



# Morphological diversity of *Lasiodiplodia* sp. isolates infecting woody and fruit crops in Côte d'Ivoire

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## Abstract

This study aims to assess the morphological, cultural, and microscopic diversity of *Lasiodiplodia* sp. isolates from woody and fruit crops in Côte d'Ivoire, to better understand their variability and support integrated fungal disease management. It is a descriptive and experimental study conducted at the phytopathology laboratory of the Institut National Polytechnique Félix Houphouët-Boigny (INP-HB) in Yamoussoukro, with surveys carried out in the main agricultural areas. A total of 281 symptomatic samples were collected from seven economically important crops (cashew, mango, cacao, cola, rubber, *Polyalthia longifolia*, and banana) in 96 locations. Isolates were purified on various media (PDA, CMA, and agar), and 161 representative isolates were selected for morpho-cultural and microscopic evaluation. Characteristics studied included mycelial growth rate, colony appearance, pycnidia count, and conidia size, shape, color, and density. Statistical analyses were performed using XLSTAT (one-way ANOVA, Newman-Keuls test,  $p < 0.05$ ). The results revealed strong morpho-cultural variability depending on the host, culture medium, and agroecological zone. Radial growth rates ranged from 1.45 to 6.04 cm/d, and three colony morphotypes were identified (grayish, brown, and blackish). The mean number of pycnidia ranged from 0 to 7.00. Conidia were ovoid, ellipsoid, or oblong, measuring 4.81–18.29  $\mu\text{m} \times 2.95$ –10.53  $\mu\text{m}$ , with spore densities ranging from 0.90 to 50.00 conidia/ml. The proportion of unicellular and bicellular conidia differed depending on the isolate and host. Statistical analysis confirmed that the host and the medium significantly influenced colony and spore morphology. This morpho-cultural and microscopic diversity underscores the combined influence of the host, the environment, and agroecological conditions. The results provide an essential basis for understanding pathogenicity and implementing integrated management strategies for fungal diseases in woody and fruit crops in Côte d'Ivoire.

**Keywords:** *Lasiodiplodia* sp.; morphological diversity; woody cultures; fungal pathogens; Ivory Coast.

## 1. INTRODUCTION

Fungal diseases pose a major threat to agricultural production in tropical regions, affecting both the quality and yield of woody and fruit crops [1]. In Côte d'Ivoire, crops such as cashew (*Anacardium occidentale*), mango (*Mangifera indica*), cacao (*Theobroma cacao*), and rubber (*Hevea brasiliensis*) are important sources of income for local producers. However, the productivity of these crops is frequently limited by fungal diseases, including those caused by *Lasiodiplodia* [6].

*Lasiodiplodia* spp. is a phytopathogenic fungus widely distributed in tropical and subtropical zones, responsible for fruit rot, twig dieback, and cankers on many woody trees [12]. This genus is distinguished by its high morphological and physiological diversity, influenced by the host species, growing environment, and agroecological conditions [8,11]. In Côte d'Ivoire, despite some studies on *Lasiodiplodia theobromae*\* in cocoa and mango plantations [3],

knowledge of the morphological diversity, distribution, and pathogenic potential of isolates from different crops remains limited.

Morpho-cultural and microscopic characterization of isolates is an essential tool for understanding the variability of fungal populations and adapting integrated disease management strategies [4,15]. Indeed, the diversity of traits such as mycelial growth, pycnid production, and conidia density and morphology can reflect the genetic variability of isolates and their ability to adapt to environmental conditions [14].

Thus, this study aims to evaluate the distribution and morphological and cultural diversity of *Lasiodiplodia* sp. isolates collected from different woody and fruit crops in Côte d'Ivoire, in order to provide useful information for disease management and the improvement of agricultural productivity.

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## 2. MATERIALS AND METHODS

### 2.1. Prospecting and sample collection

Surveys were conducted during the production season in the main agro-ecological and agricultural production areas of Côte d'Ivoire. They covered several crops of economic interest, including cashew, mango, cocoa, cola, rubber, Accra Cone, and banana (Figure 1).

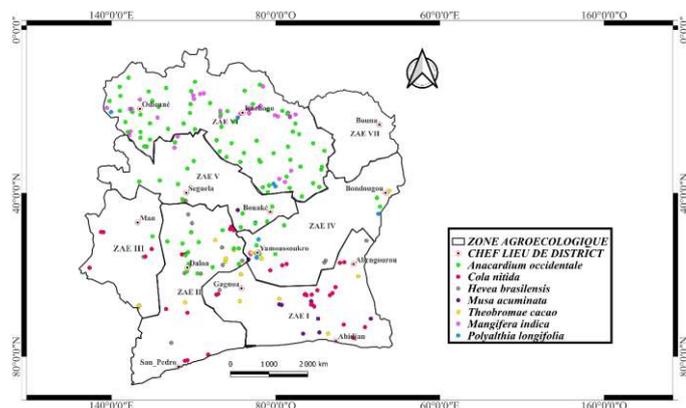


Figure 1: Sampling sites in the surveyed localities

Cocoa pods naturally affected by black rot were collected from different trees within each surveyed plot and individually packaged in labeled plastic bags (date, plot code, and sample number).

