) to investigate and update data on the epidemiology of anthracnose in mango orchards in North Côte d'Ivoire one of the main mango producing region and 2) identify the causal agents associated with the disease using molecular tools.

2 MATERIALS AND METHODS

2.1 PHYTOSANITARY SURVEY AND MANGOES SYMPTOMATIC SAMPLING

Symptomatic leaves were collected in the north agro-ecological zone of mango cultivation in Côte d'Ivoire in the period of January to March 2022 in the localities of Korhogo and Ferkessedougou. The first stage of this survey was carried out in the first quarter of the year and consisted of collecting data on the incidence and aggressiveness of the disease. Within each orchard, the experimental plot was cleared of 10 m from border to avoid border effects. From this new boundary of the plot, five elementary plots in the shape of a square of 30 m on each side were delimited. One elementary plot was drawn in the center of the plantation and the other four at each end of the plantation. The prospection consisted of observing the mango trees on the basis of the symptoms present on their leaves and fruits in each elementary plot. On each mango tree, the observations were made by subdividing the tree into four parts according to the four cardinal points (Afouda et al., 2013). In each part (North, South, East, West), the observation was made in a quadra of 1 m^2. All the leaves within this quadra were observed and counted, thus giving a number of symptomatic leaves and total leaves. Leaf observation allowed the incidence and severity of diseases to be assessed. Each side of the tree was considered as a replicate. Thus, four replicates were made per tree.

DISEASE INCIDENCE ASSESSMENT

The incidence was calculated using the formula below (Cooke, 2006):

I = (P/N) x 100

I = Incidence; P = Number of diseased leaves**; N =** Total number of leaves.

A scale adapted from that of Narasimhudu (2007) was used to qualify the level of disease incidence (Table 1).

DISEASE SEVERITY ASSESSMENT

The severity or amount of disease on each leaf for anthracnose disease was visually assessed using the Silué et al. (2018) scoring scale described in Table 2. The disease severity index or disease severity rating threshold was calculated using the Soro et al. (2020) used equation below:

$$Is = \sum \left(\frac{Xi \times ni}{N \times Z} \right) \times 100$$

Is: Severity index**; Xi:** severity i of disease on leaf**; ni:** number of severity sheets i**; N:** total number of leaves observed**; Z:** highest severity scale **(9).**

Table 1. Incidence scale

Scale	Incidences (%)	Qualification of the level of incidence
0	0	No symptoms e
1	1-10	Low incidence
2	11-20	Moderate incidence
3	21-30	Medium incidence
4	31-50	Incidence forte
5	>50	Very high

Table 2. Severity scale

Grade	Severity	Characteristics of symptoms
0	0	No symptoms
1	1-5	Mild infection
3	6-10	Moderate infection
5	11-25	Mildly severe infection
7	26-50	Severe infection
9	5 > 0	Very severe infection

2.2 Collection Of Symptomatic Mangoes

Collections of symptomatic mango were carried out in the North of Côte d'Ivoire (Korhogo and Ferkessedougou) during the mango harvest period from April to June 2023. In each orchard, a device in the shape of an equilateral triangle with sides of 100 m was used. Each vertex of the triangle constituted the sample collection point. Therefore, three samples of symptomatic mango leaves were collected per locality.

2.3 Fungal Isolation

For fungal isolation, symptomatic fruit and leaves from the margins of the lesions were used. The pathogens were isolated and cultured on potato dextrose agar (PDA) using a modifed (ref) protocol. To obtain pure cultures, mycelial plugs were transferred to fresh PDA plates and incubated at 25°C with a 12-hour light/12-hour dark photoperiod for 5 days. Each single spore was transferred to a new PDA plate and incubated under the same conditions. Each resulting colony represented a purified isolate for further analysis.

2.4 Morphological Characteristics

The colony characteristics of all isolates grown on PDA were compared. Conidial morphology was studied using a compound microscope and documented with photographs. The isolates were visually inspected two weeks after the pure strains were cultured. The identification process utilized the keys from Botton et al. (1990) and Champion (1997). Macroscopic identification focused on traits such as growth rate, shape, color, thallus texture, and colony relief of each isolate. For microscopic identification, a small fragment of mycelium was carefully taken from the edge of a 15-day-old culture and examined under an optical microscope. The observed mycelia and spores were then described.

2.5 DNA Extraction And PCR Amplification

The extraction of total DNA was carried out from the mycelium of each isolates using the CTAB method as described by Doyle and Doyle (1990). The isolated DNA was then quantified and stored at -20°C. The ITS region, were amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'TCCTCCGCTTA TTGATATGC-3') for the amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics analysis (White ET AL 1990). PCR was conducted using a MiniAmp thermal cycler from Applied Biosystems and OneTaq 2X Master Mix (NEB), following the manufacturer's guidelines. The PCR products were analyzed by electrophoresis on a 1% agarose gel, which was stained with SyberSafe solution. DNA sequencing was carried out by Eurofin Biotech in Germany. The sequences obtained were reviewed for accuracy using chromatograms in MEGA v. 7 (Tamura et al., 2013).

2.6 PHYLOGENETIC ANALYSES

The obtained sequences were phylogenetically studied with the NCBI BLAST (https://blast.ncbi.nlm.nih.gov) by comparing their counterpart's 18S rRNA sequences in the GenBank database. MEGA version 7.0. Software was used to modify the DNA sequence, Sequence alignments were performed using ClustalW alignment implemented in MEGA version 7 and were manually adjusted to allow maximum sequence similarity (Kumar, Stecher, and Tamura 2016). To demonstrate the relationships between homologous species, the Neighbour-Joining method using Molecular Evolutionary Genetics Analysis (MEGA 7.0) was used to create the phylogenetic tree

