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Research Article
Morphometric Analysis of Isolated Conidia of Various Species of *Colletotrichum* sp. from Avocado and Mango in Côte d'Ivoire

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Abstract

Background and Objective: Fruits are important sources of income for farmers. However, they are subject to many diseases caused mainly by fungi, of which the genus *Colletotrichum*. The genus *Colletotrichum* is an important and frequent causal agent of post-harvest infections of fruits. Although this genus can infect a wide range of hosts, one or more species of *Colletotrichum* may be found on the same host. Based on morphological characteristics, this study aimed to differentiate 49 isolates of *Colletotrichum* sp., obtained from avocados and mangoes from the main production areas in Côte d'Ivoire. **Materials and Methods:** In this study, the growth rate of *Colletotrichum* isolates was evaluated on three culture media (potato dextrose agar (PDA), malt agar and corn meal agar (CMA) media), as well as their sporulation. The size of the spores was measured and their shapes were noted. Descending hierarchical classification (DHC) was used to group the isolates by size. A comparison of isolates obtained from the two fruits was performed by Principal Component Analysis (PCA) based on all morphological characteristics studied. **Results:** The study revealed a very high morphological variability in the conidia of *Colletotrichum* isolates. DHC grouped avocado isolates into 11 classes and mango isolates into four classes. Spore shape analysis revealed a majority of spores with either both ends rounded or one end rounded and the other sharp. Growth and sporulation of the isolates were best on the malt medium. PCA revealed that mango isolates were distinct from avocado isolates. **Conclusion:** This study showed that most isolates had cylindrical or ellipsoidal spores with rounded or one sharp end, with better growth and sporulation on the malt medium. However, these isolates were distinct and reported to have characteristics of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*.

Key words: Mango, avocado, *Colletotrichum*, morphometry, growth, sporulation, Côte d'Ivoire

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits are often subject to various post-harvest deteriorations that limit their production and cause many losses. These deteriorations are mainly due to pathogens, the most important of which are the microscopic fungi[1]. The *Colletotrichum* genus is one of the most common fungi since one or more species are practically present on all crops worldwide[2]. This fungus causes anthracnose disease, characterized by black necrotic lesions either showing or not the pathogen's fruiting bodies (bright orange acervuli)[3]. The *Colletotrichum* genus is considered one of the top 10 most important plant pathogens globally due to its scientific and economic importance[2]. It attacks several hosts and the anthracnose caused represents the main post-harvest disease for many fruits such as mango, plum, papaya, banana and avocado[4]. This genus has been reported among the many fungi identified on various fruits in the United States[5], Senegal and Cameroon on mango[6], Cameroon on avocado and papaya[4,7], Côte d'Ivoire in post-harvest alterations of fruits[8,9]. In Thailand, it has also been revealed on various fruits, including mango, papaya, jujube, pink apple[10] and other parts of the world[11]. However, this genus has a very high morphological and pathogenic diversity[8]. Several species can cause fruit rots as a single species can infect several hosts[4,12]. Identifying *Colletotrichum* spp., is therefore essential in developing effective control measures against this pathogen. This identification is based mainly on differences in morphological characters such as colony colour, conidial size and shape, radial growth[4,13] and sporulation. Some authors have used the shape and size of conidia to distinguish several species[14]. These authors have successfully used morphological characters such as conidial shape to identify and distinguish *Colletotrichum* species and pathogens of strawberries.

The objective of this study was to identify and distinguish through morphological characteristics (size, shape, growth and sporulation of conidia), isolates of *Colletotrichum* sp., obtained from avocado and mango fruits from several major production areas in Côte d'Ivoire, as very few studies have specifically focused on these two particular hosts.

MATERIALS AND METHODS

Study area and fruits and fungal materials: The study was conducted over the period from March, 2017 to May, 2019. Avocado and mango fruits were sampled from orchards in the main Ivorian agroecological production zones. The fruits were transported in sterile and hermetically sealed containers. They were received at the Laboratory of Phytopathology and Plant Biology related to the Department of Agriculture and Animal Resources located at the Institut National Polytechnique Félix Houphouet-Boigny (INP-HB) in Yamoussoukro, Côte d'Ivoire (West Africa).

The fungal material consisted of 34 *Colletotrichum* isolates from avocado samples (Hall, Pollock, Lula, Booth 8 and local varieties) and 15 *Colletotrichum* isolates from mangoes (Amelie, Kent, Keitt, Brook and various local varieties named 'Assabonou', 'Greffe', 'Camerounaise', 'Séguéla', 'Mademoiselle', 'Ravia' and 'Apple'). Avocado fruits were sourced from the following Ivorian localities: Bouafle, Gagnoa, Oume, Sakassou, Sinfra, Toumodi, Yamoussoukro and Zuenoula located in the center of Côte d'Ivoire, Aboisso, Azaguie Tiassale in the South, Guezon, Man and San-Pedro are located in the west, Touba in the Northwest, Abengourou and Transua in the East of Côte d'Ivoire. The mangoes were sourced from the central locations of Bouake, Toumodi, Yamoussoukro and Daloa, as well as the Northern locations of Ferkessedougou, Korhogo, Odienne and Touba.

Fungal isolation and pure culture: Fruits showing typical symptoms were surface disinfected with 70°C alcohol and flamed with a gas burner. Subsequently, pieces of infected tissue were cut from the pericarp of the fruits and placed in plates containing PDA (Potato Dextrose Agar) medium. After 3-4 successive subcultures on the PDA medium, pure cultures of fungi were obtained and stored in the dark at 27°C.

Identification of fungal isolates: The pure cultures obtained after three successive subcultures were used for identification based on macroscopic and microscopic (400×) morphological characteristics: Colouration or pigmentation of the mycelium, presence or absence of septum and morphology of the spores. An Amscope light microscope equipped with a camera and the determination keys of Botton *et al.*[15] and Agrios[3] were used for the identification. In some cases, microscopic observations have required staining infected tissues with cotton blue, as McClenny[16] described.

Growth media-based characterization: This characterization was based on the mycelial growth rate of *Colletotrichum* sp., isolates on three different culture media: PDA (potato dextrose agar), malt agar (maltose certified 12.75 g, dextrin 2.75 g, glycerol 2.35 g, peptone 0.78 g and bacteriological agar 15.0 g) and CMA (corn meal agar) media. To perform this characterization, mycelial discs (4 mm diameter) of seven-day-old *Colletotrichum* isolate cultures were aseptically

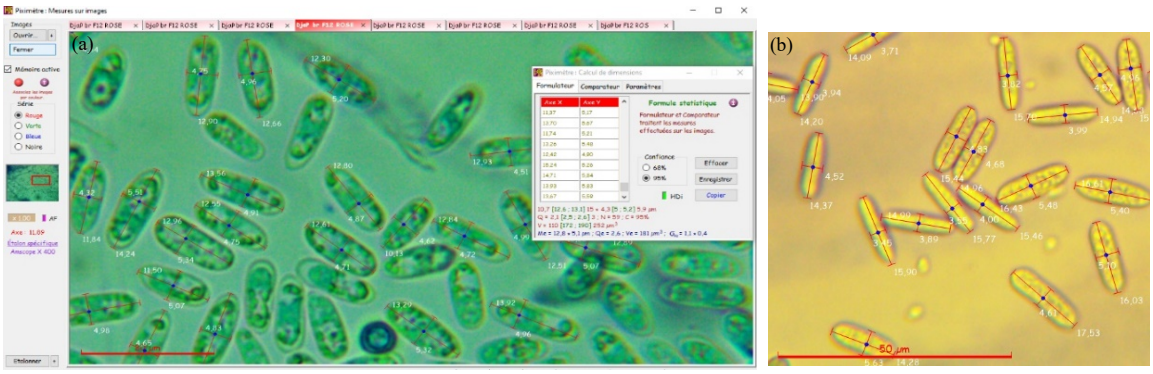

Fig. 1(a-b): Views of *Colletotrichum* sp., spore size measurements using Piximeter 5.9 software, (a) Piximeter images during conidia measurement and (b) Conidia measured in length and width