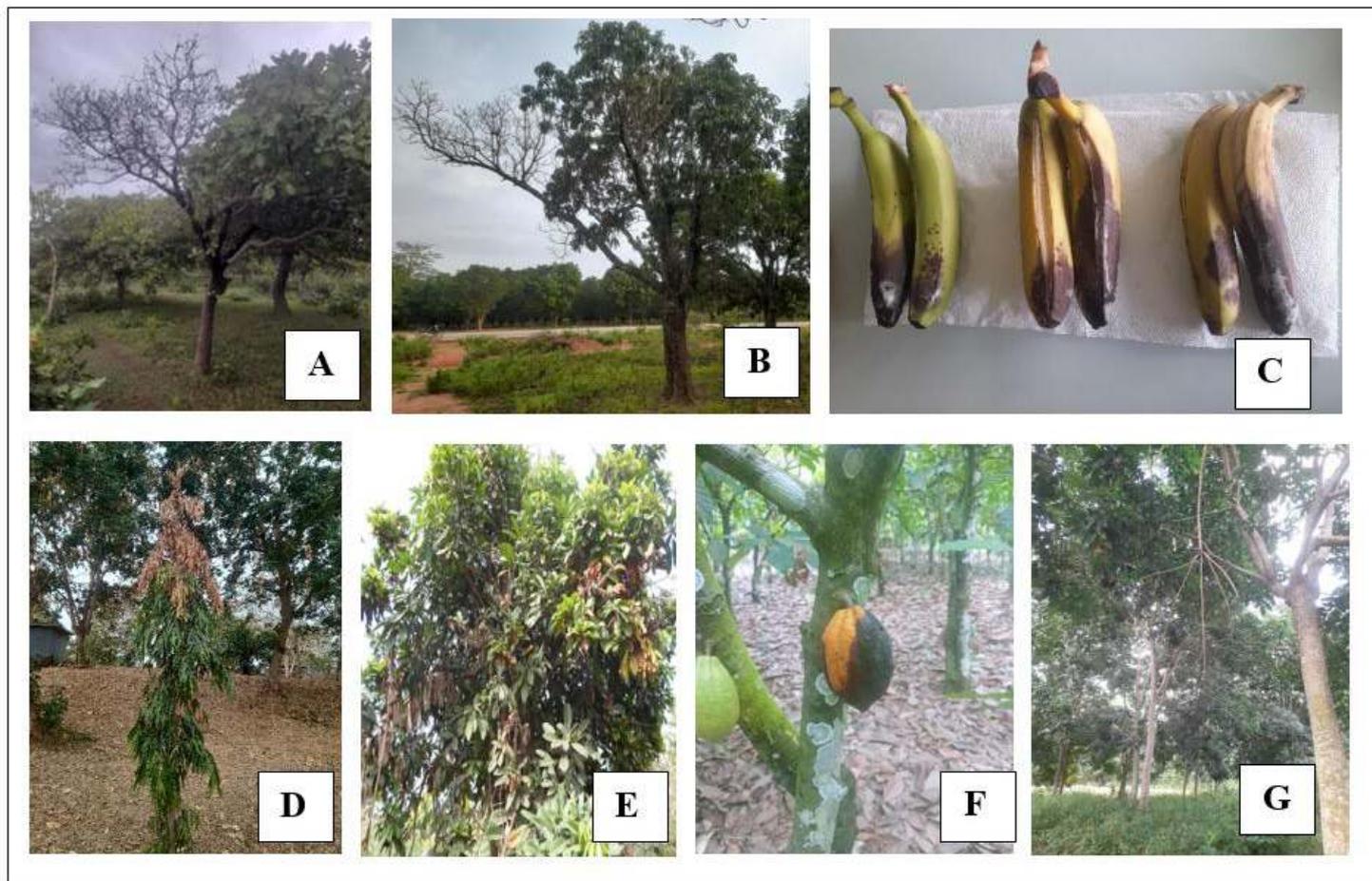
The banana samples, harvested at physiological maturity, from industrial plantations were obtained in Zambakro, Abengourou, Tiassalé, Dabou, Béoumi, Azaguié, and Agboville. A detailed breakdown of the samples by crop, location, and region is presented in Table 1.

The collected samples were kept in plastic bags and transported to the phytopathology laboratory of the INP-HB in Yamoussoukro.

Plant material exhibiting characteristic symptoms, including dieback, cracking with exposure of internal tissues, gummy exudations, and rot were collected in the field. A total of 281 samples were collected from several locations distributed across different production regions, depending on the crops studied (Table 1). The collection covered 96 locations for cashew, 50 for cola (*Cola nitida*), 38 for cocoa (*Theobroma cacao*), 34 for mango (*Mangifera indica*), 29 for rubber (*Hevea brasiliensis*), 11 for Accra Cone (*Polyalthia longifolia*), and 11 for banana (*Musa acuminata*), distributed respectively across 7 to 15 regions depending on the crop.

Table 1: Location of collection areas for woody plants and fruits in Côte d'Ivoire

Crops	Sample number	Localities	Region
Cashew	100	96	13
Mango	35	34	8
Cocoa	38	38	10
Cola	50	50	15
Rubber	30	29	10
Accra Cone	16	11	10
Banana	12	11	7



A: *Anacardium occidentale* B: *Mangifera indica* C: *Musa acuminata* D: *Polyalthia longifolia* E: *Cola nitida* F: *Theobroma cacao* G: *Hevea brasiliensis*

Figure 2: Plants and fruits affected by drying out or rotting

## 2.2. Isolation and purification of fungi

The isolation and purification of fungi were carried out in the laboratory according to protocols adapted to the plant organs studied. For stem samples, the method described by [19] was applied with slight modifications. Symptomatic fragments of mango, rubber, cola, Accra Cone, and cashew stems ( $\approx 1$  cm) were superficially disinfected in a 50% sodium hypochlorite solution for 1 min, then rinsed three times with sterile distilled water before being placed on PDA medium amended with citric acid.

The harvested bananas were washed, rinsed with sterile distilled water, and kept at 28°C. Ripening was homogenized by immersion in an ethylene solution ( $2 \text{ ml}\cdot\text{L}^{-1}$ ) according to [9]. After lesions appeared, the fruits were disinfected with 70% alcohol, and fragments taken from the growth front were inoculated onto PDA medium.

For cocoa, subcortical tissue fragments were collected from necrotic lesions of pods affected by black rot, after disinfection with 96% alcohol and flaming, and then placed on PDA medium [5]. Petri dishes were incubated in the dark for 3 days at  $28 \pm 2$  °C. Isolates were purified by subculturing at the mycelial growth front [5].

## 2.3. Laboratory identification

The final stage of the study consisted of identifying the pathogens associated with fruit dieback and rot. Isolates were characterized based on morphological criteria, including macroscopic observation of colonies (color, general appearance, and presence of pycnidia) and microscopic examination of conidia (morphology, color, and size). Species identification was performed by referring to the descriptions and taxonomic keys proposed by [16].

## 2.4. Morphological characterization of the isolates

In total, 161 isolates representative of the different sampling areas were selected for the evaluation of morpho-cultural parameters. The study was carried out on three culture media: PDA, MEA and agar, prepared according to the protocols described by [18] for PDA and agar media, and by [13] for the MEA medium [8].

For each isolate, a 4–5 mm diameter mycelial explant, taken from the growth front of a 5-day-old culture on PDA, was placed in the center of Petri dishes containing the different media. Each isolate was subcultured into five dishes (replicates) and incubated at  $28 \pm 2$  °C under a 12 h/12 h light/dark cycle, as described by [16].

Morphological and cultural traits were assessed based on mycelial growth rate, as well as mycelium color and texture. Radial growth was measured daily by drawing two perpendicular axes on the back of each Petri dish and then calculating the average of the two diameters until the dish was fully filled [7]. The average growth of each isolate was determined from the five replicates. The diameter (in mm) of each isolate was measured in two perpendicular directions, and the average colony diameter was calculated using equation 1.

$$Ddn = \frac{d1 + d2}{2} \text{ [Equation 1]}$$

where D is the average diameter of the isolate in a box, d1 and d2 are the measurements of the two perpendicular diameters. Growth was measured daily until full culture expansion using equation 2:

$$CM = \frac{[(Dd1 - Dd0) + (Dd2 - Dd1) + \dots + (Ddn - Ddn-1)]}{n} \text{ [Equation 2]}$$

where CM is the daily mycelial growth rate, Ddn is the average diameter growth on the day of measurement. Dd0 = initial diameter of the mycelium disc, which is 5 mm [8]. The appearance of colonies and pigmentation, as well as vegetative and reproductive structures, were described after 10 days of incubation.

