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RESEARCH ARTICLES

Pathogenicity Evaluation of *Lasiodiplodia* spp. as the Causal Agent of Cashew Gummosis (*Anacardium occidentale* L.) in Côte d'Ivoire

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Abstract

Cashew gummosis is an emerging phytopathological threat showing an expanding distribution across Côte d'Ivoire. Elucidating the primary etiological agent is a critical prerequisite for developing targeted disease-management strategies. To this end, a total of 105 fungal isolates comprising the genera *Lasiodiplodia*, *Pestalotia*, and *Ceratocystis*, which were recovered from symptomatic gummosis lesions were evaluated.Two distinct inoculation protocols were used: mechanically wounded and non-wounded cortical tissue applications. The bioassays showed that macroscopic symptoms developed exclusively following the wounded inoculation protocol, confirming that structural tissue compromise was necessary for fungal entry. Furthermore, symptoms typical of gummosis were confined to seedlings inoculated with *Lasiodiplodia* isolates. Successful re-isolation of the fungus from newly induced lesions satisfied Koch's postulates, confirming that this genus is the causative agent of cashew gummosis in Côte d'Ivoire. Agglomerative hierarchical cluster analysis based on virulence parameters categorized the pathogenic *Lasiodiplodia* population into three distinct phenotypic clusters. Consequently, this study establishes *Lasiodiplodia* spp. as the principal pathotype driving cashew gummosis in Côte d'Ivoire and highlights intrageneric pathogenic diversity within the genus.

Keywords: agroecological zones, cashew, pathogenicity, virulence

Introduction

The cashew tree (*Anacardium occidentale* L.), native to tropical America, is a perennial plant in the family Anacardiaceae (Dendena & Corsi, 2014). Within the genus *Anacardium*, this plant is the only species cultivated for economic purposes. The cashew nut is the most economically and nutritionally important part of the plant (Dendena & Corsi, 2014).

The cashew tree was introduced to Africa by Portuguese explorers, and its cultivation is now practiced in several countries across Africa,

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Asia, and the Americas (Dendena & Corsi, 2014). The cashew tree was introduced in Côte d'Ivoire in the 1950s and 1960s as part of a reforestation and soil conservation strategy in savannah zones (Lebailly et al., 2012). Since 2015, Côte d'Ivoire has been the world's leading producer of cashew nuts. In 2023, the country produced more than 1 million tons of raw cashew nuts, accounting for about 25% of global production (Food and Agriculture Organization of the United Nations, 2023). In Côte d'Ivoire, cashews have become the country's second most important agricultural product, second only to cocoa (Babatunde et al., 2023).

Despite its importance to the Ivorian economy, cashew nut production is threatened by numerous diseases and pests (Silué et al., 2017). Gummosis is among the most damaging diseases in cashew plantations in Côte d'Ivoire. It occurs on the trunks and branches of cashew trees and can affect more than 50% of the cashew trees in a plantation. Gummosis is characterized primarily by the oozing of gum from infected areas. The underlying infected tissues appear dark and cracked. Sap flow can be interrupted when the conducting vessels become obstructed. This causes leaf yellowing and eventual defoliation, or the death of affected trees (Cissé et al., 2024). Gummosis is also among the most damaging diseases (Muntala et al., 2020; Wonni et al., 2017). In Brazil, yield losses caused by this disease can reach 30% (Coutinho et al., 2017). Gummosis is most often attributed to *Lasiodiplodia* spp., which are ascomycete fungi in the family Botryosphaeriaceae (Phillips et al., 2013). *Lasiodiplodia* spp. cause vascular diseases by developing in the xylem vessels and producing enzymes to kill their hosts' tissues (Obrador-Sánchez & Hernandez-Martínez, 2020). *Lasiodiplodia* species are characterized by rapid growth on PDA culture medium. The mycelium develops and completely covers the Petri dish within three days (Rangel-Montoya et al., 2021). Unlike in Burkina Faso, where studies have identified six species of *Lasiodiplodia* associated with cashew exudation disease (Dianda et al., 2025), diagnostic survey conducted in Côte d'Ivoire by Cissé et al. (2024) identified three genera of fungi associated with this cashew disease. These were the genera *Lasiodiplodia*, *Pestalotia*, and *Ceratocystis*. However, no pathogenicity tests were conducted in that study, even though such tests are essential, according to Koch's postulates, for determining whether an organism is responsible for the observed pathology. This study was conducted to identify the pathogens responsible for cashew gummosis in Côte d'Ivoire and to assess their diversity. It used an approach based on pathogenicity and virulence tests of isolates of *Lasiodiplodia*, *Pestalotia*, and *Ceratocystis* on cashew trees.