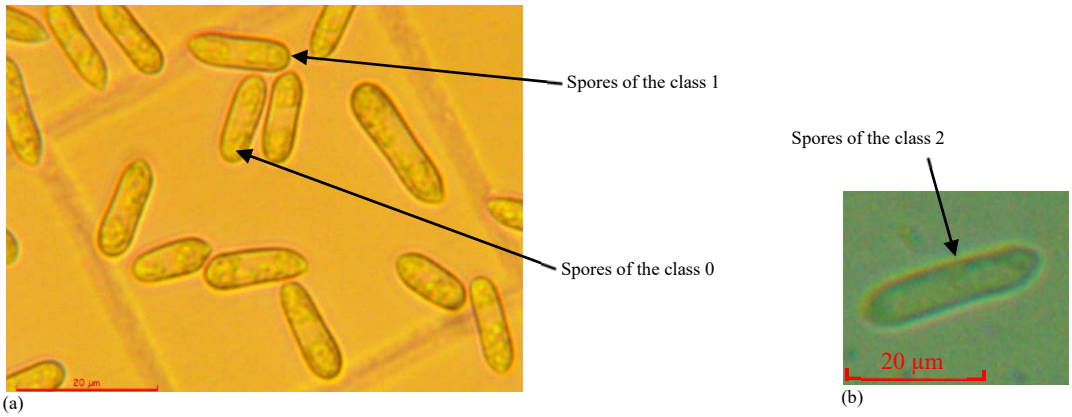

Fig. 2(a-b): Spores shapes of *Colletotrichum* sp., isolates, (a) Spores of the class 0 (conidia with both ends rounded) and 1 (conidia with one rounded end and the other sharp) and (b) Spore of class 2 (conidia with both sharp ends)

transferred to the centre of PDA using a perforating punch (cookie cutter), malt agar and CMA plates. The plates were sealed and incubated at 27±2°C for seven days. Six replicates were performed for each isolate and culture medium in a complete randomization experimental design[17]. Fungal colony diameter measurements were performed daily using a graduated ruler. The colony diameter was measured in two perpendicular directions on the reverse side of the plates every day after the incubation period. The daily gain in radial growth was calculated according to the formula of Sofi *et al.*[18]:

$$Cr = C_n - C_{n-1} \quad (1)$$

Where:

Cr : Daily gain in radial growth (mm)
C_n : Radial growth measured on a given day (mm)
C_{n-1} : Radial growth measured the day before (mm)

Spore characterization: Spores' characterization consisted of morphometric evaluations as described by Wokocha *et al.*[19]. These evaluations involved measuring the length and width of conidia from seven-day-old PDA cultures of *Colletotrichum* spp., isolates. Images of 100 random conidia were obtained with a light microscope equipped with a micrometre and calibrated at 400X magnification. The experiment was repeated three times[4,8]. The average length and width of the respective conidia were calculated using Piximeter software 5.9 (Fig. 1a-b). Piximeter is a metrology software whose domain is the measurement of spatial dimensions (length, width, height, or thickness) of any objects[20]. This software uses the laws of statistics and probability to calculate the characteristics of an entire population from a sample. The expressed measurements are average values.

The shapes of the 100 conidia were also noted and allowed to group into different classes (Fig. 2a-b) according to the method of Martinez *et al.*[21]:

Class 0 : Conidia with both rounded ends
Class 1 : Conidia with one rounded end and the other sharp
Class 2 : Conidia with both sharp ends

The average proportion of conidia in each class was calculated using the following formula of Ching'anda *et al.*[22]:

$$P_i = \frac{n_i}{T} \times 100 \quad (2)$$

Where:

P_i : Proportion of conidia belonging to class i

n_i : Number of conidia belonging to class i

T : Total number of conidia observed

Spores' concentration of *Colletotrichum* isolates cultures: The spore concentration of *Colletotrichum* sp., isolates was determined on seven-day-old cultures. To do this, 5 mL of distilled water was added to each culture plate and the surface of the plates was scraped with a Pasteur pipette. Then, a drop was taken and placed on a Malassez slide. The number of spores in each culture medium was determined by counting the spores in the diagonals. This count was repeated twice. The spore concentration Q of the isolates was finally calculated as follows[23,24]:

$$Q = 2n \times 10^6 \text{ spore/mL} \quad (3)$$

n : Average number of spores counted

Data analysis: Data obtained on the size (length and width) of *Colletotrichum* sp., isolates' conidia and their growth and sporulation were subjected to a One-way Analysis of Variance (One-way ANOVA) using STATISTICA version 7.1 software. In case of significant differences, the comparison of means was performed at the 5% threshold, using the Newman and Keuls Test. Moreover, a descending hierarchical classification (DHC) was used to classify the isolates by size. A Principal Component Analysis (PCA) was also used to classify *Colletotrichum* sp., isolates through a morphological data matrix according to the method described by Dembele[25]. This analysis allowed grouping by fruit, *Colletotrichum* sp., isolates with similar spores' size and shape, growth and sporulation and clustering of all *Colletotrichum* sp., isolates from both fruits according to the same criteria.

RESULTS

Spores size and shape of *Colletotrichum* sp., isolates from avocado: The measured lengths ranged from 10.3±1.36-17.1±2.40 µm, whereas the widths ranged from 4±0.47-4.8±0.72 µm (Table 1). Analysis of the variance of lengths and widths revealed significant differences ($p = 0.00001$). A comparison of the means revealed that the isolate with the smallest size was from the locality of Bayota (KA11) in the department of Gagnoa (Centre Côte d'Ivoire). The largest sizes were observed in isolates from Guezon (KA09) in the department of Gagnoa and Koninfla (KA36) in the department of Sinfra (Centre Côte d'Ivoire). KA is the code given to *Colletotrichum* isolates obtained from avocado samples. Moreover, the number after the code represents the rank of the number of isolates.

CDH gave the following dendrogram (Fig. 3) and allowed the classification of *Colletotrichum* sp., isolates into 11 groups (Table 1).

The *Colletotrichum* isolates analyzed, three types of cylindrical conidia were noted based on their tips: Class 0 conidia, class 1 conidia and class 2 conidia. *Colletotrichum* sp., isolates produced either class 0 and 1 conidia or all class 0, 1 and 2 conidia. Isolates producing two types of conidia made them in proportions ranging from 34-88% for those in class 0 and 12-66% for those in class 1 (Table 1). An exception was found in isolates KA28 and KA09, which obtained a high proportion of class 1 conidia (51 and 66%, respectively). Isolates producing all three classes of conidia got ratios ranging from 34-70% for class 0, 16-58% for class 1 and 3-9% for class 2 conidia.

Mycelial growth and sporulation of *Colletotrichum* sp., isolates obtained from avocados: The radial growth of the isolates varied on day 7 of culture from 26.0±1.5-66.0±12.8 mm on the PDA medium. On CMA and MALT media, it varied from 27.0±12.5-77±4.0 mm and 31±1.2-73.0±6.1 mm, respectively. The mean of this radial growth was 60 mm on the MALT medium, whereas those on CMA and PDA medium were 52 and 50 mm, respectively.

ANOVA indicated a significant difference between isolates ($p = 0.0001$), between media ($p = 0.0001$) and in the interaction between isolates and media ($p = 0.0001$) at day 7 of culture (Table 2). A comparison of means on day 7 showed that the radial growth of isolate KA02 (26 mm) was the smallest in the PDA medium and the largest was from isolate KA15 (66 mm). On the CMA medium, the growth of isolates KA08 and KA02 were the smallest, 27 and 33 mm, respectively. The largest growth was of isolate KA31 (77 mm). Isolates KA02, KA08 and KA28 expressed the smallest growths of 31, 32 and 37 mm on the MALT medium. KA20 expressed the largest growth. Regardless of medium, isolates KA02 and KA08 expressed the lowest growths. CMA and Malt media show

Table 1: Size and shape distribution of *Colletotrichum* sp., isolates obtained from avocados

Groups	Code of Isolates	Origin of the isolates	Colony colour on PDA medium	Mean length± Standard error (µm)	Mean width± Standard error (µm)	Conidia morphology (%)			
							Two rounded ends	One rounded end and one sharp end	Two sharp ends
1	KA14	Toumodi	Grey	16.94±0.22	5.17±0.46	Cylindrical	70	30	0
	KA09	Gagnoa	Grey orange			Cylindrical	34	66	0
	KA36	Sinfra	Grey			Cylindrical	58	42	0
2	KA18	Oume	Grey	16.21±0.13	4.55±0.04	Cylindrical	59	38	3
	KA40	Tiassale	Grey			Cylindrical	75	25	0
3	KA15	Toumodi	Black	16.16±0.09	5.34±0.28	Cylindrical	67	27	6
	KA21	Sakassou	Grey			Cylindrical	62	31	7
4	KA06	Transua	Black	15.52±0.25	4.81±0.21	Cylindrical	70	26	4
	KA29	Aboisso	Grey			Ellipsoidal	46	47	7
	KA32	Touba	Grey white			Cylindrical	83	17	0
5	KA01	Abengourou	Brown	15.32±0.16	4.67±0.06	Cylindrical	57	43	0
	KA02	Abengourou	Grey white			Cylindrical	85	15	0
	KA03	Transua	Brown			Cylindrical	79	21	0
	KA04	Transua	Black			Cylindrical	74	26	0
	KA10	Gagnoa	Black			Cylindrical Ellipsoidal	54	46	0
6	KA20	Sakassou	Grey orange	14.75±0.16	5.11±0.17	Cylindrical	75	25	0
	KA26	Yamoussoukro	White			Ellipsoidal	71	29	0
7	KA08	Azaguie	Grey	14.29±0.29	4.69±0.23	Ellipsoidal	55	36	9
	KA13	Bouafle	Greenish grey			Cylindrical	48	48	4
	KA17	Oume	Grey			Cylindrical	74	26	0
	KA22	Sakassou	Brown			Cylindrical	77	23	0
	KA27	Yamoussoukro	Grey			Ellipsoidal	61	39	0
	KA34	Sinfra	Greenish grey			Cylindrical	60	40	0
8	KA25	Zuenoula	Grey	13.74±0.26	5.31±0.01	Cylindrical	82	18	0
	KA31	Aboisso	Grey-orange			Cylindrica Ellipsoidal	81	19	0
9	KA07	Azaguie	Black	13.58±0.18	4.09±0.05	Cylindrical	46	47	7
	KA38	San-Pedro	Grey			Cylindrical	34	58	8
10	KA19	Guezon	Grey	12.67±0.04	4.62±0.11	Ellipsoidal	74	26	0
	KA28	Yamoussoukro	Grey			Ellipsoidal	49	51	0
11	KA11	Gagnoa	Grey white	10.64±0.50	3.70±0.47	Ellipsoidal	69	29	3
	KA12	Man	Grey			Cylindrical	52	48	0