## 2.5. Microscopic characterization of isolates

Microscopic characterization was performed to assess conidia morphology, considering size, color, the presence of septa, and spore density. To estimate conidial concentration, the mycelium of each 27-day-old *Lasiodiplodia* sp. isolate was superficially scraped with a sterile spatula and then flooded with 10 ml of sterile distilled water to detach the conidia [5]. Spore concentration was determined by light microscopy (40 $\times$ ) from a drop of suspension placed on a Malassez cell, and photographs were taken with a digital camera connected to the microscope. Each measurement was repeated five times. The size (length and width) of 30 conidia per isolate was measured, and the number of cells per conidium was counted [2, 8].

The average number of conidia per millilitre was calculated according to the formula of [5] (Equation 3).

$$N = \frac{1}{25\sum(ni)} * 10^5 \text{ [Equation 3]}$$

N: average number of conidia per square; ni: number of conidia in the square; 25: number of squares considered; and  $10^5$ : the factor that reduces the volume of one square ( $0.01 \text{ mm}^3$ ) to one milliliter of solution. The morphological characteristics of the spores were compared to those described in the literature [14, 8].

## 2.6. Statistical analyses

Data were analyzed using XLSTAT software version 2021. A one-way analysis of variance (ANOVA) was performed to compare *Lasiodiplodia* sp. isolates based on their morpho-cultural and microscopic characteristics, at a significance level of 5%. The parameters studied included mycelial growth, conidial size, and conidial concentration. Differences between homogeneous groups were then determined using the Newman-Keuls test.

## 3. RESULTS

### 3.1. Morphological characteristics of isolates from cultures

#### 3.1.1. Macroscopic observation

Observation of *Lasiodiplodia* sp. colonies 3 and 14 days after subculturing revealed three distinct morphotypes (Figure 3). On day three, the mycelium was whitish, sparse, or cottony on the front surface of the Petri dishes. This appearance gradually evolved to cover the entire dish. From day five onward, the initial coloration changed to grayish (morphotype A), brown (B), or blackish (C), with a cottony appearance and a downy texture.

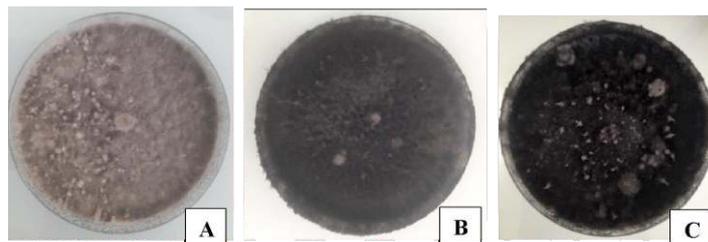


Figure 3. Different morphotypes within the isolates of *Lasiodiplodia* sp.

### 3.1.2. Mycelial Growth

Statistical analysis revealed significant differences between the isolates ( $P < 0.05$ ). The mean mycelial growth of the *Lasiodiplodia* sp. isolates, presented in Table 2, showed considerable variation depending on the original culture and the type of culture medium. The mean radial growth rate ranged from 1.45 to 6.04 cm/day. In general, the isolates showed slower growth on agar compared to PDA and CMA media, with PDA exhibiting the highest growth rate, followed by CMA, then agar.

Isolates from *Hevea brasiliensis* and *Musa acuminata* exhibited the fastest growth rates across all three media, while those from *Anacardium occidentale* showed high growth only on PDA. Isolates from *Theobroma cacao* displayed the weakest radial growth on all media. These results indicate that the mycelial growth of *Lasiodiplodia* sp. isolates is jointly influenced by host plant type and culture medium.

### 3.1.3. Appearance of the colonies

Analysis of colony appearance revealed significant variations depending on the culture medium and host plant ( $P < 0.05$ ). Isolates from *Mangifera indica* exhibited the greatest morphological diversity on PDA (1.54), while those from *Theobroma cacao* showed the maximum diversity on CMA. *Polyalthia longifolia* isolates remained homogeneous on both PDA and CMA (1.00), as did those from *Hevea brasiliensis* and *Musa acuminata*, which were homogeneous on CMA and PDA, respectively. On agar, no significant differences were observed between isolates ( $P > 0.05$ ), indicating uniformity in colony appearance on this medium (Table 2).

### 3.1.4. Number of pycnidia

The mean number of pycnidia in the isolates varied significantly depending on the culture medium and host plant ( $P < 0.05$ ) (Table 2). In general, agar produced the fewest pycnidia compared to PDA and CMA media. *Polyalthia longifolia* isolates produced the highest number of pycnidia across the three media. For *Hevea brasiliensis*, the mean number of pycnidia was highest on PDA (6.35) and CMA (3.27). Conversely, *Musa acuminata* and *Cola nitida* isolates showed the lowest numbers on PDA and CMA (0.66 and 0.00, respectively).

**Table 2: Mycelial growth and colony appearance of *Lasiodiplodia* sp according to different crops on culture media**

Isolates derived from	CM_PDA	CM_CMA	CM_Agar	AsCl_PDA	AsCl_CMA	AsCl_Agar	NPyc_PDA	NPyc_CMA	NPyc_Agar
<i>Anacardium occidentale</i>	6.09±0.12c	3.99±0.15bc	2.87 ± 0.11 b	1.09±0.02a	1.02 ± 0.01 a	1.49±0.00a	2.15±0.39a	0.31±0.19ab	0.04±0.29a
<i>Cola nitida</i>	5.15±0.14b	3,570,17b	1.74 ± 0.13a	1.19±0.02b	1.06 ± 0.01 b	1.49±0.00a	0.66±0.45a	0±0.22a	0±0.33a
<i>Hevea brasiliensis</i>	5.94±0.20c	4.82 ± 0.24d	3.08±0.18bc	1.32±0.03c	1.00±0.01a	1.49±0.00a	6.35 ± 0.63 b	2.72 ± 0.31c	3.2 ± 0.46 b
<i>Mangifera indica</i>	5.58±0.17bc	3.92±0.21bc	2.57 ± 0.16 b	1.45±0.03d	1.04±0.01ab	1.49±0.00a	1.30±0.56a	1.043±0.27b	0.26±0.41a
<i>Musa acuminata</i>	6,040,28c	5.34 ± 0.34d	3.62 ± 0.26c	1.00±0.04a	1.02±0.02ab	1.49±0.00a	0.55±0.90a	0.8±0.44ab	0±0.66a
<i>Polyalthia longifolia</i>	5.60±0.30bc	4.63±0.37cd	2.55 ± 0.27 b	1.00±0.05a	1.00±0.02a	1.49±0.00a	7.00 ± 0.95 b	6.5±0.46d	3.25 ± 0.70 b
<i>Theobroma cacao</i>	4.63 ± 0.20a	2.92 ± 0.24a	1.45 ± 0.18a	1.22±0.03b	1.11±0.01c	1.49±0.00a	1.33 ± 0.63a	1.11± 0.31 b	0±0.46a
F	<b>9.0272</b>	<b>8.9576</b>	<b>17.1178</b>	<b>22.8213</b>	<b>5.4045</b>	<b>0.0272</b>	<b>14.4768</b>	<b>33.5860</b>	<b>9.4478</b>
Pr > F	<b>&lt; 0.0001</b>	<b>0.9999</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>				

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. CM: Mycelial growth, AsCl: Colony appearance, NPyc: Pycnid number.