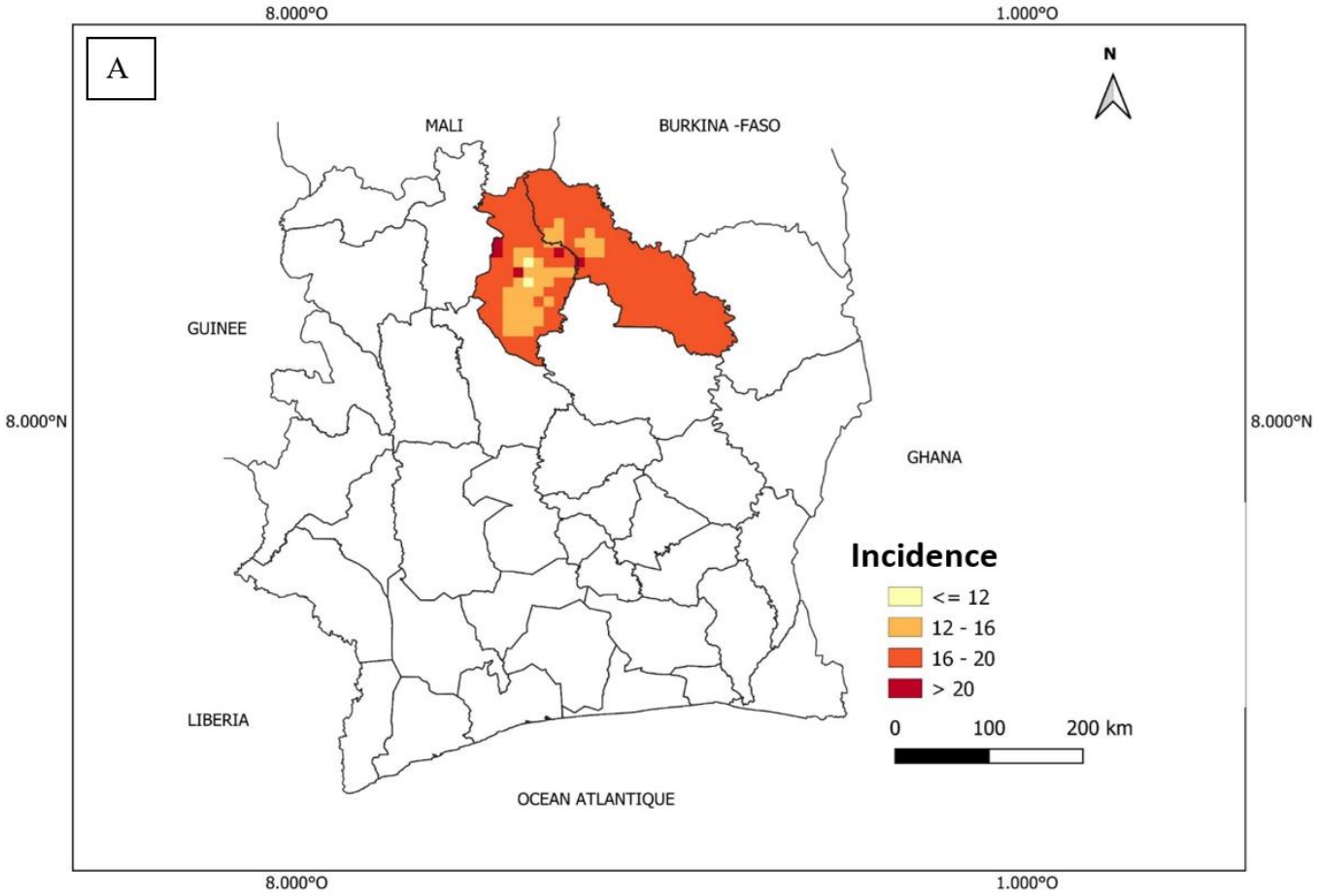
2.7 STATISTICAL ANALYSIS

Statistica version 7.1 was employed for the statistical analysis. The indices for disease prevalence and symptom severity collected in this study were analyzed statistically. A test for homogeneity of variance was conducted to guide the selection of subsequent tests. If no significant difference was found ($P > 0.05$), parametric tests were used. If a significant difference was detected ($P < 0.05$), non-parametric tests were applied for mean comparisons, followed by Fisher's LSD test to evaluate the extent of the differences.

3 RESULTS

3.1 DISEASE SURVEYS AND COLLECTION OF DISEASED LEAVES AND MANGOES FRUITS

A disease survey was carried out in the mangoes-growing regions within Côte d'Ivoire in the pre-harvest and harvest periods of the year 2022-2023. Anthracnose was reported on leaves and fruits. And the pathogen was verified by our isolations from symptomatic leaves and fruit. In total, 41 infected samples fruits (of which 17 mangoes were harvested in Korhogo, 22 mangoes in Ferké. The locations of orchards surveyed, which were used for the incidence and severity analyses, are recorded in Figure 1.