KA: Code initials of isolates obtained from avocado samples

greater variability in the radial growth of isolates, up to 11 distinct homogeneous groups. PDA medium showed only eight (8) distinct homogeneous groups (Table 2).

Colletotrichum sp., isolates from avocados grown on PDA, CMA and MALT media showed different sporulation on the three culture media.

The spore concentration of *Colletotrichum* isolates varied in all culture media from 0.3×10^6-44.4×10^6 spores/mL (Table 3). On PDA and CMA media, the concentrations were 0.4×10^6-20×10^6 and 0.3×10^6-37×10^6 spores/mL, respectively. On the MALT medium, the concentration was 0.4×106-44×106 spores/mL. Isolates KA02, KA09, KA10, KA12, KA13, KA17, KA20, KA22, KA36, KA37 and KA38 produced fewer spores on all three media (Table 3). The greatest amounts of spores came from isolates KA29 on CMA and MALT media, KA31 on MALT media and KA06 on PDA media. Statistical analysis showed a significant difference between isolates ($p = 0.0001$), between culture media ($p = 0.0001$) and between isolate-culture media interactions ($p = 0.0001$). The spore concentrations of isolates on PDA and MALT media did not differ. MALT medium allowed isolates to produce a large number of spores. PDA and Malt media showed greater variability in the spore concentrations of *Colletotrichum* isolates, up to eight distinct homogeneous groups. The CMA medium showed only five distinct homogeneous groups (Table 3).

Interaction of studied traits of *Colletotrichum* sp., isolates from avocados: Principal Component Analysis (PCA) was performed on *Colletotrichum* isolates obtained from avocados based on morphological characters. The first three components (F1, F2 and F3) with eigenvalues greater than 1 explain, respectively 31.79, 21.48 and 16.13% of the variability, i.e., a cumulative variance of 69.40%. Factor 1 is defined by the

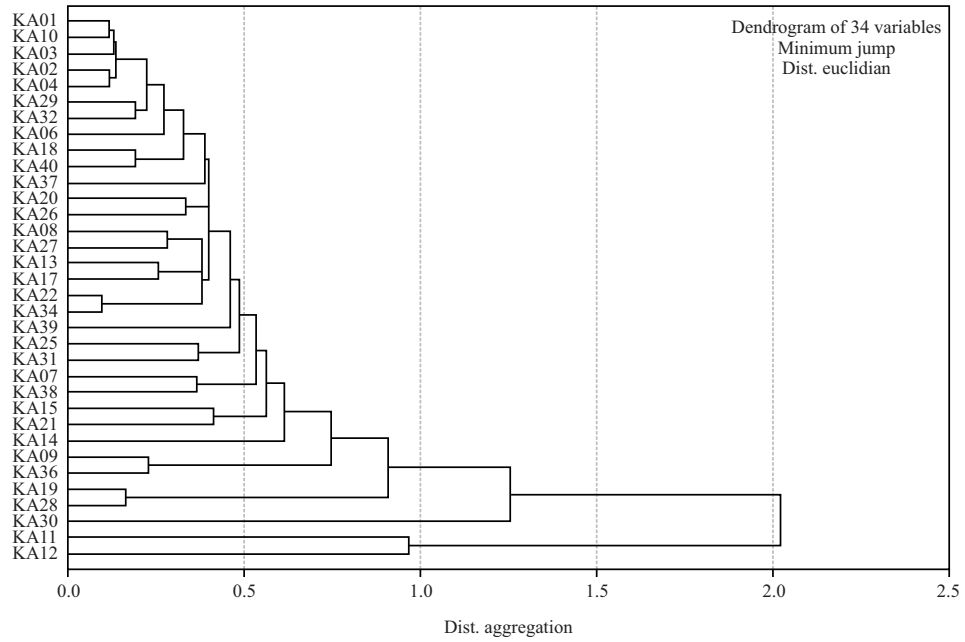

Fig. 3: Descending hierarchical classification of *Colletotrichum* isolates from avocado samples

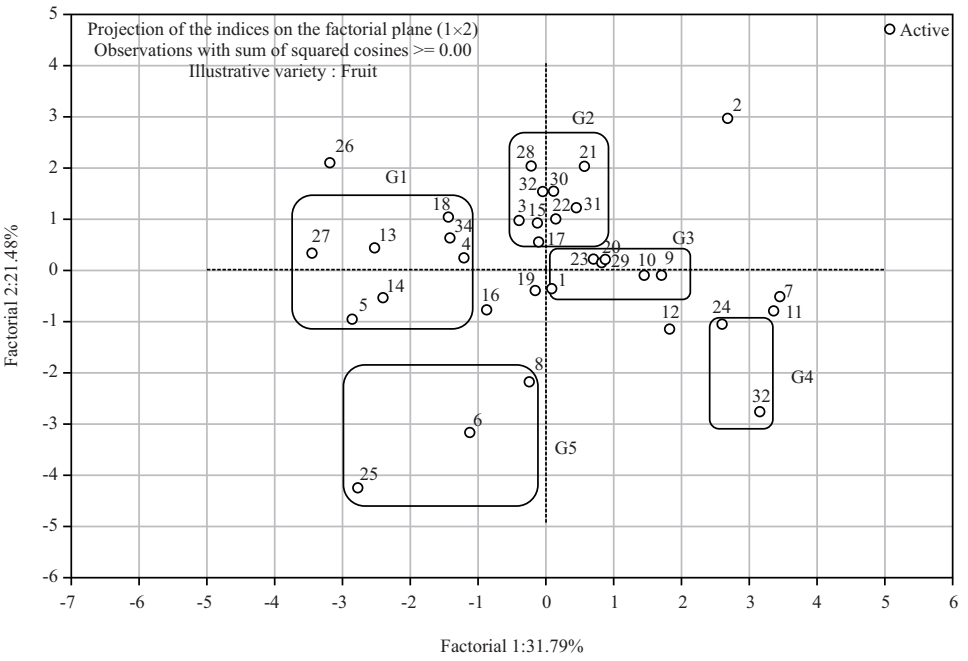

Fig. 4: Projection of avocado isolates of *Colletotrichum* sp., according to PCA principal components 1 and 2

mycelial growth of isolates on CMA and MALT media and sporulation on MALT media. Factor 2 is composed of a spore tip shape, class 0, 1 and 2. Factor 3 includes mycelial growth in the PDA medium and the sporulation of isolates in the PDA and CMA media. Projection of variables and isolates onto the two planes resulted in Gi groupings (Fig. 4):

- Cluster G1 includes seven isolates (KA31, KA14, KA15, KA06, KA20, KA40 and KA04). These isolates are characterized by a dominance of spores with rounded ends (class 0), relatively weak growth (42-66 mm) on the PDA medium and strong sporulation on the MALT medium. Their size (lengths × widths) was 14-16 × 4 -5 µm

Table 2: Radial growth on day 7 of growing *Colletotrichum* sp., isolates obtained from avocados