**Table 3: Mycelial growth and colony appearance *Lasiodiplodia* sp., from different agroecological zones on different growing media**

ZAE	CM_PDA	CM_CMA	CM_Agar	AsCl_PDA	AsCl_CMA	AsCl_Agar	NPyc_PDA	NPyc_CMA	NPyc_Agar
Zone I	5.25 ± 0.15ab	3.84 ± 0.19a	2.23 ± 0.15 b	1.17±0.02b	1.11±0.01a	1.49±0.00a	0.58±0.49b	0.74 ± 0.26 b	0±0.36b
Zone III	4.80 ± 0.27 b	2.77 ± 0.33b	2.13 ± 0.26 b	1.32 ± 0.04a	1.00±0.02c	1.49±0.00a	0.65±0.86b	0.59 ± 0.45 b	0.09 ± 0.62b
Zone II	5.64 ± 0.13a	4.09 ± 0.16a	2.48 ± 0.13ab	1.25±0.02a	1.00±0.01c	1.49±0.00a	1.78 ± 0.43 b	0.54 ± 0.22 b	0.02 ± 0.31 b
Zone IV	5.61 ± 0.18a	4.24 ± 0.23a	2.18 ± 0.18 b	1.00±0.03c	1.07 ± 0.01 b	1.49±0.00a	6.73 ± 0.59a	2.83 ± 0.31a	4.04 ± 0.43a
Zone V	5.92 ± 0.19a	3.96±0.23a	2.85±0.18a	1.12 ± 0.03 b	1.01±0.01c	1.49±0.00a	1.76 ± 0.60 b	0.55 ± 0.31 b	0±0.44b
Zone VI	5.84 ± 0.140a	4.10 ± 0.17a	2.66 ± 0.13ab	1.27±0.02a	1.03±0.01c	1.49±0.00a	2.17±0.44b	1.22 ± 0.23 b	0.15±0.32b
F	<b>3.7634</b>	<b>3.1260</b>	<b>2.5836</b>	<b>11.6544</b>	<b>11.5692</b>	<b>0.0163</b>	<b>14.6938</b>	<b>8.6040</b>	<b>14.9156</b>
Pr > F	<b>0.0022</b>	<b>0.0083</b>	<b>0.0248</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.9999</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. CM: Mycelial growth, AsCl: Colony appearance, NPyc: Pycnid number.

On CMA, *Musa acuminata* isolates produced slightly more pycnidia than on PDA. No pycnidia were observed on agar for the isolates of *Musa acuminata*, *Theobroma cacao* and *Cola nitida*.

## 3.2. Morphological characteristics of isolates according to agroecological zones

### 3.2.1. Mycelial Growth

Table 3 presents the average mycelial growth of *Lasiodiplodia* sp. isolates from different agroecological zones and cultured on three media. The average growth rate ranged from 2.13 to 9.92 cm/day, with values generally lower on agar than on PDA and CMA. Radial growth was dependent on both the agroecological zone and the culture medium. On PDA, isolates showed the highest growth rates, followed by CMA, then agar. Isolates from agroecological zone 3 exhibited the lowest growth rates on all media, while those from zone 5 showed the fastest growth on PDA and agar. Isolates from zone 4 showed maximum growth only on CMA.

### 3.2.2. Appearance of the colonies

The morphological diversity of the colonies varied with the culture medium and agroecological zone (Table 3). On PDA, isolates from zones II, III, and VI exhibited the highest diversity, while on CMA, the maximum diversity was observed for isolates from zone 1. On agar, no significant difference was detected between the isolates ( $P > 0.05$ ), indicating morphological uniformity on this medium.

### 3.2.3. Number of pycnidia

The mean number of pycnidia in the isolates varied according to the culture medium and the agroecological zone (Table 3). In general, the values were lower on agar than on PDA and CMA. Isolates from zone 4 had the highest number of pycnidia across all three media. Conversely, those from zones 1 and 3 produced the fewest pycnidia, respectively on PDA (0.58) and CMA (0.55). On PDA and CMA, isolates from *Musa acuminata* and *Cola nitida* showed the lowest numbers (0.66 and 0.00, respectively). No pycnidia were observed on agar for isolates from zones 1 and 3.

### 3.3. Microscopic characteristics according to cultures

#### 3.3.1. Microscopic characteristics

Microscopic observation of *Lasiodiplodia* sp. isolates revealed inter-isolate variations in the conidia. Two main colors were observed: brownish and hyaline. Based on their shape, the conidia were classified as ovoid, oblong, or ellipsoid. Some conidia exhibited irregular longitudinal striations and rings at the septa. The conidia were generally unicellular or bicellular (Figure 4).

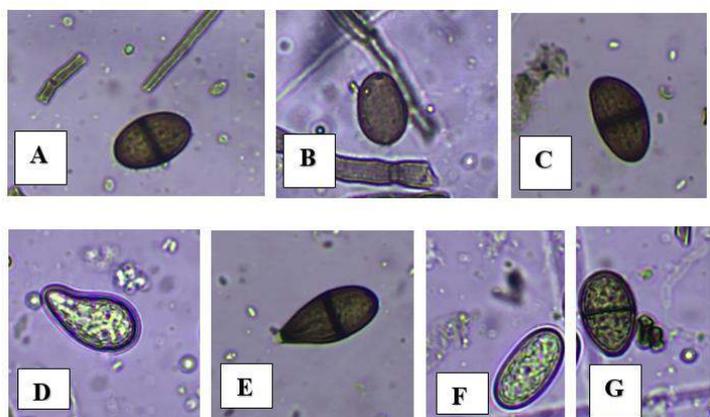


Figure 4. Different conidia within the isolates of *Lasiodiplodia* sp. studied

**HAS-** Ovoid, brownish, bicellular; **B** - Ovoid, brownish, unicellular; **C** - Ellipsoid, brownish, bicellular; **D** - Oblong, hyaline, unicellular; **E** - Oblong, brownish, bicellular; **F** - Ellipsoid, hyaline, unicellular; **G** - Ellipsoid, hyaline, bicellular

#### 3.3.2. Spore density and conidia dimensions

The spore densities and conidia dimensions of the isolates are presented in Table 4. Statistical analysis showed significant differences between the isolates on PDA and CMA ( $P < 0.05$ ), but none produced conidia on agar by day 28 of culture.