Materials and Methods

Plant Material

The plant material consisted of 12-month-old cashew seedlings of the 'Tout venant' genotype. Two-month-old seedlings were transplanted from the nursery to a new site. The seedlings remained there until they were 12 months old. These seedlings were used for pathogenicity and virulence testing.

Fungal Material

The fungal material consisted of three genera: *Lasiodiplodia*, *Pestalotia*, and *Ceratocystis*. These fungi were isolated from gummosis samples in the study by Cissé et al. (2024). The fungal genera were isolated in the following proportions: 100 *Lasiodiplodia* isolates, 2 *Pestalotia* isolates, and 3 *Ceratocystis* isolates.

Culture of Fungal Isolates

The fungal isolates associated with cashew gummosis in Côte d'Ivoire were subcultured on potato dextrose agar (PDA) medium supplemented with 0.25 g/L of chloramphenicol. The inoculated Petri dishes were then sealed and incubated at laboratory ambient temperature, which ranged from 25 to 28 °C (Dembélé et al., 2020). The morphology of the isolates was observed on Petri plates over four days to ensure

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their purity. Pure cultures of fungi were used for pathogenicity and virulence testing. The fungi used in this study were identified by Cissé et al. (2024) using the identification guide by Barnett and Hunter (1972).

Pathogenicity and Virulence Tests of Fungal Isolates

In vivo pathogenicity assays were conducted on 12-month-old cashew seedlings maintained under a controlled environment in a screenhouse, with a mean recorded temperature of 29.35 °C and an average relative humidity of 78%. The bioassays were conducted at the Jean Lorougnon Guédé University, experimental facility (Latitude: 6°54'35'' N; Longitude: 6°26'14'' W). The inoculated parts of the cashew trees consisted of branches and trunks. The types of inoculation used were wounded and non-wounding inoculation. Inoculation began by disinfecting the inoculation sites with 70% ethanol, as shown in Figure 1a. Wounded inoculation involved creating a wound with a knife, as illustrated in Figure 1b. The wounds were inoculated after placing 5 mm diameter mycelial discs (176.7 mm^3), taken from four day-old pure cultures, as shown in Figure 1c. To prevent moisture loss during the infection process, the inoculation site was covered with cotton wool moistened with sterile distilled water and then secured with plastic wrap, as shown in Figures 1d–1e (Ismail et al., 2012). The plastic wrap and cotton were removed 14 days after inoculation. Non-wounded inoculation was performed by placing mycelial discs directly on the bark without prior wounding.The control cashew trees were inoculated with plain PDA disks.

The trials were conducted in a completely randomized design, with four inoculated cashew trees per isolate. The parameters used to assess isolate virulence were incubation period, length, width, and observed lesion area. With the exception of the incubation period, which was recorded throughout the trials, the other parameters were measured 30 days after inoculation using a measuring tape. The extent of the lesions was estimated using the following formula:

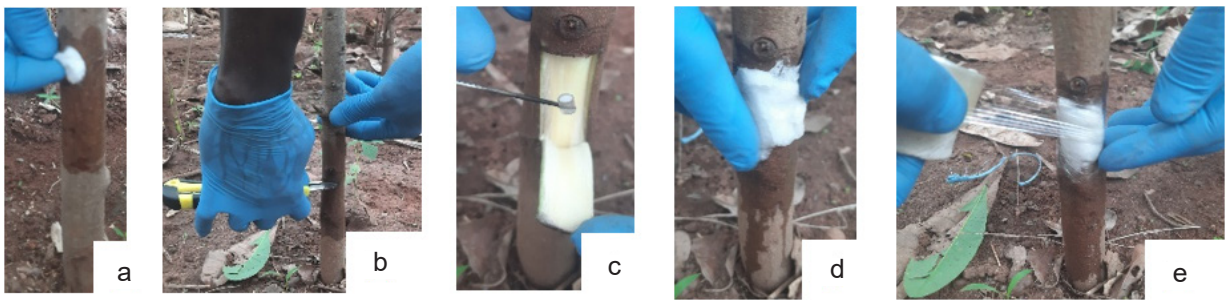
Area = length × width

Multivariate Analysis and Distribution of Pathogenic Fungal Isolates of Cashew

The multivariate analysis was conducted to assess diversity among fungal isolates causing cashew gummosis. This diversity was assessed by considering the virulence parameters of the isolates, as determined in this study: incubation period, symptom length, width, and area after inoculation. The multivariate analyses performed included principal component analysis (PCA) followed by agglomerative hierarchical clustering (AHC). The classes resulting from the AHC were characterized, and the specific isolates for each class were identified. The distribution of fungal

Figure 1

Steps in Wounded Inoculation of a Cashew Trunk under Shelter



Note. a = disinfection of the trunk, b = creating a wound, c = depositing the inoculum in the wound, d = covering the inoculation point with damp cotton, e = ligating the inoculation point with stretch film.

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isolates causing cashew gummosis was mapped across the agroecological zones. QGIS version 3.16.4 was used for this purpose, taking into account the longitude and latitude of the orchard of origin for each isolate.

Statistical Analysis

Statistical analysis was performed using R software version 4.3.2. (R Core Team, 2023). Data on the virulence of fungal isolates were subjected to the Shapiro-Wilk and Levene tests to assess normality and homogeneity of variance, respectively. Subsequently, an analysis of variance (ANOVA) was conducted for each parameter; when significant differences were detected, the Student-Newman-Keuls post hoc test was used to compare means. Multivariate analysis was performed using the FactorMineR package. The HCPC function in this package was used to perform agglomerative hierarchical clustering of the isolates.

Results and Discussion

Pathogenicity of Fungi Associated with Cashew Gummosis

Pathogenicity tests of isolates of *Lasiodiplodia*, *Pestalotia*, and *Ceratocystis* revealed that only *Lasiodiplodia* isolates caused symptoms on inoculated cashew seedlings after wounding. The virulence of *Lasiodiplodia* isolates may stem from their ability to neutralize antimicrobial compounds. As Rangel-Montoya et al. (2023) pointed out, the production of suberin, lignin, and phytoalexins by the host plant to compartmentalize the pathogen is likely insufficient against *Lasiodiplodia*. The pathogen's melanin may neutralize the host's antimicrobial activity and further degrade phenolic compounds. The symptoms that appeared, as shown in Figure 2, were gum exudation on the branches and stems, the presence of brown and necrotic lesions around the inoculation sites and in the vicinity of the pith, and wilting of the cashew trees. Previous studies are consistent with these results. These symptoms include bark necrosis, vascular discoloration, defoliation, apical dieback, and gummosis (Endes & Kayim, 2022; Verma et al., 2021). The genus *Lasiodiplodia* has been associated with various diseases in many plant species worldwide. It has been reported to cause leaf spot on *Cynometra malaccensis* in Thailand (Gomdola et al., 2020), on apple trees in China, causing trunk and branch cankers (Sha et al., 2021), on mango, causing post-harvest stem-end rot (Gariba et al., 2025). The gum production observed during the pathogenicity test represents a tree response. Indeed, Fontaine et al. (2015), in their study on vine physiology, observed that plants attempted to resist pathogens associated with esca disease and vine decline by establishing biochemical barriers. These substances, in the form of tyloses and gummosis, block the plant's vascular system to limit the spread of hyphae.

The fungi re-isolated from the symptoms observed on the inoculated plants initially exhibited white aerial mycelium that later turned black, and they grew rapidly on PDA medium. Additionally, the conidia were hyaline when immature and, when mature, brown, with one septum, thick walls, and longitudinal striations. These morphological characteristics are identical to those of the parental *Lasiodiplodia* isolates used during inoculation. Thus, Koch's postulates were fulfilled, confirming that *Lasiodiplodia* spp. are responsible. This result is consistent with the findings of several previous studies. Indeed, Coutinho et al. (2017) and Dianda et al. (2025) associated *Lasiodiplodia* spp. with cashew gummosis in Brazil and Burkina Faso, respectively.