		Radial growth (mm) on medium				
Isolates	Provenances	PDA	CMA	MALT	F	p-value
KA02	Abengourou	26±1.54^{aA}	33±1.80^{aB}	31±1.20^{aB}	35.35	0.000002
KA08	Azaguie	35±14.86^{bA}	27±12.50^{aA}	32±19.02^{aA}	0.372	0.7
KA03	Transua	37±1.07^{bA}	47±1.11^{bcdeB}	53±2.44^{bcdC}	137.17	0.00001
KA25	Zuenoula	37±1.17^{bA}	50±1.03^{bcdefB}	50±1.72^{bcB}	175.96	0.00001
KA28	Yamoussoukro	38±0.92^{bA}	41±2.30^{bcB}	37±1.28^{aA}	9.73	0.002
KA12	Man	39±7.01^{bA}	40±3.29^{bA}	56±7.76^{cdeA}	7.89	0.0046
KA36	Sinfra	41±.1.20^{bcA}	50±2.29^{bcdefB}	59±1.17^{cdefghC}	194.66	0.0001
KA19	Guezon	41±0.98^{bcA}	55±1.83^{efghB}	59±2.36^{cdefghC}	162.42	0.0001
KA38	San-Pedro	42±2.57^{bcA}	45±3.20^{bcdB}	47±1.03^{bB}	8.14	0.004
KA31	Aboisso	42±12.01^{bcA}	77±4.0^{kB}	72±5.57^{jkB}	34.03	0.000003
KA10	Gagnoa	42±2.65^{bcA}	44±3.24^{bcdA}	55±2.58^{cdeB}	37.75	0.000001
KA13	Bouafle	49±2.56^{cdB}	43±3.91^{bcA}	56±0.82^{cdeC}	32.23	0.000004
KA11	Gagnoa	50±2.66^{cdeA}	49±4.74^{bcdefA}	58±2.72^{cdefB}	12.6	0.00062
KA06	Transua	51±5.63^{defA}	67±2.92^{ijB}	70±1.22^{hijkB}	44.48	0.00001
KA04	Transua	51±3.4^{defA}	58±2.74^{fghiB}	60±1.02^{defghiB}	18.9	0.00008
KA01	Abengourou	52±2.04^{defA}	54±4.60^{defgA}	58±5.85^{cdefgA}	3.54	0.055
KA21	Sakassou	52±2.58^{defA}	48±6.83^{bcdefA}	70±1.02^{ijkB}	46.92	0.0001
KA37	San-Pedro	52±5.16^{defB}	41±1.18^{bcA}	59±1.53^{cdefghC}	52.46	0.0001
KA26	Yamoussoukro	53±2.15^{defB}	48±1.96^{bcdefA}	58±1.75^{cdefgC}	40.94	0.000001
KA34	Sinfra	53±5.97^{defAB}	48±6.19^{bcdefA}	57±1.33^{cdefB}	4.74	0.025
KA40	Tiassale	54±2.09^{defA}	61±4.08^{ghijB}	65±1.88^{efghijkC}	25.3	0.000016
KA17	Oume	54±4.20^{defA}	55±5.40^{efghA}	68±14.45^{fghijkB}	3.97	0.041
KA39	Tiassale	55±3.96^{defgAB}	51±4.32^{cdefA}	57±3.68^{cdefC}	3.98	0.041
KA32	Touba	55±3.20^{defgB}	42±4.54^{bcA}	65±1.56^{efghijkC}	74.56	0.00001
KA29	Aboisso	55±2.24^{defgA}	70±1.29^{jB}	69±1.11^{hijkB}	157.8	0.0001
KA09	Gagnoa	55±1.01^{defgA}	66±7.83^{ijB}	72±3.40^{jkB}	16.7	0.0002
KA07	Azaguie	55±4.01^{defgA}	62±6.82^{ghijB}	69±3.06^{ghijkC}	11.13	0.001
KA14	Toumodi	56±2.62^{defgA}	61±4.22^{ghijB}	63±3.85^{defhijkB}	6.4	0.0098
KA22	Sakassou	57±2.70^{defgB}	44±3.79^{bcdA}	64±1.08^{efghijkC}	94.71	0.0001
KA27	Yamoussoukro	58±2.18^{defghB}	46±2.59^{bcdeA}	61±0.94^{defghijC}	96.15	0.0001
KA30	Aboisso	60±10.18^{efghA}	67±5.72^{jA}	65±1.88^{efghijkA}	1.95	0.18
KA20	Sakassou	61±5.20^{fghA}	65±10.47^{hijA}	73±6.12^{kA}	3.52	0.056
KA18	Oume	64±5.96^{ghA}	64±8.23^{hijA}	72±10.66^{jkA}	1.56	0.24
KA15	Toumodi	66±12.82^{hA}	61±12.01^{ghijA}	64±4.92^{efghijkA}	0.40	0.675
F		23.66	28.55	24.21		
p-value		0.0001	0.0001	0.0001		

Significant differences between isolates for a given growth medium is shown by lower case letters and significant differences between growth media for a given isolate are shown by upper case letters

- Cluster G2 is composed of nine isolates (KA32, KA39, KA37, KA19, KA25, KA22, KA03, KA17 and KA26). These isolates are characterized by spores with rounded ends (class 0), their growth is average on CMA and MALT media. Their size (average length × width) is 13-15×4-5 µm
- Grouping G3 is composed of KA01, KA11, KA10, KA27, KA34 and KA36. This group's spores are mostly class 0, with low growth (below 60 mm) and low sporulation. The sizes (lengths×widths) are 14-17×5 µm
- The G4 grouping includes two isolates (KA28 and KA38). Here, spore lengths and widths were 13×4-5 µm with rounded and sharp tips (class 1) and mycelial growth is weak (below 50 mm)
- G5: KA29, KA07 and KA09. The spores are class 1, the growth of these isolates is average on CMA and MALT media and are very fertile on the latter medium. Their dimensions (lengths×widths) are 14-17×4-5 µm.

Spores' sizes and shapes of *Colletotrichum* sp., isolates from mango: Measurement of spore lengths and widths of the different *Colletotrichum* sp., isolates revealed that the dimensions (lengths×widths) of these isolates ranged from 13.1±1.85-16.7±1.66×4.4±0.56-4.6±0.31 µm (Table 4). Analysis of the variance of lengths and widths revealed significant differences (p = 0.00001). A comparison of the means revealed that the isolate with the smallest length was from the locality of Bouake (KM03). The longest *Colletotrichum*

Table 3: Spores concentration on day 7 of growing *Colletotrichum* sp., isolates obtained from avocados

		Number of spores (10^6 spores/mL) per medium				
Isolates	Provenances	PDA	CMA	MALT	F	p-value
KA02	Abengourou	0.4 ± 0^{aA}	0.80 ± 0.53^{aA}	0.40 ± 0^{aA}	1.71	0.26
KA13	Bouafle	0.47 ± 0.12^{aA}	0.80 ± 0.20^{aA}	0.60 ± 0.2^{aA}	2.71	0.14
KA12	Man	0.53 ± 0.12^{aA}	0.47 ± 0.12^{aA}	0.40 ± 0^{aA}	1.50	0.3
KA34	Sinfra	0.6 ± 0.2^{aA}	1.27 ± 0.23^{abA}	3.07 ± 1.03^{abB}	12.78	0.0069
KA10	Gagnoa	0.6 ± 0.2^{aA}	0.33 ± 0.12^{aA}	0.60 ± 0.2^{aA}	2.28	0.183
KA18	Oume	0.67 ± 0.12^{aA}	1.33 ± 0.50^{abAB}	1.87 ± 0.46^{aB}	6.78	0.03
KA21	Sakassou	0.67 ± 0.46^{aA}	1.2 ± 0.20^{abAB}	1.60 ± 0.2^{aB}	6.73	0.0293
KA37	San-Pedro	0.8 ± 0.2^{aA}	0.47 ± 0.12^{aA}	1.53 ± 0.23^{aB}	25.13	0.0012
KA36	Sinfra	0.8 ± 0.35^{aA}	0.60 ± 0^{aA}	0.40 ± 0^{aA}	3.00	0.125
KA09	Gagnoa	0.87 ± 0.23^{aA}	0.67 ± 0.12^{aA}	1.40 ± 0^{aB}	19.40	0.0024
KA38	San-Pedro	1 ± 0.2^{aB}	0.60 ± 0.2^{aA}	0.53 ± 0.12^{aA}	6.14	0.035
KA26	Yamoussoukro	1.13 ± 0.23^{aA}	2.1 ± 0.12^{abB}	5.07 ± 0.76^{abC}	59.39	0.00011
KA22	Sakassou	1.13 ± 0.12^{aA}	0.67 ± 0.46^{aA}	1.27 ± 0.57^{aA}	1.6	0.28
KA20	Sakassou	1.4 ± 0.2^{aB}	0.40 ± 0^{aA}	1.07 ± 0.42^{aB}	10.94	0.0099
KA39	Tiassale	1.47 ± 0.23^{aA}	0.40 ± 0^{aA}	9.73 ± 1.10^{bcB}	185.43	0.000004
KA17	Oume	1.47 ± 0.23^{aB}	0.60 ± 0.2^{aA}	1.07 ± 0.31^{aAB}	9.07	0.02
KA25	Zuenoula	1.67 ± 0.42^{aA}	8.8 ± 0^{cB}	1.53 ± 0.46^{aA}	402.31	0.0001
KA32	Touba	2.47 ± 0.46^{abB}	0.93 ± 0.31^{abA}	1.27 ± 0.12^{aA}	18.29	0.0028
KA11	Gagnoa	3.4 ± 0.35^{abcAB}	2.5 ± 0.6^{abA}	3.80 ± 0.53^{abB}	5.75	0.040
KA08	Azaguie	3.73 ± 8.33^{abcdB}	1.2 ± 0.72^{abA}	0.40 ± 0^{aA}	22.5	0.0016
KA28	Yamoussoukro	3.73 ± 0.76^{abcdA}	16.07 ± 2.6^{dB}	4.20 ± 1.11^{abA}	49.37	0.0002
KA29	Aboisso	4.93 ± 0.64^{abcdeA}	36.93 ± 6.3^{eB}	44.4 ± 4.3^{hB}	67.85	0.00008
KA40	Tiassale	5.93 ± 1.17^{abcdeB}	0.73 ± 0.23^{aA}	17.13 ± 2.10^{deC}	108.25	0.00002
KA30	Aboisso	7.2 ± 0.2^{bcdefB}	4.47 ± 1.10^{abA}	13.13 ± 0.61^{cdC}	108.6	0.00002
KA19	Guezon	7.47 ± 2.0^{cdefA}	9.67 ± 0.50^{cA}	9.33 ± 2.14^{bcA}	1.43	0.31
KA04	Transua	8 ± 0.4^{cdefA}	11.60 ± 2.16^{cAB}	15.53 ± 2.66^{deB}	10.71	0.0104
KA01	Abengourou	8.1 ± 4.1^{cdefB}	1.1 ± 0.42^{abA}	3.13 ± 1.10^{abA}	12.91	0.007
KA15	Toumodi	8.67 ± 1.17^{defB}	2.60 ± 1.05^{abA}	17.87 ± 2.91^{deC}	48.40	0.0002
KA14	Toumodi	9.53 ± 3.78^{efB}	0.73 ± 0.12^{aA}	26.80 ± 4.70^{gC}	43.44	0.0003
KA03	Transua	9.73 ± 2.21^{efA}	10.87 ± 0.81^{cA}	20.73 ± 2.84^{efB}	24.15	0.0013
KA31	Aboisso	11.87 ± 8.53^{fgA}	4.67 ± 1.02^{bA}	43.33 ± 9.14^{hB}	24.2	0.0013
KA07	Azaguie	14.6 ± 3.47^{gB}	2.40 ± 0.8^{abA}	25.67 ± 5.22^{fgC}	30.56	0.0007
KA06	Transua	20.13 ± 1.29^{hB}	3.3 ± 0.95^{abA}	24.80 ± 7.81^{fgB}	18.19	0.003
F		18.20	83.73	58.84		
p-value		0.0001	0.0001	0.0001		