Spore density ranged from 7.04 to 50.00 conidia/ml on PDA and from 0.90 to 10.88 conidia/ml on CMA. *Musa acuminata* isolates exhibited the highest densities on PDA (50.00 conidia/ml) and CMA (10.88 conidia/ml), while *Cola nitida* (0.90 conidia/ml on PDA) and *Anacardium occidentale* (7.04 conidia/ml on CMA) isolates exhibited the lowest densities.

Regarding conidia dimensions, the average length ranged from 8.30 to 18.29  $\mu\text{m}$  on PDA and from 4.81 to 16.37  $\mu\text{m}$  on CMA, with the largest conidia observed in *Hevea brasiliensis* isolates and the smallest in *Cola nitida* and *Anacardium occidentale* isolates. The average conidia width followed the same trend, ranging from 5.18 to 10.53  $\mu\text{m}$  on PDA and from 2.95 to 9.74  $\mu\text{m}$  on CMA.

#### 3.3.3. Diversity and proportion of spore color

The diversity and proportion of spore colors in *Lasiodiplodia* sp. isolates are presented in Table 5. Two main colors were observed: brown and hyaline. The mean conidia color ranged from 0.00 to 1.25, with significant differences between isolates on PDA and CMA. On PDA, the mean color ranged from 0.44 in *Cola nitida* to 1.25 in *Hevea brasiliensis*, while on CMA, it ranged from 0.22 in *Anacardium occidentale* to 1.27 in *Hevea brasiliensis*. *Hevea brasiliensis* isolates exhibited the highest mean color on both media, while *Cola nitida* and *Anacardium occidentale* isolates showed the lowest values.

Regarding spore proportions, *Hevea brasiliensis* and *Mangifera indica* isolates showed a high proportion of brown and hyaline conidia on PDA, with brown conidia being predominant. On CMA, *Polyalthia longifolia* and *Mangifera indica* isolates produced mainly hyaline conidia, while *Hevea brasiliensis* isolates showed the highest percentage of brown conidia. Finally, some isolates (*Theobroma cacao*, *Polyalthia longifolia*, *Cola nitida*) produced more hyaline conidia on CMA than on PDA. These results show that the type of medium significantly influences the staining and spore proportions of the isolates.

Table 4: Spore density and conidia dimensions *Lasiodiplodia* sp. depending on different cultures on different culture media

Host plants	DSps_PDA	DSps_CMA	LonC_PDA	LonC_CMA	LarC_PDA	LarC_CMA
<i>Anacardium occidentale</i>	8.80±2.21a	0.90±0.78a	15.09 ± 0.65 b	4.81 ± 0.60a	8.87±0.38cd	2.95±0.36a
<i>Cola nitida</i>	7.04±2.52a	1.06±0.89a	8.30 ± 0.74a	5.83 ± 0.69a	5.18±0.44a	3.78 ± 0.41ab
<i>Hevea brasiliensis</i>	23.16±3.57b	5.66 ± 1.26 b	18.29±1.05c	16.37±0.98b	10.53±0.62d	9.74±0.58c
<i>Mangifera indica</i>	10.10±3.16a	1.15±1.12a	17.56±0.93c	7.62 ± 0.86a	10.34±0.55d	4.83 ± 0.52 b
<i>Musa acuminata</i>	50±5.05c	10.88±1.79c	12.56±1.49ab	7.81±1.38a	7.982 ± 0.88 cd	4.63 ± 0.83ab
<i>Polyalthia longifolia</i>	10.14 ± 5.36ab	2.05±1.90ab	11.24±1.58ab	5.99±1.47a	6.61 ± 0.94bc	3.37 ± 0.88ab
<i>Theobroma cacao</i>	14.23 ± 3.57ab	12.06±1.26c	10.32±1.05a	5.99±0.98a	5.89 ± 0.62ab	3.48 ± 0.58ab
<b>F</b>	<b>12.0010</b>	<b>14.8229</b>	<b>17.5146</b>	<b>18,1444</b>	<b>15,4699</b>	<b>17,4298</b>
<b>Pr &gt; F</b>	<b>&lt; 0.0001</b>					

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. DSps: Spore density, LonC: Conidia length, LarC: Conidia width.

Table 5: Diversity and proportion of conidia color *Lasiodiplodia* sp depending on different cultures on different culture media

Host plants	CouC_PDA	CouC_CMA	PCHy_PDA	PCHy_CMA	PCBr_PDA	PCBr_CMA
<i>Anacardium occidentale</i>	0.93±0.04b	0.22 ± 0.05a	4.20 ± 1.11a	0.08±1.06a	59.65±2.71c	16.93±2.37a
<i>Cola nitida</i>	0.44±0.05a	0.34±0.06a	3.13 ± 1.27a	5.12 ± 1.21ab	30.15±3.10a	19.87±2.71a
<i>Hevea brasiliensis</i>	1.25±0.07c	1.27±0.09c	16.64 ± 1.79 b	7.79 ± 1.71ab	61.13±4.38c	64.42 ± 3.84 b
<i>Mangifera indica</i>	1.21 ± 0.06c	0.77±0.08b	16.31±1.58b	8.69 ± 1.52 b	61.94±3.88c	26.08±3.39a
<i>Musa acuminata</i>	0.77 ± 0.10 b	0.41 ± 0.13a	3.70±2.54a	0.00 ± 2.43a	51.85 ± 6.20 bc	33.33 ± 5.43a
<i>Polyalthia longifolia</i>	0.81 ± 0.10 b	0.49 ± 0.14ab	1.43 ± 2.69a	11.62 ± 2.57 b	48.56±6.57bc	13.37±5.76a
<i>Theobroma cacao</i>	0.76±0.07b	0.47±0.09a	4.73±1.79a	6.12 ± 1.71ab	40.51 ± 4.38ab	21.65±3.84a
<b>F</b>	<b>21,3937</b>	<b>17,3932</b>	<b>14,3703</b>	<b>6,7765</b>	<b>12,3838</b>	<b>21,2358</b>
<b>Pr &gt; F</b>	<b>&lt; 0.0001</b>					

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test., CouC: Colour of conidia, PCHy: Proportion of hyaline conidia, PCBr: Proportion of brown conidia.