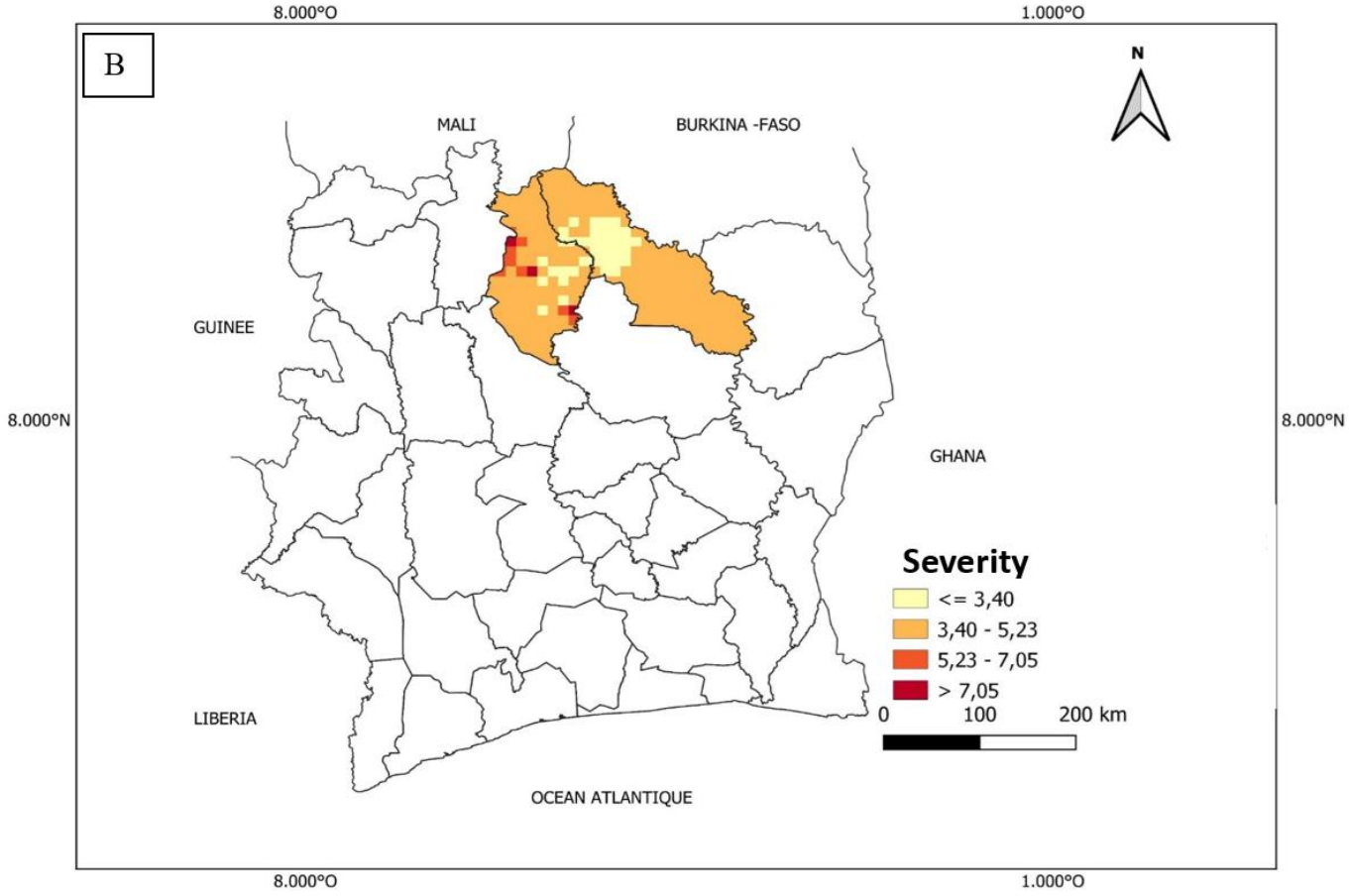


Fig. 1. Map of the distribution (incidence and severity) of anthracnose in Ferké and Korogho mango orchards

3.2 Incidence And Severity Assessment

The incidence of disease symptoms observed on mangoes orchards ranged from 14.14% in the locality of Ferkéssedougou, to 14.99% in Korogho. Thus, a significant difference was observed between the incidence and the severity of the disease in these two localities. On the other hand for disease severity values, for Korogo and Ferké, the severity of the anthracnose disease, was significantly high 3.8% to 5.13%, respectively (Table 3)

Table 3. Anthracnose incidence and severity in 02 localities in North Côte d'Ivoire

Locality	Incidence de l'anthracnose (%)	Sévérité de l'anthracnose (%)
Korhogo	14,9958824	3,80647059
Ferkessedougou	14,1483333	5,13

3.3 Different Anthracnose Disease Symptomes Observed On Mangoes Leaves And Fruits

Visual anthracnose symptoms on the mangoes fruits were small brown to black spots, and some rot (Figure 2).

Fig. 2. Different symptoms of anthracnose observed on leaves and fruits of mangoes

Fungal isolates were morphologically and phylogenetically identified as *Colletotrichum sp.*

A total of 41 isolates of *Colletotrichum spp*. were obtained from fruits samples. On PDA media, colonies typically exhibited Orange and weak mycelium, Rose and aerial mycelium very dance, Ivory white and a cottony and light brown mycelium (Figure 3). Conidia were fusiform, measuring 12.5 to 18.4 × 3.5 to 5.4 mm (Figure 4). All 41 isolates from mango were provisionally identified as part of the *C. gloeosporioides* species complex based on their morphological and cultural characteristics.

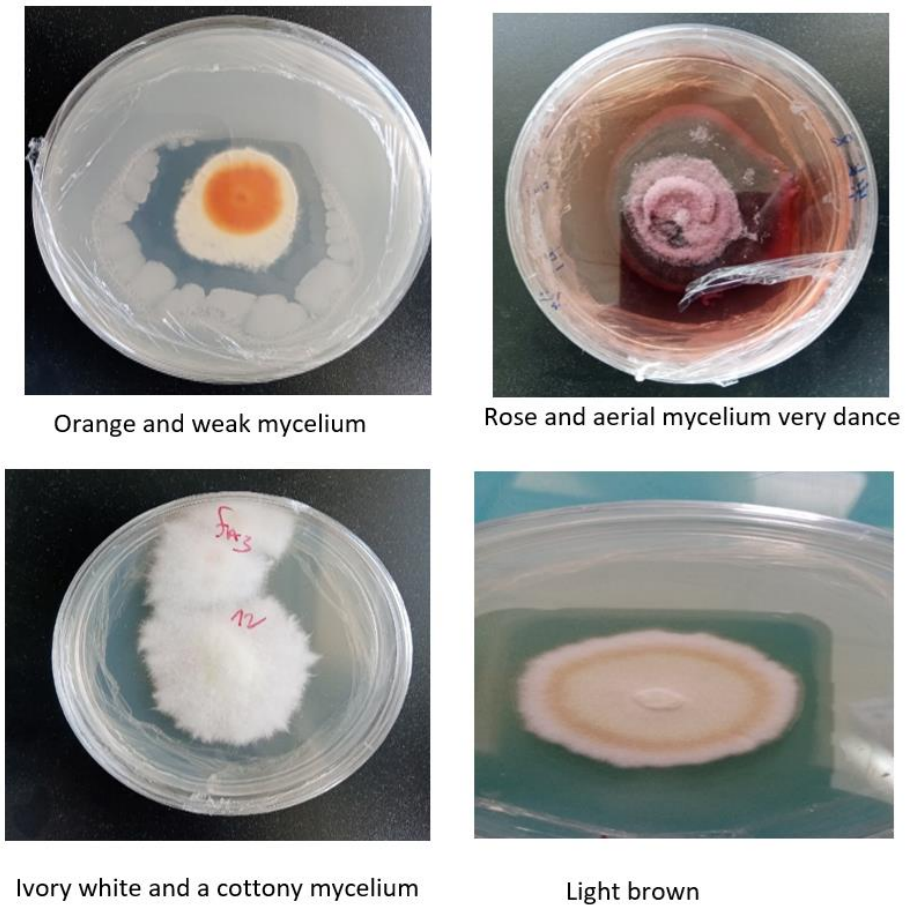


Fig. 3. Isolates of Colletotrichum gloesporides on PDA

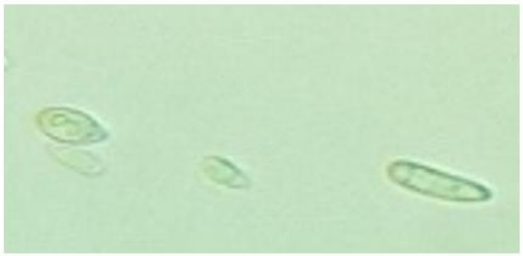


Fig. 4. Conidia form as isolated on PDA

Colletotrichum gloesporides and *Fusarium concentricum* were identified as causal agents

Of the 41 morphologically characterized isolates 12 isolates were identified as morphological groups and used for phylogenetic analysis. The phylogeny provided sufficient information to distinguish the Colletotrichum species associated with symptoms of mango anthracnose in Côte d'Ivoire. 11 isolates were nested within the clade of different isolate species of *C. gloeosporioides* with a high level of similarity, ranging from 96% to 99 %. And 01 isolate was similar to *Fusarium concentricum* with 100% similarity (Table 4).