Significant differences between isolates for a given growth medium is shown by lowercase letters and significant differences between growth media for a given isolate are shown by upper case letters

Table 4: Size and shape distribution of *Colletotrichum* sp., isolates obtained from mango

							Conidia morphology (%)		
Groups	Code of Isolates	Colony colour on PDA medium	Origin of the isolates	Mean length± Standard error (µm)	Mean width± Standard error (µm)		Two rounded ends	One rounded end and one sharp end	Two sharp ends
1	KM01	Grey	Bouake	16.59±0.13	4.76±0.29	Cylindrical	64	27	9
	KM04	Grey	Bouake			Cylindrical	44	52	4
	KM07	Grey	Daloa			Cylindrical	53	42	5
2	KM02	Grey-Orange	Bouake	15.82±0.06	3.35±0.24	Ellipsoidal	45	48	7
	KM12	Brown	Yamoussoukro			Cylindrical	40	60	0
	KM15	Brown	Korhogo			Ellipsoidal	64	36	0
3	KM05	Brown	Daloa	15.28±0.30	4.57±0.10	Cylindrical	76	19	5
	KM06	Grey	Daloa			Cylindrical	53	42	5
	KM10	Grey	Odienne			Cylindrical	54	41	5
	KM13	Brown	Touba			Cylindrical	44	48	9
	KM11	Grey	Yamoussoukro			Cylindrical	73	27	0
4	KM03	Grey	Bouake	13.77±0.51	4.49±0.50	Cylindrical	60	40	0
	KM08	Grey	Ferkessedougou			Cylindrical	39	50	11
	KM09	Grey	Ferkessedougou			Cylindrical	40	46	15
	KM14	Brown	Toumodi			Ellipsoidal	81	19	0

KM: Code initials of isolates obtained from mango samples

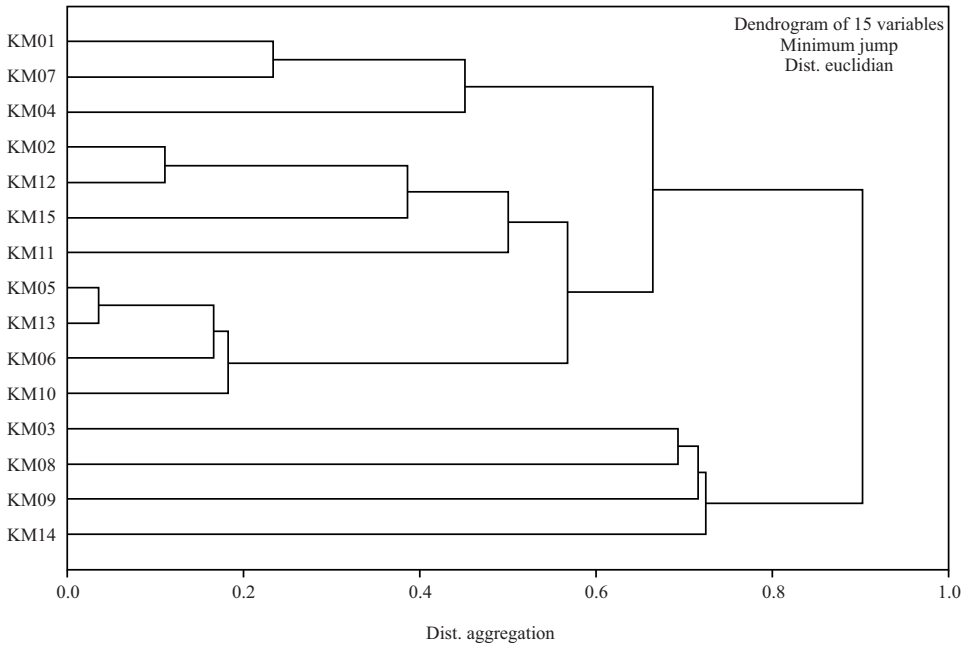

Fig. 5: Descending hierarchical classification of *Colletotrichum* isolates from mango samples

spores were observed on isolates from Daloa (KM07). The smallest and largest widths were observed in Ferkessedougou (KM09) and Korhogo (KM15), respectively. The dendrogram (Fig. 5) and the distance matrix allow us to group the *Colletotrichum* sp., isolates into four categories (Table 4).

Analysis of the shape of *Colletotrichum* sp., isolates showed the production of three cylindrical spore types from all isolates except five isolates that produced only two spore types (Table 4). The proportion of class 0 spores ranged from 35-81%, class 1 spores ranged from 18-60% and class 2 spores ranged from 4-15%. Some isolates produced predominantly class 0 spores, these are: KM07 (52%), KM06 (53%), KM10 (54%), KM03 (60%), KM01 (64%), KM15 (64%), KM11 (73%), KM05 (76%) and KM14 (81%). Other isolates produced predominantly class 1 spores. These were: KM09 (46%), KM13 (48%), KM02 (48%), KM08 (50%), KM04 (52%) and KM12 (60%). KM, followed by a number, is the code given to mango isolates.

Mycelial growth and sporulation of *Colletotrichum* sp., isolates from mango: The average radial growth of *Colletotrichum* sp., isolates obtained from mango was higher on the MALT medium than on PDA and CMA media. These mycelial growths of *Colletotrichum* sp., isolates ranged from 17.3±1.0-66.9±4.7 mm in the PDA medium (Table 5). On CMA and MALT media, the growth of *Colletotrichum* sp., isolates ranged from 21.4±1.2-68.4±3.8 and 23.6±1.5-67.0±3.1 mm, respectively. Thus, the KM11 isolate appeared to have the smallest growth in all three culture media. On MCA and MALT isolate KM06 expressed the lowest radial growths. Isolate KM12 expressed the largest growth in all three culture media.

Statistical analysis showed a significant difference ($p = 0.0001$) between the culture media, between isolates ($p = 0.0001$) and between isolates-culture media ($p = 0.002$). Comparing the mean mycelial growths of each isolate in the three media revealed a significant difference in only six isolates. The average growth of the other nine isolates was not different in the three culture media. In addition, isolate KM11 expressed the lowest radial growth on the PDA medium and the highest growth was from isolate KM12 on CMA. However, the mycelial growths of the isolates were found to be different in the three culture media. PDA and CMA media revealed greater variability in radial growths of *Colletotrichum* isolates up to six distinct homogeneous groups compared to MALT media which showed only three distinct homogeneous groups *Colletotrichum* sp., isolates from mangoes showed different sporulation on the three culture media PDA, CMA and MALT.