### 3.3.4. Diversity and proportion of spore shape

The diversity of conidia shapes, according to whether the tip was pointed or rounded, ranged from 0.34 to 1.88, generally higher on PDA than on CMA (Table 6). Isolates from *Hevea brasiliensis* exhibited the greatest diversity, while those from *Cola nitida* and *Anacardium occidentale* had the lowest values.

Ellipsoid conidia were predominant across all media, followed by ovoid and oblong conidia. Ovoid conidia were most common in *Hevea brasiliensis* (32.36% on PDA; 30.50% on CMA), while oblong conidia were frequent in *Musa acuminata* (8.67% on PDA) and *Polyalthia longifolia* (8.94% on CMA). The highest proportion of ellipsoid conidia was observed in *Mangifera indica* on PDA (54.70%) and *Hevea brasiliensis* on CMA (33.20%).

### 3.3.5. Diversity and proportion of spore type

Table 7 presents the number of cells in the conidia of *Lasiodiplodia* sp. isolates, which are either unicellular or bicellular. The diversity of spore types ranged from 0.22 to 1.18, with a general dominance of bicellular conidia over PDA and CMA.

The mean number of cells was highest in *Hevea brasiliensis* on CMA (1.08) and in *Mangifera indica* on PDA (1.11), while the lowest values were observed in *Cola nitida* on PDA (0.38) and *Anacardium occidentale* on CMA (0.22). The proportion of unicellular conidia was highest in *Mangifera indica* (22.39% on PDA; 13.04% on CMA) and lowest in *Musa acuminata* (0% on CMA) and *Polyalthia longifolia* (1.14% on PDA). Conversely, bicellular conidia predominated in *Hevea brasiliensis*, while their proportion was lowest in *Theobroma cacao*. Some isolates (*Cola nitida*, *Polyalthia longifolia*, *Theobroma cacao*) produced more unicellular conidia on CMA than on PDA, suggesting that culture medium influenced spore cell type.

**Table 6: Diversity and proportion of conidia shape *Lasiodiplodia* sp. depending on different cultures on different culture media**

Host plants	AsCi_PDA	AsCi_CMA	PCOV_PDA	PCOV_CMA	PCOb_PDA	PCOb_CMA	PCEl_PDA	PCEl_CMA
<i>Anacardium occidentale</i>	1.35±0.06bc	0.34±0.05a	9.60±1.45c	1.94±1.10c	3.13 ± 0.58a	0.79±0.50a	51.08±2.38ab	14.28±2.08c
<i>Cola nitida</i>	0.54±0.07a	0.38±0.06a	7.48±1.66c	7.38 ± 1.25 b	3.22 ± 0.066a	0.81±0.57a	22.61±2.72c	16.79±2.38c
<i>Hevea brasiliensis</i>	1.86 ± 0.10d	1.88 ± 0.08 b	32.36±2.35a	30.50 ± 1.78a	4.88±0.93a	8.51 ± 0.81b	40.58 ± 3.85b	33.20±3.36a
<i>Mangifera indica</i>	1.46±0.09c	0.43 ± 0.07a	20.93±2.08b	9.05 ± 1.57 b	2.70 ± 0.83a	1.63±0.71a	54.70±3.40a	18.87±2.97bc
<i>Musa acuminata</i>	1.30 ± 0.15bc	0.69±0.12a	20.66 ± 3.32b	2.16±2.51bc	8.67±1.32b	0.12±1.15a	26.23 ± 5.44c	31.00± 4.76ab
<i>Polyalthia longifolia</i>	0.93 ± 0.16 b	0.53±0.13a	6.76±3.52c	4.64 ± 2.67bc	0.25±1.40a	8.94 ± 1.22b	42.97±5.77ab	12.97±5.05c
<i>Theobroma cacao</i>	1.00 ± 0.10 b	0.55±0.08a	17.66 ± 2.35b	4.30 ± 1.78bc	0.92±0.93a	3.45 ± 0.81a	25.84±3.85c	19.94±3.36bc
F	<b>21,2712</b>	<b>43.3669</b>	<b>17.7107</b>	<b>33.6248</b>	<b>5.1369</b>	<b>18.2834</b>	<b>16.8215</b>	<b>5.2669</b>
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test., AsCi: Conidia appearance, PCOV: Proportion of ovoid conidia, PCOb: Proportion of oblong conidia, PCEl: Proportion of ellipsoid conidia.

**Table 7: Diversity and proportion of conidia type *Lasiodiplodia* sp. from different cultures on different culture media**

Host plants	NCCo_PDA	NCCo_CMA	PCUn_PDA	PCUn_CMA	PCBi_PDA	PCBi_CMA
<i>Anacardium occidentale</i>	0.94±0.04cd	0.22 ± 0.03a	3.64±1.51a	0.08±1.27a	60.18±2.68c	16.93±2.29a
<i>Cola nitida</i>	0.38±0.05a	0.243 ± 0.04a	11.80±1.73ab	12.34 ± 1.45 b	21.52±3.06a	12.65±2.62a
<i>Hevea brasiliensis</i>	1.11±0.07 of	1.08±0.05c	13.09±2.44ab	6.23 ± 2.05a	64.68±4.34c	65.99±3.71c
<i>Mangifera indica</i>	1.18±0.06e	0.30±0.05a	22.39±2.16c	13.04 ± 1.45 b	55.87±3.84bc	21.73 ± 3.28ab
<i>Musa acuminata</i>	0.72 ± 0.10 b	0.41 ± 0.08ab	18.05 ± 3.46 bc	0±2.90a	37.5±6.13b	33.33 ± 5.25 b
<i>Polyalthia longifolia</i>	0.81 ± 0.10bc	0.34±0.03a	1.43 ± 3.67a	1.94±3.07a	48.56±6.51bc	23.05±5.57ab
<i>Theobroma cacao</i>	0.80 ± 0.07bc	0.51 ± 0.05 b	4.32 ± 2.44a	5.72 ± 2.05a	39.98±4.34b	22.05±3.71ab
F	22.0270	30.5419	11.5501	10.3011	19.9922	26.6345
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. NCCo: Number of cells per conidium, PCUn: Proportion of unicellular conidia, PCBi: Proportion of bicellular conidia.

### 3.3.6. Spore density and sizes

Table 8 presents the spore densities and mean conidia sizes of *Lasiodiplodia* sp. isolates according to agroecological zones and culture media. Statistical analysis revealed significant differences between isolates on PDA and CMA ( $p < 0.05$ ). No isolate produced conidia on agar by day 28.

Spore density ranged from 6.56 to 32.45 conidia/ml on PDA and from 0.56 to 8.33 conidia/ml on CMA. Isolates from zone 5 exhibited the highest density on PDA (32.45 conidia/ml), while those from zone I reached the maximum on CMA (8.33 conidia/ml). The lowest densities were observed in isolates from zones III (6.56 conidia/ml) and VI (0.56 conidia/ml) on PDA and CMA, respectively.