Table 4. Identification of isolates from the ITS region of the rRNA

Code	Locality	N °acession	Species	S.C	SI
Isolat 1	Ferké	KF000080.1		90%	96%
Isolat 3	Ferké	HQ636422.1 https://www.ncbi.nlm.nih.gov/nucleotide/HM138672.1?report=genbank&log$=nucltop&blast_rank=1&RID=ETYHT1D0016		100%	97%
Isolat 7	Ferké	KF000080.1		99%	97%
Isolat 8	Ferké	KF000080.1		96%	99%
Isolat 9	Korhogo	JF288537.1		97%	99%
Isolat 12	Ferké	HQ636422.1			
Isolat 13	Korhogo	HQ636422.1			
Isolat 4	Ferké	JF288537.1			
Isolat 8-1	Ferké	JF288537.1			
Isolat 15	Korhogo	JF288537.1	***Colletotrichum gloeosporioides***		
Isolat 17	Ferké	HQ636422.1			97%
Isolat 7-1	Ferké	MG489962.1	***Fusarium concentricum***		

S.C (%): Sequence coverage rate SI. (%): Similarity rate

Phylogenetic analysis was conducted on all identified sequences after BLAST belonging to the genus *Colletotrichum* and *Fusarium*. The phylogenetic tree of *Colletotrichum* species based on the comparison of rDNA sequences, is presented in Figure 5. This tree shows the relationship between the closest strains of the genus *Colletotrichum* and *Fusarium* extracted from the NCBI database.

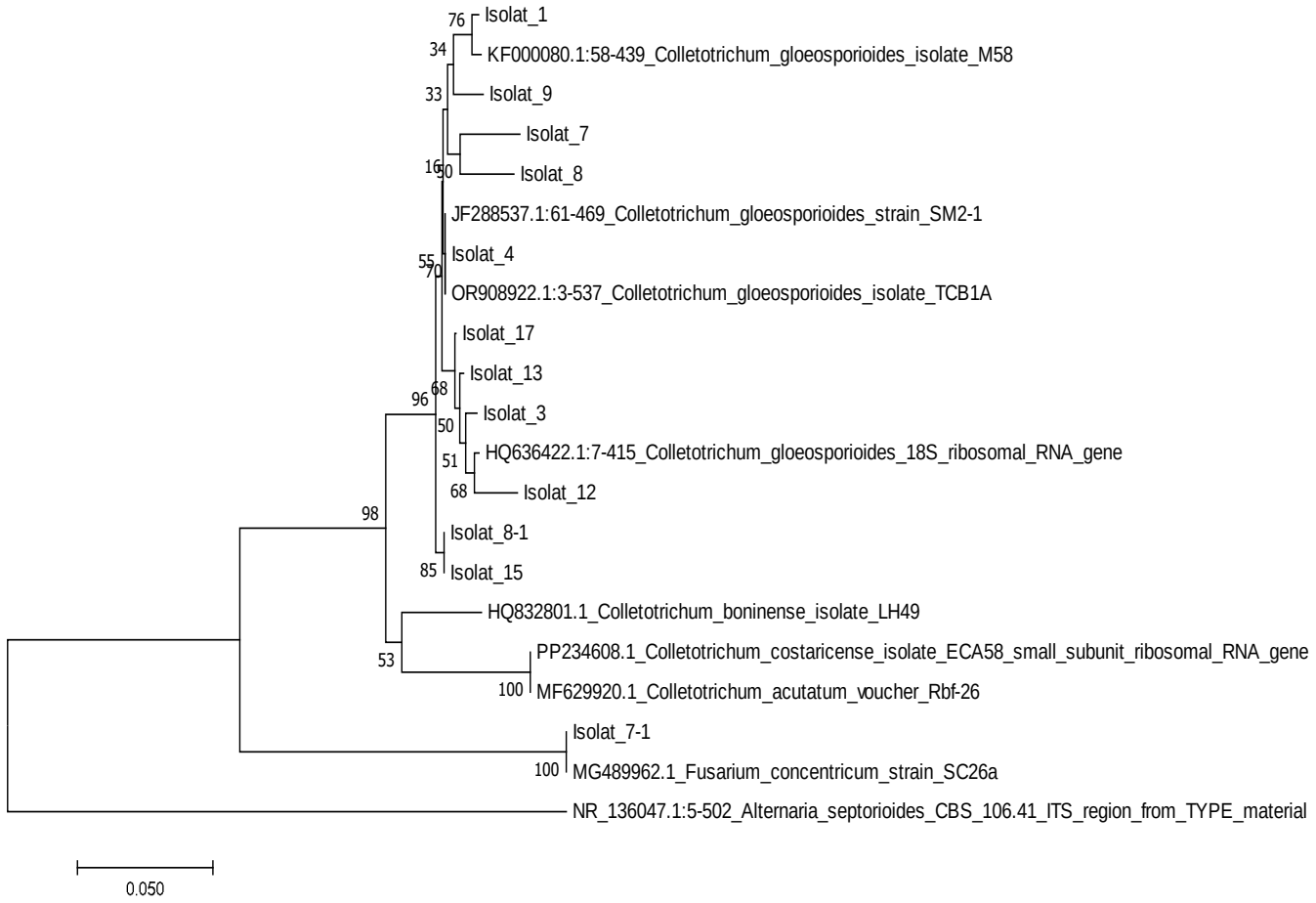


Fig. 5. Phylogenetic tree showing the phylogenetic position of pathogenic Colletotrichum and Fusarium species isolated from mangoes fruits in Côte d'Ivoire, compared with ITS sequence data available on GenBank. The tree is rooted with Alternaria_septoriodes strain

4 DISCUSSION

This study investigated on both the epidemiology of anthracnose in mango orchards in North Côte d'Ivoire one of the main mango producing region and the identification on the causal agents. Concerning the epidemiological surveys have revealed the effective presence of anthracnose in the North of Côte d'Ivoire with an incidence of symptoms of the disease varying from 14.14% in the locality of Ferkéssedougou, to 14.99% in Korogho, significantly high severity rate of 3.8% to 5.13%, in the cities of Korhogo and Ferké respectively. This showed that anthractone was highly present in North Côte d'Ivoire. Indeed a study, highlighted a predominance of the disease in orchards in northern Côte d'Ivoire of the major type in terms of severity index and incidence to the detriment of the marginal type (conducted in 2019 by Dio et al and by Brou et al in 2021).