The number of spore/mL in the three culture media ranged from 0.4×10^6-10.9×10^6. MALT medium allowed good sporulation of *Colletotrichum* sp., isolates compared to PDA and CMA media. Indeed, the spore concentrations of the isolates were 0.4×10^6-8.5×10^6 spores/mL and 0.4×10^6-10.9×10^6 spores/mL on PDA and CMA media, respectively (Table 6). On the MALT medium, the concentrations ranged from 0.4×10^6-9.3×10^6 spores/mL.

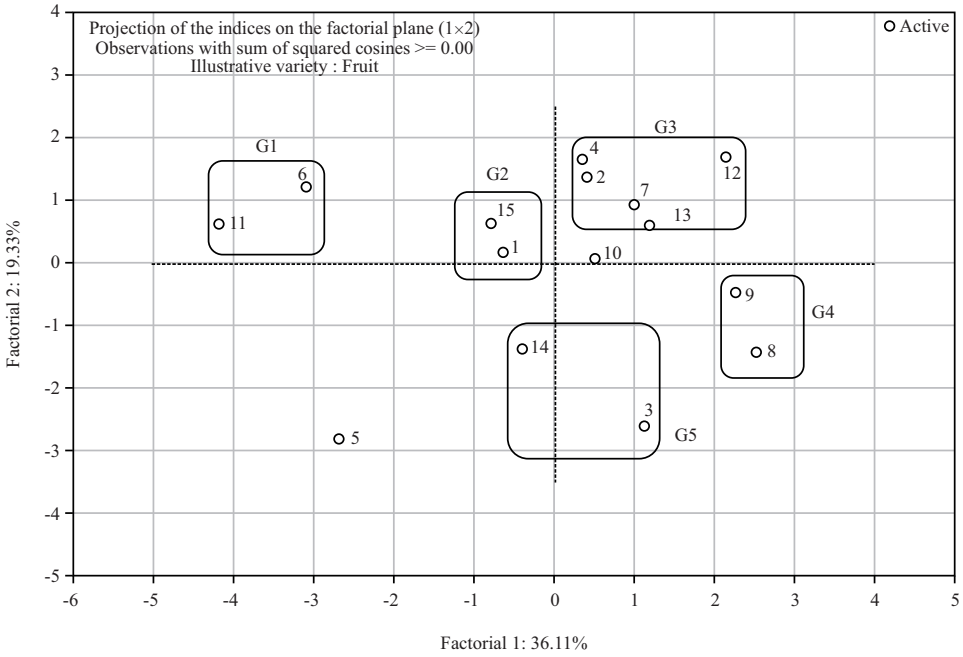

Fig. 6: Projection of *Colletotrichum* sp., isolates obtained from mangoes according to PCA principal components 1 and 2

Statistical analysis of spore counts showed a significant difference between isolates (p = 0.0001), between media (p = 0.0003) and between isolates-media (p = 0.0001). The lowest sporulation was from isolate KM111 on the PDA medium and the isolate that sporulated the most was KM12 on the CMA medium. However, the spore counts of the isolates were higher on the MALT medium. CMA, PDA and MALT media revealed variability in spore concentration of *Colletotrichum* isolates up to five and three distinct homogeneous groups, respectively (Table 6).

Correlation of studied characters of *Colletotrichum* sp., isolates from mango: Principal Component Analysis (PCA) was performed on *Colletotrichum* isolates obtained from mangoes based on morphological characters. The first three components (F1, F2 and F3) with eigenvalues greater than 1 explain, respectively, 36.11, 19.33 and 18.16% of the variability, i.e., a cumulative variance of 73.60%. Factor 1 is defined by the mycelial growth of the isolates on the three culture media. Factor 2 comprises the concentration of isolates on MALT and the average length. Factor 3 includes conidial width and spore tip shape class 2. Projection of the variables and isolates onto the two planes resulted in clusters noted Gi (Fig. 6):

- Cluster G1 is characterized by isolates with very low mycelial growth (less than 30 mm) but had large spore sizes (15-16×5 µm) with mostly rounded tips (class 0). Fertility was relatively high on PDA medium and low on CMA and MALT media. There were two isolates: KM06 and KM11
- G2 cluster consisted of two isolates: KM15 and KM01. This group is characterized by a relatively weak growth (55 mm) on the three culture media, large spore sizes (16-17×5-6 µm) and dominance of class 0 spores
- Cluster G3 included KM12, KM13, KM02, KM07 and KM04. These were isolates with large spore widths (5 µm), the dominance of class 1 spores and relatively high mycelial growth on CMA and MALT media
- Cluster G4 contained isolates KM09 and KM08. These isolates had high mycelial growth (58-67 mm) on all three media but small spore sizes (14×4 µm) with three spore types, including the dominance of class 1 spores. They also had low sporulation on the PDA medium
- Cluster G5 consisted of two isolates, KM14 and KM03. These isolates had good mycelial growth on PDA and MALT media (61-66 mm) but small spore sizes (13-14×4-5 µm) with mostly rounded tips (class 0) and good fertility on MALT media.

Correlation of characters of *Colletotrichum* sp., isolates obtained from the two fruits: A PCA revealed four factors. These factors (F1, F2, F3 and F4) explain, respectively 27.8, 24.2, 16.00 and 10.6% of the variability, i.e. a cumulative variance of 78.6%. Factor 1 is defined by the mycelial growth of isolates on CMA and MALT media. Factor 2 is influenced by

Table 5: Radial growth on day 7 of growing *Colletotrichum* sp., isolates obtained from mangoes

		Mycelial growth (mm) on medium				
Isolates	Provenances	PDA	CMA	MALT	F	p-value
KM11	Yamoussoukro	17.25 ± 1.04^{aA}	21.42 ± 1.20^{aB}	23.58 ± 1.50^{aC}	39.19	0.0001
KM06	Daloa	27.25 ± 2.48^{bA}	26.08 ± 1.56^{aA}	27.17 ± 0.82^{aA}	0.80	0.47
KM05	Daloa	40.50 ± 2.32^{cA}	40.17 ± 5.34^{bA}	46.58 ± 16.14^{bA}	0.82	0.46
KM02	Bouake	50.33 ± 1.51^{dA}	52.33 ± 6.19^{dA}	52.25 ± 13.37^{bcA}	0.11	0.90
KM15	Korhogo	52.42 ± 4.87^{dA}	49.00 ± 2.02^{cdA}	53.58 ± 3.18^{bcA}	2.69	0.1
KM01	Bouake	52.92 ± 2.63^{dA}	49.92 ± 14.57^{cdA}	51.08 ± 15.98^{bcA}	0.09	0.92
KM04	Bouake	53.33 ± 4.29^{dB}	42.50 ± 2.79^{bcA}	59.08 ± 1.46^{bcC}	45.09	0.0001
KM13	Touba	54.33 ± 1.69^{dA}	51.50 ± 3.18^{cdA}	63.17 ± 2.62^{cB}	33.61	0.0001
KM03	Bouake	60.75 ± 5.41^{dB}	54.83 ± 5.59^{deA}	66.33 ± 1.25^{cB}	9.58	0.0001
KM14	Toumodi	61.33 ± 7.39^{eA}	57.17 ± 5.69^{deA}	66.25 ± 5.86^{cA}	3.07	0.08
KM07	Daloa	63.00 ± 1.48^{eA}	63.83 ± 1.86^{efA}	62.17 ± 2.14^{bcA}	1.22	0.32
KM10	Odienne	64.25 ± 2.36^{eA}	55.33 ± 10.23^{deA}	52.33 ± 20.57^{bcA}	1.3	0.3
KM09	Ferkessédougou	65.00 ± 1.90^{eA}	62.67 ± 6.34^{efA}	63.08 ± 0.92^{cA}	0.62	0.55
KM08	Ferkessédougou	66.42 ± 6.27^{eB}	57.58 ± 4.40^{deA}	67.00 ± 3.13^{cB}	7.32	0.01
KM12	Yamoussoukro	66.92 ± 4.73^{fA}	68.42 ± 3.79^{fA}	66.42 ± 1.16^{cA}	0.51	0.61
F		85.64	28.04	13.98		
p-value		0.0001	0.0001	0.0001		

Significant differences between isolates for a given growth medium is shown by lowercase letters and significant differences between growth media for a given isolate are shown by upper case letters

Table 6: Spores concentration at day 7 of growing *Colletotrichum* sp., isolates obtained from mangoes