The mean conidia length ranged from 8.68 to 17.68  $\mu\text{m}$  on PDA and from 4.17 to 11.22  $\mu\text{m}$  on CMA, while the mean width ranged from 5.26 to 10.26  $\mu\text{m}$  on PDA and from 2.58 to 6.50  $\mu\text{m}$  on CMA. The largest dimensions were recorded in isolates from zones 5 and 2 on PDA and CMA, respectively, and the smallest in isolates from zone IV.

**Table 8: Density and sizes of *Lasiodiplodia* sp. spores depending on the geographical origin of the isolate and host plant**

ZAE	DSps_PDA	DSps_CMA	LonC_PDA	LonC_CMA	LarC_PDA	LarC_CMA
Zone I	11.26 ± 2.80 b	8.33±1.00a	10.77±0.83b	7.61 ± 0.77 b	6.44 ± 0.49 b	4.65 ± 0.46 b
Zone III	6.56 ± 4.86 b	0.8±1.73bc	9.64 ± 1.44 b	6.27 ± 1.35bc	5.95 ± 0.85 b	4.34 ± 0.81 bc
Zone II	14.94±2.43b	3.19 ± 0.86 bc	15.10±0.72a	11.22 ± 0.67a	8.97±0.42a	6.50±0.40a
Zone IV	9.36 ± 3.35 b	5.06 ± 1.20 b	8.68 ± 0.99 b	4.17±0.93c	5.26 ± 0.59 b	2.58±0.56c
Zone V	32.45±3.44a	1.36±1.22bc	17.68±1.02a	5.03 ± 0.95bc	10.26±0.60a	2.84 ± 0.57bc
Zone VI	6.76 ± 2.49 b	0.56±0.89c	15.01±0.74a	5.36± 0.69 bc	8.79 ± 0.43a	3.65 ± 0.41 bc
F	<b>8.4211</b>	<b>8.2396</b>	<b>13.4114</b>	<b>11.7638</b>	<b>11.8669</b>	<b>9.6602</b>
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. DSps: Spore density, LonC: Conidia length, LarC: Conidia width.

### 3.3.7. Diversity and proportion of spore color

Table 9 presents the diversity and proportions of conidia colors in *Lasiodiplodia* sp. isolates according to agroecological zones and culture media. Two main colors were observed: brown and hyaline, with a range of average coloration from 0.57 to 1.16.

Statistical analysis showed significant differences in PDA and CMA ( $p < 0.05$ ). In PDA, the mean color ranged from 0.57 to 1.16, with isolates from zone V exhibiting the highest value and those from zone 3 the lowest. In CMA, values ranged from 0.25 to 0.71, with a maximum for isolates from zone III and a minimum for those from zone V.

Regarding the proportion of conidia, the proportion of hyaline conidia was highest in isolates from zone VI on PDA (11.79%) and zone II on CMA (8%), while isolates from zone V produced no hyaline conidia on CMA. For brown conidia, the highest proportions were observed in isolates from zones 5 (70.10% on PDA) and 2 (39.72% on CMA), while isolates from zones IV and VI had the lowest values (35.12% on PDA and 16.05% on CMA).

**Table 9: Diversity and proportion of conidia color *Lasiodiplodia* sp. depending on the geographical origin of the isolate and host plant**

ZAE	CouC_PDA	CouC_CMA	PCHy_PDA	PCHy_CMA	PCBr_PDA	PCBr_CMA
Zone I	0.58±0.05c	0.49 ± 0.07ab	7.45±1.43b	4.67±1.34a	35.87±3.40c	28.65 ± 3.09 b
Zone III	0.57±0.10c	0.45 ± 0.13ab	0.58 ± 2.48 b	7.99±2.32a	39.41±5.90c	22±5.35bc
Zone II	1.02 ± 0.57ab	0.71±0.06a	6.52 ± 1.24 b	5.27±1.16a	58.43 ± 2.95 b	39.72 ± 2.67a
Zone IV	0.65±0.06c	0.34 ± 0.09ab	3.66 ± 1.71 b	0.49 ± 1.60 b	35.125 ± 4.07c	18.54 ± 3.69 bc
Zone V	1.16±0.07a	0.25±0.09ab	4.97±1.75b	0±1.64b	70.10±4.17a	20±3.78bc
Zone VI	0.95±0.05b	0.51 ± 0.06ab	11.79±1.27a	7.63 ± 1.19a	53.99±3.02b	16.05±2.74c
F	<b>14.2552</b>	<b>4,1861</b>	<b>5.2373</b>	<b>4.6529</b>	<b>13.3561</b>	<b>9.4277</b>
Pr > F	<b>&lt; 0.0001</b>	<b>0.0009</b>	<b>&lt; 0.0001</b>	<b>0.0003</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test., CouC: Colour of conidia, PCHy: Proportion of hyaline conidia, PCBr: Proportion of brown conidia.

### 3.3.8. Diversity and proportion of spore shape

Table 10 presents the diversity and proportion of conidial shapes in *Lasiodiplodia* sp. isolates. Spore tips were pointed or rounded, allowing for the identification of three categories of isolates. Spore diversity ranged from 0.25 to 1.82, being higher on PDA than on CMA. The highest values were observed for isolates from zones V (1.82 on PDA) and 3 (1.10 on CMA), while isolates from zones II and VI exhibited the lowest values.

Regarding the proportion of shapes, ellipsoid conidia consistently predominated across all media and zones. Isolates from zone II exhibited the highest rates of ovoid (20.16% on PDA; 12.53% on CMA) and oblong (5.58% on PDA; 5.19% on CMA) conidia. The highest proportion of ellipsoid conidia was observed on PDA for zone V (54.32%) and on CMA for zone II (27.26%). Oblong conidia were the least frequent, with minimal values in isolates from zones VI and V, and almost absent in some areas on CMA.

These results confirm that, regardless of the agroecological zone or environment, ellipsoid conidia are predominant compared to ovoid and oblong conidia.