This disease can be attributed to the use of planting material already infected with the pathogens responsible for anthracnose. This practice is a key factor in orchard contamination (CPLEACP/PIP, 2013b). The large number of mango orchards in this production zone favors the rapid development of this disease. The presence of anthracnose in the orchards of the study area is supported by Akem (2006) and Onyeani et al. (2012) who in their work have shown that the disease is present in all mango production areas worldwide. It is the most important mango pathology (Arauz, 2000). Symptoms are most pronounced on fruit. This is due to the loss of fruit resistance during ripening. During ripening, there are hormonal changes, biochemical modifications and increased sensitivity in the fruit. Recently, using morphological characteristics, *C. gloeosporoides* was identified as the causal agent of mango anthracnose disease in Côte d'Ivoire (Nguetta et al. 2013). However, if we were to efficiently manage anthracnose disease in Côte d'Ivoire, it is important to have clear information on the causal agent diversity since several isolates of *C. gloeosporoides* can be involved in the spread of this disease (Dofuor et al. 2023). In this study, the ITS phylogeny allowed the identification of *C. gloeosporoides* as one of the mango anthracnose causal agent in North Côte d'Ivoire confirming the previous findings. The ITS phylogeny allowed to discriminate the *C. gloeosporoides* strains showing an

intraspecific diversity. High intraspecific variation of pathogenic *C. gloeosporides* has already been evidenced in Water Yam fields in the Lesser Antilles (Dentika et al 2023).

Many other studies have demonstrated the association of several complexes of the genus Colletotrichum, with anthracnose in mango, among which, the gloeosporioides complex composed of others of the species *C. asianum* [Prihastuti, Cai & Hyde]; *C. fructicola* [Prihastuti, Cai & Hyde]; *C. gloeosporioides* [Penz. & Sacc.]; *C. theobromicola* [Delacr.]; *C. siamense* [Phoulivong, Cai & Hyde]; *C. tropicale* [Rojas, Rehner & Samuels]; *C. queenslandicum* [Weir & Johnst.] and *C. grossum* [Y.Z. Diao, Can. Zhang, L. Cai & X.L. Liu] (Jayawardena et al. 2016; Manzano León et al. 2018; Marin-Felix et al. 2017; Mo et al. 2018; Qin et al. 2019; Sharma et al. 2013; Shivas et al. 2016).

Our study only evidenced the presence of the *C. gloeoosporides* complex in North Côte d'Ivoire. This study also evidenced that *Fusarium Concentrichum* was associated with symptoms of mango anthracnose in Ferkessedougou. This is a first report of the presence of *Fusarium Concentrichum* as anthracnose causal agent in Côte d'Ivoire. Until now, *C. gloeosporioides* was identified as the only species associated with mango anthracnose in Côte d'Ivoire (Dio et al 202; N'guetta et al 2013). *Fusarium Concentrichum* has been already reported elsewhere in China as a causal agent

Fusarium species are commonly associated with diseases of several tropical crops, such as yam, mango, banana, papaya, pineapple, and avocado (Zakaria, L 2023; Thayne et al 2024). The most common and economically significant Fusarium species associated with diseases of major fruit crops include *F. oxysporum* and *F. solani,* which are widely distributed in these areas. *Fusarium concentricum* Nirenberg & O' Donnell (Ascomycota: Hypocreales) is a fungal species known to infect plants (Qiu et al 2023). This Fusarium species has previously been identified as a fungal pathogen of shoot blight on Podocarpus macrophyllus in China (Qin et al 2021) and also the causal agent of Fusarium leaf spot on pecan (Carya illinoinensis) in southeastern China (Saibin et al 2023). Thus, this study represents the first report of *F. Concentrichum* associated with mango in Côte d'Ivoire.

To set up a management system for these microbial populations in mango orchards in northern Côte d'Ivoire and even beyond, it will be necessary to expand the prospecting area to other orchards located in other cities in the country, and establish pathogenicity tests. This will provide more details on the intraspecific or extraspecific diversity of the strains of *Colletotrichum gloeosporides* and *Fusarium constentrichum* present, the levels of virulence, and the establishment of control methods, like several studies conducted on pathogenic fungi such as Ganoderma Boninense in oil palm (Zakaria, L 2023).

ACKNOWLEDGMENTS

The author expresses their gratitude to the C2D FRUITIER PROJECT for their support and for providing for the for the execution of this work

REFERENCES

[1] Afouda, L. C. A., Zinsou, V., Balogoun, R. K., Onzo, A. & Ahohuendo, B. C., (2013). Inventaire des agents pathogènes de l'anacardier (Anacardium occidentale L.) au Bénin. Bulletin de la Recherche Agronomique du Bénin, 73: 13-19.

[2] Arauz, L.F. (2000) Mango Anthracnose: Economic Impact and Current Options for Integrated Management. Plant Disease, 84, 600-611. http://dx.doi.org/10.1094/PDIS.2000.84.6.600

[3] Botton, B., Breton, A., Fevra, M., Gauthier, S., Guy, P., Larpent, J.P., Reymond, P., Sanglier, J.J., Vayssier, Y. and Veau, P. (1990) Moisissures utiles et nuisibles. Importance industrielle. Masson, Paris, 41-220.

[4] Akem C.N. (2006). Mango Anthracnose Disease: Present Statut and Future Research Priorities. Plant pathology, 5: 266-273.