		Number of spores (10^6 spores/mL) per medium				
Isolates	Provenances	PDA	CMA	MALT	F	p-value
KM15	Korhogo	0.4 ± 0^{aA}	1.4 ± 0.53^{abcdB}	1.87 ± 0.31^{aB}	13.54	0.01
KM14	Toumodi	0.47 ± 0.12^{aA}	0.47 ± 0.12^{aA}	0.6 ± 0.2^{aA}	0.80	0.49
KM01	Bouake	0.47 ± 0.12^{aA}	1.47 ± 0.7^{cdA}	0.73 ± 0.12^{aA}	4.64	0.06
KM13	Touba	0.53 ± 0.12^{aA}	0.53 ± 0.12^{aA}	0.53 ± 0.12^{aA}	0.00	1.00
KM03	Bouake	0.53 ± 0.12^{aA}	0.73 ± 0.23^{abcA}	9.27 ± 6.02^{cB}	6.17	0.04
KM04	Bouake	0.73 ± 0.31^{aA}	0.4 ± 0^{aA}	1.47 ± 0.23^{aB}	18.27	0.003
KM12	Yamoussoukro	0.8 ± 0.2^{aA}	0.47 ± 0.12^{aA}	0.6 ± 0^{aA}	4.75	0.06
KM11	Yamoussoukro	0.87 ± 0.31^{aA}	0.6 ± 0.2^{abA}	0.4 ± 0^{aA}	3.70	0.09
KM02	Bouake	0.87 ± 0.23^{aA}	0.73 ± 0.23^{abcA}	0.8 ± 0.35^{aA}	0.18	0.84
KM09	Ferkessédougou	1.1 ± 0.64^{abA}	1.6 ± 0.53^{dA}	0.67 ± 0.31^{aA}	2.51	0.16
KM08	Ferkessédougou	1.2 ± 0.4^{abA}	0.47 ± 0.12^{aA}	8.47 ± 1.33^{cB}	90.4178	0.00033
KM05	Daloa	1.27 ± 0.42^{abA}	10.87 ± 0.53^{eC}	5.4 ± 0.7^{bB}	252.45	0.00002
KM07	Daloa	1.6 ± 0.2^{abB}	0.4 ± 0^{aA}	0.47 ± 0.12^{aA}	76.75	0.00053
KM10	Odienne	2.2 ± 0.53^{bB}	1.13 ± 0.31^{abcdA}	0.67 ± 0.23^{aA}	14.38	0.005144
KM06	Daloa	8.47 ± 1.5^{cB}	1.1 ± 0.31^{abcdA}	1.53 ± 0.61^{aA}	53.67	0.00015
F		47.53	197.39	10.09		
p-value		0.0001	0.0001	0.0001		

Significant differences between isolates for a given growth medium is shown by lowercase letters and significant differences between growth media for a given isolate are shown by upper case letters

spore tip shape, class 0 and class 1. Factor 3 includes the spore concentration of *Colletotrichum* isolates on CMA and MALT media. Factor 4 is defined by the average length of isolates and the average width of isolates to a lesser extent. Projection of the variables and isolates onto the two planes allowed the classification of *Colletotrichum* sp., isolates into five groups (Fig. 7 and 8).

The 1st group consisted solely of isolates from avocado. This group consisted of six isolates (KA29, KA15, KA06, KA14, KA31 and KA30). These isolates were characterized by very good mycelial growth on CMA and MALT media (>60 mm) and strong sporulation on MALT media (13×10^6-44×10^6 spores/mL). Their size (length × width) was 14-17 × 5-6 µm and both spore ends were mostly rounded (class 0).

The 2nd group consisted of four isolates (KA20, KA40, KA04 and KM14), one of which (KM14) was from mangoes. These isolates all produced two types of spores, dominated by spores with rounded ends (class 0). Mycelial growth is average on the culture media (57-65 mm). Their size (lengths × widths) was 14-16 × 5 µm.

The 3rd group consisted of 11 isolates, three of which were from the avocado samples. The isolates were characterized by relatively good growth (between 54-59 mm) on the three culture media. Their fertility was low in all three growth media. The size (length × width) of these isolates was 15 × 5 µm and the spores were predominantly class 1.

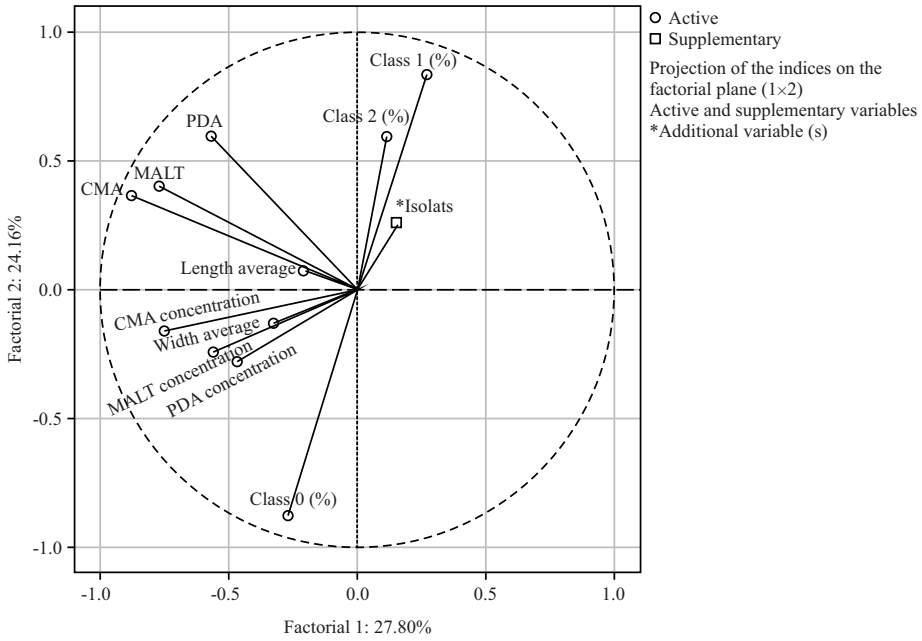

Fig. 7: Projection of variables in the factorial plane 1-2 of the PCA of *Colletotrichum* sp., isolates obtained from both fruits

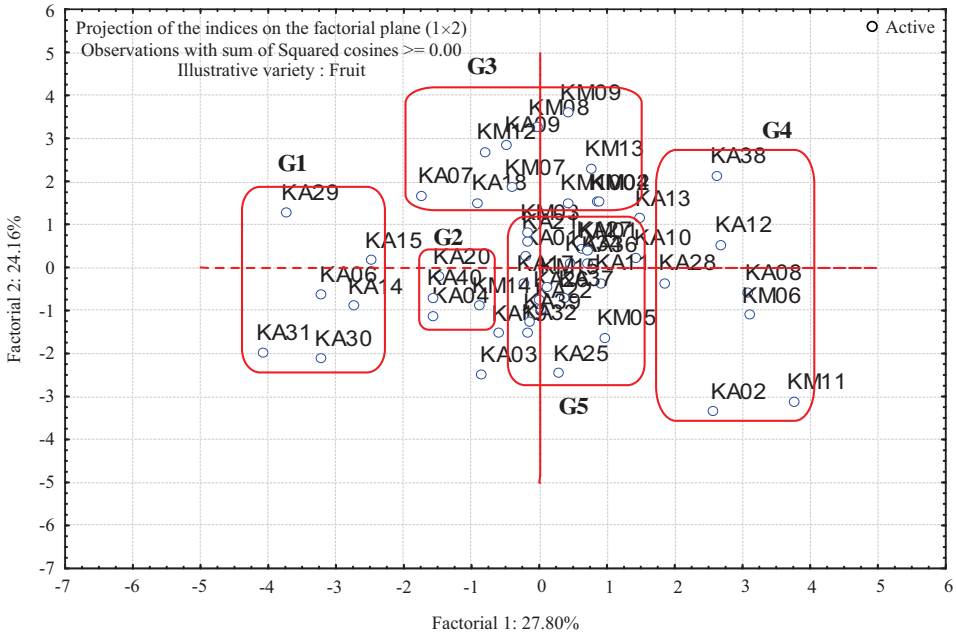

Fig. 8: Projection of *Colletotrichum* sp., isolates obtained from the two fruits in the factorial plane 1-2 of the PCA

The 4th group comprised isolates characterized by low growth on all three growth media (>40 mm) and low fertility. Both spore ends were mostly rounded. The spore size of these isolates was small.

The 5th group included 17 isolates, four of which were from mango. The spore size of these isolates was medium on MALT medium and the spore ends were rounded. Sporulation was relatively low.