**Table 10: Diversity and proportion of conidia shape *Lasiodiplodia* sp. depending on the geographical origin of the isolate and host plant**

ZAE	AsCi_PDA	AsCi_CMA	PCOV_PDA	PCOV_CMA	PCOb_PDA	PCOb_CMA	PCEl_PDA	PCEl_CMA
Zone I	0.9 ± 0.08d	0.60 ± 0.07 b	14.66±1.89ab	9.23 ± 1.49 abc	3.71 ± 0.72ab	2.34 ± 0.65 b	24.96±2.99c	21.69 ± 2.61ab
Zone III	0.70±0.14d	0.40 ± 0.12bc	13.06±3.29ab	12.5±2.58ab	0.138±1.25c	0±1.12b	26.79 ± 5.18c	17.5±4.53ab
Zone II	1.41 ± 0.07b	1.1±0.06a	20.16±1.64a	12.53±1.29a	5.58±0.62a	5.19±0.56a	39.27 ± 2.59 b	27.26±2.26a
Zone IV	0.76 ± 0.10d	0.52 ± 0.08bc	12.92±2.27ab	4.15±1.78cd	0.09±0.86c	0.66 ± 0.77 b	25.07±3.57c	11.37±3.16b
Zone V	1.82 ± 0.10a	0.37±0.08bc	16.28±2.32ab	1.31±1.82d	4.38 ± 0.88ab	0.20 ± 0.79 b	54.32 ± 3.66a	18.47±3.20ab
Zone VI	1.17±0.07c	0.25±0.06	9.71 ± 1.68 b	6.09±1.32 bcd	2.22 ± 0.64bc	2.52 ± 0.57 b	53.90±2.66a	13.81±2.32b
F	<b>17.0448</b>	<b>20.6259</b>	<b>4.2181</b>	<b>7.2282</b>	<b>7.5836</b>	<b>8.3285</b>	<b>18,2822</b>	<b>5.0860</b>
Pr > F	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.0008</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.0001</b>

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test., AsCi: Conidia appearance, PCOV: Proportion of ovoid conidia, PCOb: Proportion of oblong conidia, PCEl: Proportion of ellipsoid conidia.

### 3.3.9. Diversity and proportion of spore type

Table 11 presents the diversity and proportion of cell types of conidia in *Lasiodiplodia* sp. isolates, which were either unicellular or bicellular. Diversity ranged from 0.20 to 1.17, being higher in PDA than in CMA, with a general predominance of bicellular conidia. The mean number of cells per conidium ranged from 0.52 to 1.17 in PDA and from 0.20 to 0.66 in CMA, with the highest values observed for isolates from zones V (PDA) and III (CMA). The proportion of unicellular conidia was highest in isolates from zones I and II on PDA (14.03%) and CMA (14.00%), and lowest in isolates from zones V (0.00% on CMA) and IV (5.70% on PDA). Conversely, bicellular conidia were predominant, with highest proportions in isolates from zone V on PDA (66.17%) and zone II on CMA (40.34%), and the lowest in isolates from zones I (PDA) and VI (CMA).

**Table 11: Diversity and proportion of conidia type *Lasiodiplodia* sp depending on the geographical origin of the isolate and host plant**

ZAE	NCCo_PDA	NCCo_CMA	PCUn_PDA	PCUn_CMA	PCBi_PDA	PCBi_CMA
Zone I	0.59±0.05c	0.41 ± 0.04b	14.03±1.94a	11.10±1.60a	29.23 ± 3.41c	22.22 ± 3.02 b
Zone III	0.52±0.09c	0.27±0.08bc	10.50 ± 3.37ab	14±2.78a	29.41 ± 5.91c	16±5.23b
Zone II	0.96±0.04b	0.66±0.04a	6.92 ± 1.68 b	4.65 ± 1.39 bc	58.07±2.95ab	40.34±2.61a
Zone IV	0.59±0.06c	0.34±0.05bc	5.70 ± 2.32 b	0.34±1.92c	32.39 ± 4.07c	18.71 ± 3.61 b
Zone V	1.17±0.07a	0.25±0.05bc	8.82 ± 2.38ab	0±1.96c	66.17±4.18a	20±3.70b
Zone VI	0.92± 0.05b	0.20±0.04c	13.13 ± 1.73ab	8.22 ± 1.42ab	52.65 ± 3.03b	15.46 ± 2.68 b
F	<b>14.8660</b>	<b>14.5020</b>	<b>2.9126</b>	<b>7.9281</b>	<b>17.4569</b>	<b>11.0164</b>
Pr > F	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.0129</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>

Within the same column, values followed by the same letters are not statistically different at the 5% significance level according to the Newmann-Keuls test. NCCo: Number of cells per conidium, PCUn: Proportion of unicellular conidia, PCBi: Proportion of bicellular conidia.

#### 4. Discussion

The results show that *Lasiodiplodia* sp. isolates exhibit morphological variations depending on the host, geographic origin, and culture medium. The nature and provenance of the explant influence mycelial growth, colony appearance, and the number of pycnidia, confirming the observations of [8], who showed that fungi develop and sporulate differently depending on the medium and the isolate's origin. Media low in carbohydrates but rich in plant compounds can also modulate morphological characteristics [4].

Microscopic analysis revealed that the conidia are initially hyaline, unicellular, ellipsoid to oblong, thick-walled, and granular in content, becoming bicellular, dark brown, with sizes of 17–30 × 6–15 µm and longitudinal striations at maturity, in agreement with [5, 6]. The presence of pycnidial paraphyses and striations distinguishes *Lasiodiplodia* sp. from related genera [12]. However, some isolates produced unicellular or dark-brown conidia without pycnidia, suggesting the existence of different morphotypes influenced by the explant, environment, or isolate's genetics. Spore density varies independently of the number of pycnidia, as reported by [4]. Unlike [5], only unicellular and bicellular conidia were observed here, although aging can produce 1 to 3 septa [17].

Variations in color, shape, and the number of cells per conidia reflect the influence of culture type, medium age, environmental conditions, and agroecological zone, as well as the genetic diversity of the isolates, corroborating the findings of [11]. Overall, morpho-cultural and microscopic parameters vary according to agroecological zones, crops, and environments, reflecting the adaptation of isolates to local conditions and the pathogen's genetic diversity, in agreement with [15, 10]. In Côte d'Ivoire, environmental variations across agroecological zones [3] and cultural factors, such as pH, light, glucose, and temperature, influence the growth and sporulation of isolates [14].

#### 5. Conclusion

This study revealed that *Lasiodiplodia* sp. isolates exhibit a great morpho-cultural and microscopic diversity, influenced by the host, the agroecological zone, and the type of culture medium. The observed variations concern mycelial growth, colony appearance, the number of pycnidia, as well as the density, color, shape, size, and number of conidial cells. Bicellular ellipsoid conidia predominate, although some remain unicellular or dark brown without pycnidia depending on the isolate and the culture conditions. These results highlight the adaptation of the isolates to local conditions and reflect the genetic diversity of the pathogen. Understanding these morphological and microscopic variations is essential for the accurate identification of *Lasiodiplodia* sp. and for the development of suitable management strategies in different crops and agroecological zones. Although characteristics vary according to host species and region, the specificity and pathogenicity of these isolates remain poorly understood. Further research is therefore needed to better understand the pathosystems associated with this fungus.

**Conceptualization:** methodology, experimental design, sample collection, data analysis, and original draft preparation.

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