[5] BROU Kouassi Guy, DOUMBIA Mohamed, Doga Dabé, ORO Zokou Franck, KOUAME Brou N'guéssan Akuèlou, KOUASSI Koffi II Nazaire et DOGBO Denezon Odette (2021). Distribution of anthracnose disease in orchards in the mango production area in northern regions of Côte d'Ivoire. IJSER Volume 12, Issue 8.

[6] BROU Kouassi Guy, DOUMBIA Mohamed, Doga Dabé, ORO Zokou Franck, KOUAME Brou.

[7] Champion, R. (1997) Identifying Seed-Transmitted Fungi. INRA (National Institute of Agronomic Research). https://www.quae.com/produit/487/9782759213122/identifier-les-champignons-transmis-par-les-semences/preview?escape=false#lg=1&slide=0.

[8] Dembele, Dio D. & Elisée, Amari & Camara, Brahima & Grechi, Isabelle & Rey, Jean-Yves & Kone, Daouda (2020). Pre and postharvest assessment of mango anthracnose incidence and severity in the north of Côte d'Ivoire. International Journal of Biological and Chemical Sciences. 13. 2714. 10.4314/ijbcs.v13i6.24.

[9] Dentika P, Blazy JM, Alleyne A, Petro D, Eversley A, Penet L. High Genetic Diversity and Structure of *Colletotrichum gloeosporioides* s.l. in the Archipelago of Lesser Antilles. J Fungi (Basel). 2023 May 27; 9 (6): 619. doi: 10.3390/jof9060619. PMID: 37367555; PMCID: PMC10302672.
[10] Dentika P, Blazy JM, Alleyne A, Petro D, Eversley A, Penet L. High Genetic Diversity and Structure of *Colletotrichum gloeosporioides* s.l. in the Archipelago of Lesser Antilles. J Fungi (Basel). 2023 May 27; 9 (6): 619. doi: 10.3390/jof9060619. PMID: 37367555; PMCID: PMC10302672.
[11] Dentika P, Blazy JM, Alleyne A, Petro D, Eversley A, Penet L. High Genetic Diversity and Structure of *Colletotrichum gloeosporioides* s.l. in the Archipelago of Lesser Antilles. J Fungi (Basel). 2023 May 27; 9 (6): 619. doi: 10.3390/jof9060619. PMID: 37367555; PMCID: PMC10302672.
[12] Dinh (2002): Mostharvest loss of mango due to anthracnose and its infection biology and resistance of mango to the disease. Thesis. Pp103.
[13] Dinh, Quang (2002). Postharvest loss of mango due to anthracnose and its infection biology and resistance of mango to the disease pp 103.
[14] Dinh, Quang (2002). Postharvest loss of mango due to anthracnose and its infection biology and resistance of mango to the disease pp 103.
[15] Dio Dramane DEMBELE, Ler-N'Og n Dadé Georges Elisée AMARI, Brahima CAMARA, Isabelle GRECHI, Jean-Yves REY and Daouda KONE (2019). Pre and postharvest assessment of mango anthracnose incidence and severity in the north of Côte d'Ivoire. Int. J. Biol. Chem. Sci. 13 (6): 2726-2738. DOI: https://dx.doi.org/10.4314/ijbcs.v13i6.24.
[16] Dio Dramane DEMBELE, Ler-N'Og n Dadé Georges Elisée AMARI, Brahima CAMARA, Isabelle GRECHI, Jean-Yves REY and Daouda KONE (2019). Pre and postharvest assessment of mango anthracnose incidence and severity in the north of Côte d'Ivoire. Int. J. Biol. Chem. Sci. 13 (6): 2726-2738.
[17] Dofuor AK, Quartey NK-A, Osabutey AF, Antwi-Agyakwa AK, Asante K, Boateng BO, Ablormeti FK, Lutuf H, Osei-Owusu J, Osei JHN, Ekloh W, Loh SK, Honger JO, Aidoo OF and Ninsin KD (2023). Mango anthracnose disease: the current situation and direction for future research. Front. Microbiol. 14: 1168203. doi: 10.3389/fmicb.2023.1168203.
[18] Doyle and Doyle (1990): J. J. Doyle and J. L. Doyle, «Isolation of Plant DNA from Fresh Tissue,» Focus, Vol. 12, No. 1, 1990, pp. 13-15.
[19] Erekalo, Kassa & Banta, Yishak. (2022). Determinants of Market Participation among Dairy Producers in Southwestern Ethiopia. Research in World Economy. 3. 16-23. 10.36956/rwae.v3i1.486.
[20] Freeman S, Katan T, Shabi E. (1998). Characterization of Colletotrichum Species Responsible for Anthracnose Diseases of Various Fruits. Plant Dis. Jun; 82 (6): 596-605. doi: 10.1094/PDIS.1998.82.6.596. PMID: 30857006.
[21] *Fruitop (2008)* https://agritrop.cirad.fr/547335/1/ID547335.pdf
[22] Fruitop (2017) https://www.fruitrop.com/media/Publications/FruiTrop-Magazine/2017/fruitrop-249.
[23] Gerbaud, P. (2008). Les dossiers de fruitrop, fiche de pays producteur la Côte d'ivoire. *Fruitrop* (26 pp.).
[24] Global status and the way forward for disease management. Journal of Innovative Sciences, 8 (2): 222-235. DOI | https://dx.doi.org/10.17582/journal.jis/2022/8.2.222.235
[25] Hala K et Coulibaly F. (2006). L'étude diagnostique de l'état sanitaire du verger manguier et les acquis de la recherche agronomiques sur la lutte intégrée contre les mouches de fruits etla cochenille farineuse en Côte d'Ivoire. Rapport d'exécution technique. Appel d'offres N° 016/ FIRCA/ Filière Mangue.
[26] IQBAL, J., KIRAN, S., MUSTAFA, G., KHAN, A., RAZA, A., BIBI, F., HUSSAIN, R., BUKHARI, S., IQBAL, N., & KHAN, A. (2022). Effect of different nursery potting media on the germination and development of mango (mangifera indica l.) seedlings. *Biological and Clinical Sciences* doi.org/10.54112/bcsrj.v2022i1.136
[27] Iqbal, S., M.A. Khan, M. Atiq, N.A. Rajput, M. Usman, A. Nawaz, G.A. Kachelo, A. Akram and H. Ahmad. 2022. Mango anthracno.
[28] Jayasinghe and Fernando Jayasinghe, Chandra & Fernando, T. & Jayawardana, N. (2009). A comparative study of Colletotrichum species causing anthracnose in Hevea. Journal of the Rubber Research Institute of Sri Lanka. 89. 20. 10.4038/jrrisl.v89i0.1845.
[29] Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M, Li XH, Zhang W, Zhao WS, Yan JY (2016). Notes on currently accepted species of Colletotrichum. Mycosphere 7 (8) 1192–1260, Doi 10.5943/mycosphere/si/2c/9.
[30] Kumar S, Stecher G, and Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870-1874.
[31] Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA 7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33, 1870-1874. https://doi.org/10.1093/molbev/msw054.
[32] La Côte d'Ivoire agricole (2022): https://lacotedivoireagricole.ci/cote-divoire-situation-de-la-filiere-mangue-en-2022/.
[33] Latiffah Zakaria (2023). Basal Stem Rot of Oil Palm: The Pathogen, Disease Incidence, and Control Methods. Plant Disease Vol. 107, No. 3.