DISCUSSION

Analysis of the spore size of *Colletotrichum* sp., isolates obtained from avocado and mango revealed a variation in conidia's mean length and mean width (regardless of the groups). It is respectively from 10.64±0.50-16.97±0.22 µm and 3.70±0.47-5.17±0.46 µm for avocado and 13.77±0.51-16.59±0.13 and 3.35±0.24-4.76±0.29 for mango. These

values were close to those obtained by Xoca-Orozco *et al.*[26] for their study on avocado and Riviera-Vargas *et al.*[27] for their work on mango. For these authors, the length and width of the conidia of *Colletotrichum* sp., isolates belong to *Colletotrichum gloeosporioides* and they vary from 9-24 µm for the length and 3-4.5 µm for the width[24] or from 12-20 µm and 3.5-6 µm[25]. However, studies by Freeman *et al.*[13] and those by the Kamdoum *et al.*[4] and Phoulivong *et al.*[28] on the same fruits reveal the presence not only of *C. gloeosporioides* but also of *C. acutatum*. The spore size of *C. acutatum* ranged from 8-13×2-5 µm[27]. Colletotrichum *gloeosporioides* and *C. acutatum* are the causal agents of most tropical fruit rots[28]. Ismail *et al.*[29] also point out in their study that in addition to these two species, there were other species of this genus, namely *C. fructicola*, *C. tropica* and *C. karstii* and the newly described *C. dianesei*[30] on mangoes.

Spores produced by avocado and mango isolates are cylindrical or ellipsoidal of two or three types with a dominance of class 0 or 1. Class 0 is spores with both ends rounded and class 1 is spores with one end rounded and the other sharp. These spore types described in this study were consistent with the analysis of *Colletotrichum* conidial morphology by Martinez *et al.*[21], who showed that a single colony could contain two or three different spore types. The results obtained in this study were also consistent with the work of Sanders and Sanders and Korsten[14] and Abera *et al.*[31], who showed that *C. gloeosporioides* isolates produced predominantly cylindrical conidia with a tapered base and obtuse (rounded) top or both ends rounded. Freeman *et al.*[13] showed in their study that conidia of *C. acutatum* are elliptical and spindle-shaped with a tip at both ends[31] while those of *C. gloeosporioides* were elongated (oblong) and have an obtuse (rounded) tip. Damm *et al.*[32] showed that the best-known form of *C. acutatum* (s. Lat.) conidia is that the ends of its conidia are acute. However, other conidial forms, especially±cylindrical with a single sharp tip, are frequently encountered, especially in strains have been repeatedly transplanted, but these conidial forms can also occur in species outside *C. acutatum* species complex[30]. *Colletotrichum gloeosporioides* and *C. acutatum* are the considered species complexes[12]. *Colletotrichum gloeosporioides* is a species complex that the includes morphologically indistinguishable but genetically and biologically isolated species[33].

Mycelial growth of *Colletotrichum* sp., isolates from avocado and mango was favourable on MALT medium by 60 and 53 mm, respectively, than on the other media. Thus, the culture medium would influence the development of the fungi. Ansari *et al.*[34] and Niren and Chandra[35] showed this influence of the culture medium in their study. For these authors, the growth of fungi depends largely on the quality of the medium they grow on and this fungal growth is influenced by factors such as the source of nitrogen, carbon, pH of the substrate and temperature. Similarly, Kumara and Rawal[36] revealed that different types of media influence colony growth and morphology, pigmentation and sporulation. These results were in agreement with the results of this study since, for the same isolate grown on three different media, the growth and morphology of the colonies were different. Khanzada *et al.*[34] also showed that the MALT medium was one of the media that allowed maximum growth of the fungi, which corroborated the result of this study. This medium allowed good growth of *Colletotrichum* isolates. Hailmi *et al.*[37] showed that the CMA medium presented weaker (less favourable) growths of *Colletotrichum* isolates than the PDA medium. This corroborates the results of this study.

Radial growth of some *Colletotrichum* isolates was low on all three media. The growth rate of colonies in vitro would be one of the important characteristics of distinguishing *Colletotrichum* species, according to Than *et al.*[38]. These authors argued that isolates with the same growth rate are closely related to each other and that isolates of *C. acutatum* had the lowest growth rates and would be differentiated from *C. gloeosporioides* by their slow growth rate.

Sporulation of *Colletotrichum* isolates in this study was better on MALT medium than on PDA and CMA media on which the concentration of spores did not differ (avocado and mango). This spore concentration is strongly influenced by the growth media, as pointed out by Kumara and Rawal[36]. Similarly, Majumdar and Mandal[35] showed in their study that sporulation is a complex phenomenon controlled by the environment and genetic factors that may vary between isolates. These results differed from those of Leharwan *et al.*[39] and Es-Soufi *et al.*[40] revealed that sporulation of the *C. gloeosporioides* and *C. acutatum* was excellent in the PDA medium.

Group 1 (KA31, KA14, KA15, KA06, KA20, KA40 and KA04) and group 2 (KA32, KA39, KA37, KA19, KA25, KA22, KA03, KA17 and KA26) are characterized by a dominance of spores with rounded ends (class 0) and a respective size between 14-16×4-5 µm and 15×5 µm. Growth is above 60 mm for group 1 and 48-59 mm for group 2. Isolates from these two groups are characteristic of *Colletotrichum gloeosporioides*, characterized by rapid growth, spores with rounded ends and a size range of 9-24×3-6 µm[26,27,39]. Group 3 (KA01, KA11, KA10, KA27, KA34 and KA36), although characterized by low growth (below 60 mm) and sporulation, has mostly class 0 spores

(rounded ends) with sizes ranging from 14-17×5 µm. This group could be a subpopulation of *C. gloeosporioides*, a species complex[12]. *Colletotrichum gloeosporioides* is a species complex that includes morphologically indistinguishable but genetically and biologically isolated species[33]. Isolates (KA28 and KA38) in group 4 are characterized by spores with rounded, sharp tips (class 1), a size of 13×4-5 µm and weak mycelial growth (below 50 mm). This group would be characteristic of *C. acutatum*, which has slow growth, a size range of 8-13×2-5 µm and the presence of spores with rounded and sharp ends. In group 5 (KA29, KA07 and KA09), the spore tips are class 1, average growth on MALT and CMA media and very good fertility on MALT media. Their size is between 14-17×4-5 µm. This group could also be a subpopulation of *C. gloeosporioides*, which can sometimes have one rounded end and the other pointed[41].

On mango, there are five groups formed. Group 1 (KM06 and KM11), although having a very weak (slow) mycelial growth (less than 30 mm), is characterized by spores with rounded ends in the majority (class 0) of sizes ranging between 15-16×5 µm. Fertility is relatively high on PDA medium and low on CMA and MALT media. This group could be characteristic of *C. asianum,* which has low mycelial growth (4.67-5.5 mm/day) and spores with rounded ends of size between 7-20.3×3-5.7 µm. Group 2 (KM15 and KM01) also showed class 0 spores, relatively low growth (55 mm) on all three media, large spore sizes (16-17×5-6 µm) and low fertility on the PDA medium. *Colletotrichum gloeosporioides* is characterized by spores with rounded ends. Group 5 (KM14 and KM03) also shows the characteristics of *C. gloeosporioides*: very good mycelial growth on PDA and MALT media (61-66 mm), spores with rounded ends (class 0) but small sizes (13-14×4-5 µm) and good fertility on MALT media. Group 3, which includes KM12, KM13, KM02, KM07 and KM04, shows isolates with large spore sizes (15-17×5 µm), the dominance of class 1 spores (one rounded end and the other sharp), relatively high mycelial growth on CMA and MALT media. Group 4, composed of KM09 and KM08, showed high mycelial growth (60-66 mm) on all three media but small spore sizes (14×4 µm) with three spore types, including the dominance of class 1 spores. These groups could be subpopulations of *C. gloeosporioides*.

CONCLUSION

Morphological analysis (size, shape, growth and sporulation) of the 49 isolates of *Colletotrichum* sp., showed very high variability within isolates of each fruit but also between fruits in the different characters studied. In addition, this analysis allowed us to cluster the isolates into 11 groups for avocado and four groups for mango. These isolates within these groups were characterized by cylindrical or ellipsoidal spores with both ends rounded or one end sharp. Growth and sporulation of the isolates were strong in the MALT medium and weak in the CMA medium. However, most isolates in this study are characteristic of *Colletotrichum gloeosporioides,* although there are characteristics of *C. acutatum* and subpopulations within the *C. gloeosporioides* and *C. acutatum* complexes.

SIGNIFICANCE STATEMENT

This paper carefully examines the distinctive morphological characteristics of conidia of the fungus *Colletotrichum*, the most important pathogenic genus affecting staple fruits in Côte d'Ivoire. Forty-nine isolates of this fungus from avocados and mangoes sampled in the main production areas of Côte d'Ivoire were characterized as belonging to *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* species. These results are noteworthy because they are likely to allow the development of targeted and effective control measures against this pathogen in Côte d'Ivoire. Our manuscript is the first report on the effective identification of *Colletotrichum* species affecting common fruits that could be used to test promising control methods in Ivorian orchards.

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