[34] Manzano León, A. M., Serra-Hernández, W., García-Pérez, L., Crespo, K., and Guarnaccia, V. 2018. First report of leaf anthracnose caused by *Colletotrichum grossum* on mango (*Mangifera indica*) in Cuba. J. Plant Pathol. 100: 329. https://doi.org/10.1007/s42161-018-0040-z CrossrefWeb of ScienceGoogle Scholar.

[35] Manzano León, A. M., Serra-Hernández, W., García-Pérez, L., Crespo, K., and Guarnaccia, V. 2018. First report of leaf anthracnose caused by *Colletotrichum grossum* on mango (*Mangifera indica*) in Cuba. J. Plant Pathol. 100: 329. https://doi.org/10.1007/s42161-018-0040-z CrossrefWeb of ScienceGoogle Scholar.

[36] Marin-Felix, Y., Groenewald, J. Z., Cai, L., Chen, Q., Marincowitz, S., Barnes, I., Bensch, K., Braun, U., Camporesi, E., Damm, U., de Beer, Z. W., Dissanayake, A., Edwards, J., Giraldo, A., Hernandez-Restrepo, M., Hyde, K. D., Jayawardena, R. S., Lombard, L., Luangsa-ard, J., McTaggart, A. R., Rossman, A. Y., Sandoval-Denis, M., Shen, M., Shivas, R. G., Tan, Y. P., van der Linde, E. J., Wingfield, M. J., Wood, A. R., Zhang, J. Q., Zhang, Y., and Crous, P. W. 2017. Genera of phytopathogenic fungi: GOPHY 1. Stud. Mycol. 86: 99-216. https://doi.org/10.1016/j.simyco.2017.04.002 CrossrefWeb of ScienceGoogle Scholar.

[37] MEMINADER (2022): https://www.agriculture.gouv.ci/

[38] Mo, J., Zhao, G., Li, Q., Soalngi, G. S., Tang, L., Huang, S., Guo, T., and Hsiang, T. 2018. Identification and characterization of *Colletotrichum* species associated with mango anthracnose in Guangxi, China. Plant Dis. 102: 1283-1289. https://doi.org/10.1094/PDIS-09-17-1516-RE LinkWeb of ScienceGoogle Scholar.

[39] Munhoz T, Vargas J, Teixeira L, Staver C and Dita M (2024) Fusarium Tropical Race 4 in Latin America and the Caribbean: status and global research advances towards disease management. *Front. Plant Sci.* 15: 1397617. doi: 10.3389/fpls.2024.1397617.

[40] N. Silué, K. Abo, F. Johnson, B. Camara, M. Kone, D. Kone. (2018). Evaluation in vitro et in vivo de trois fongicides de synthese et d'un fongicide biologique sur la croissance et la severite de *colletotrichum gloeosporioides* et de *pestalotia heterornis,* champignons responsables de maladies foliaires de l'anacardier (anacardium occidentale l.) en Cote d'ivoire. Agronomie Africaine 30 (1): 107 – 122.

[41] N'guéssan Akuèlou, KOUASSI Koffi II Nazaire et DOGBO Denezon Odette (2021). Distribution of anthracnose disease in orchards in the mango production area in northern regions of Côte d'Ivoire. IJSER Volume 12, Issue 8.

[42] N'Guettia MY, Diallo HA, Kouassi N, Coulibaly F, (2013). Diversité morphologique et pathogénique des souches de Colletotrichum sp. responsable de l'anthracnose de la mangue en Côte d'Ivoire. Journal of Animal & Plant Sciences, 18 (3): 2775-2784.

[43] Nagaraja, A., Kumar, J., Jain, A.K., Narasimhudu, Y., Raghuchander, T., Kumar, B. and Gowda, H.B. (2007). Compendium of Small Millets Diseases. Project Coordination Cell, All India Coordinated Small Millets Improvement Project, UAS, GKVK Campus, Bangalore. p. 80.

[44] N'guettia, M & Kouassi, Nazaire & Diallo, H & Kouakou, Regis. (2014). Evaluation of Anthracnose Disease of Mango (Mangifera indica L.) Fruits and Characterization of Causal Agent in Côte d'Ivoire. 2319-1473.

[45] N'guettia, M & Kouassi, Nazaire & Diallo, H & Kouakou, Regis. (2014). Evaluation of Anthracnose Disease of Mango (Mangifera indica L.) Fruits and Characterization of Causal Agent in Côte d'Ivoire. 2319-1473.

[46] Onyeani C.A., Osunlaja O., Owuru O.O. et Sosanya O. (2012). First report of fruit anthracnose in mango caused by Colletotrichum gloeosporioides in Southwestern Nigeria. Int. J. Sci. Technol., 1: 30- 34.

[47] Pardo-De la Hoz, C. J., Calderon, C., Rincon, A. M., Cardenas, M., Danies, G., Lopez-Kleine, L., Restrepo, S., and Jimenez, P. 2016. Species from the *Colletotrichum acutatum, Colletotrichum boninense* and *Colletotrichum gloeosporioides* species complexes associated with tree tomato and mango crops in Colombia. Plant Pathol. 65: 227-237. https://doi.org/10.1111/ppa.12410 CrossrefWeb of ScienceGoogle Scholar.

[48] Peralta-Ruiz Y, Rossi C, Grande-Tovar CD, Chaves-López C (2023). Green Management of Postharvest Anthracnose caused by *Colletotrichum gloeosporioides*. J Fungi (Basel). 28; 9 (6): 623. doi: 10.3390/jof9060623.

[49] Qin, China & Jiang, Yanyan & Zhang, Rui & Ali, Emran & Huo, Junwei & Li, Yonggang. (2021). First Report of Fusarium concentricum Causing Shoot Blight on Podocarpus macrophyllus in China. Plant Disease. 10.1094/PDIS-07-21-1490-PDN.

[50] Qin, China & Jiang, Yanyan & Zhang, Rui & Ali, Emran & Huo, Junwei & Li, Yonggang. (2021). First Report of Fusarium concentricum Causing Shoot Blight on Podocarpus macrophyllus in China. Plant Disease. 10.1094/PDIS-07-21-1490-PDN.

[51] Qin, L. P., Yu, G. M., Zhang, Y., Su, Q., Chen, Y. L., Nong, Q., Huang, S. L., and Xie, L. (2019). First report of anthracnose of *Mangifera indica* caused by *Colletotrichum scovillei* in China. Plant Dis. 103: 1043. https://doi.org/10.1094/PDIS-11-18-1980-PDN LinkWeb of ScienceGoogle Scholar.

[52] Qiu HL, Fox EGP, Qin CS, Yang H, Tian LY, Wang DS, Xu JZ. (2023). First record of Fusarium concentricum (Hypocreales: Hypocreaceae) isolated from the moth Polychrosis cunninhamiacola (Lepidoptera: Tortricidae) as an entomopathogenic fungus. J Insect Sci. 2023 Mar 1; 23 (2): 2. doi: 10.1093/jisesa/iead008. PMID: 36916278; PMCID: PMC10011878.

[53] Saibin Lv, Yuanping Mo, Huiling Wang, Zuhan Xie, Kai Guo, Zhengjia Wang, Chulong Zhang, and Lihong Xiao (2023). First Report of *Fusarium concentricum* as a Causal Agent of Fusarium Leaf Blotch on Pecan (*Carya illinoinensis*) in Southeast China. Plant Disease 107: 8, 2549.

[54] Sharma, G., Kumar, N., Weir, B. S., Hyde, K. D., and Shenoy, B. D. (2013). The ApMat marker can resolve Colletotrichum species: A case study with Mangifera indica. Fungal Divers. 61: 117-138.
[55] Shivas, R. G., Tan, Y. P., Edwards, J., Dinh, Q., Maxwell, A., Andjic, V., Liberato, J. R., Anderson, C., Beasley, D. R., Bransgrove, K., Coates, L. M., Cowan, K., Daniel, R., Dean, J. R., Lomavatu, M. F., Mercado-Escueta, D., Mitchell, R. W., Thangavel, R., Tran-Nguyen, L. T. T., and Weir, B. S. 2016. Colletotrichum species in Australia. Australas. Plant Pathol. 45: 447-464.
[56] Shivas, R. G., Tan, Y. P., Edwards, J., Dinh, Q., Maxwell, A., Andjic, V., Liberato, J. R., Anderson, C., Beasley, D. R., Bransgrove, K., Coates, L. M., Cowan, K., Daniel, R., Dean, J. R., Lomavatu, M. F., Mercado-Escueta, D., Mitchell, R. W., Thangavel, R., Tran-Nguyen, L. T. T., and Weir, B. S. (2016). *Colletotrichum* species in Australia. Australas. Plant Pathol. 45: 447-464. https://doi.org/10.1007/s13313-016-0443-2 CrossrefWeb of ScienceGoogle Scholar.
[57] Sibirina, Soro & Souleymane, Sanogo & Mariam, Ouattara & Nakpalo, Silue & Kone, Daouda & Justin, Kouadi. (2020). Analyse descriptive et facteurs agronomiques d'avant-garde de l'état sanitaire des vergers anacardiers (Anacardium occidentale L.) en Côte d'Ivoire Soro Sibirina. European Scientific Journal ESJ. 16. 10.19044/esj.2020.v16n30p72.
[58] Tamura, K., Stecher, G., Peterson, D., et al. (2013) MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. The Society for Molecular Biology and Evolution, Oxford University, Oxford.
[59] Tarekegn, K., & Kelem, F. (2022). Assessment of Mango Post-Harvest Losses along Value Chain in the Gamo Zone, Southern Ethiopia. *International Journal of Fruit Science, 22* (1), 170–182. https://doi.org/10.1080/15538362.2021.2025194.
[60] Tarekegn, N., Abate, B., Muluneh, A. (2022) Modeling the impact of climate change on the hydrology of Andasa watershed. *Model. Earth Syst. Environ.* 8, 103–119. https://doi.org/10.1007/s40808-020-01063-7.
[61] Tarnowski and Ploetz (2008): Tarnowski TLB, Ploetz RC. First Report of *Colletotrichum capsici* Causing Postharvest Anthracnose on Papaya in South Florida. Plant Dis. 2010 Aug; 94 (8): 1065. doi: 10.1094/PDIS-94-8-1065B. PMID: 30743449.
[62] Tarnowski, T.L. and Ploetz, R., (2008). Assessing the role of Colletotrichum gloeosporioides and C. acutatum in mango anthracnose in south Florida. Phytopathology, 98: 155.
[63] Thayne Munhoz Jorge Vargas; Jorge Vargas Luiz Teixeira Luiz Teixeira Charles Staver Charles Staver Miguel Dita; Miguel Dita (2023). Fusarium Tropical Race 4 in Latin America and the Caribbean: status and global research advances towards disease management. Plant Pathogen Interactions. Volume 15 - 2024 | https://doi.org/10.3389/fpls.2024.1397617.
[64] Thayne Munhoz; Thayne Munhoz, Jorge Vargas; Jorge Vargas uiz TeixeiraLuiz Teixeira Charles StaverCharles Staver Miguel Dita Miguel Dita5 (2024). Fusarium Tropical Race 4 in Latin America and the Caribbean: status and global research advances towards disease management. Sec. Plant Pathogen Interactions Volume 15 – 2024.
[65] White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editor. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press Inc; 1990. pp. 315–322. [Google Scholar].
[66] White, T.J. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols, a Guide to Methods and Applications, 315-322.
[67] Zakaria L (2023). Fusarium species associated with diseases of major tropical fruit crops. Horticulturae 9 (3): 322.
[68] Zakaria, L. *Fusarium* Species Associated with Diseases of Major Tropical Fruit Crops. *Horticulturae* 2023, *9,* 322. https://doi.org/10.3390/horticulturae9030322.