There was no gum production and no lesion formation after inoculation without wounding. However, *Lasiodiplodia* spp. were re-isolated from asymptomatic green-branch explants. The re-isolation of the fungus from asymptomatic explants following inoculation without wounding suggests that the fungus can enter the plant by means other than mechanical wounds and remain latent for a period. This result is consistent with the findings of several authors. According to Abbas (2018), plant pathogens enter through wounds or lenticels

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on branches, twigs, and the trunk. Moreover, this result shows the endophytic nature of the fungus. According to Cardoso et al. (2009), fungi in the genus *Lasiodiplodia* can persist in a plant without apparent symptoms for some time and eventually become pathogenic when conditions become favorable. These findings suggest that growers should minimize wounds because trees that appear healthy are potential sources of the disease.

Virulence and Distribution of *Lasiodiplodia* Isolates by Agroecological Zone of Cashew Cultivation in Côte d'Ivoire

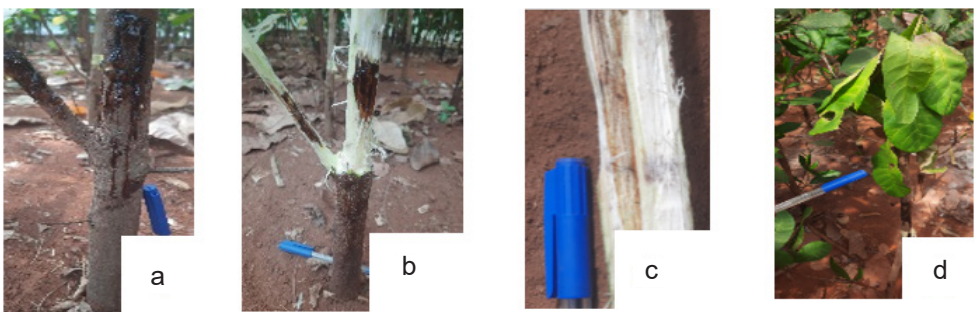
Evaluations conducted following inoculations revealed that the virulence of *Lasiodiplodia* isolates on cashew seedlings varied from one isolate to another, as well as according to the agroecological zones of origin, as illustrated in Table 1. The variation in virulence indicates genetic or physiological differences among isolates. *Lasiodiplodia theobromae* and *Lasiodiplodia hormozganensis* have been shown to be more virulent than other species (Munirah et al., 2017; Netto et al., 2014).

More *Lasiodiplodia* isolates were obtained in agroecological zones II and VI. Analysis of variance revealed that the agroecological zone of origin does not influence the virulence of *Lasiodiplodia* isolates responsible for cashew gummosis in Côte d'Ivoire. Indeed, no significant differences were observed ($p > 0.05$) among the agroecological zones for each of the four virulence parameters of the isolates, namely the length, width, surface area of canker lesions, and incubation duration. The aggressiveness of the isolates appears to be related to the fungi's intrinsic nature. Indeed, according to Li et al. (2014), the severity of gummosis caused by *Lasiodiplodia* spp. on peach trees depends on the fungal species and the season of infection.

The dendrogram from agglomerative hierarchical clustering, shown in Figure 3, revealed three classes of *Lasiodiplodia* infecting cashew trees in Côte d'Ivoire. All four variables determined in this study allowed the isolates to be discriminated. The overall means were 4.62, 1.37, 18.73, and 26.71 for the incubation period, width, length, and lesion area, respectively. Class 1 comprised 48% of the isolates and contained the least virulent isolates. These isolates had shorter incubation periods and smaller lesion widths, lengths, and areas than the overall means. Typical isolates of this class are P-GU681, H-BE221, H-ZO231, Br-BA562, and T-DI678. Class 2 consisted of six isolates, or 6%. The specific isolates in this class were H-TO671, H-BE219, M-BO26, H-BO236, and Ge-BO553. This group is characterized by isolates with a long incubation period. Finally, class 3 comprised 46 isolates (46%) that caused cankerous lesions. The isolates in this class were more virulent on cashew seedlings. The typical isolates of this

Figure 2

Characteristic Symptoms of Gummosis Observed on Cashew Plants Starting 14 Days After Inoculation



Note. a = gum dripping from the trunk and branch 14 days after inoculation, b = cankers at the inoculation sites, c = vascular browning, d = wilting of an inoculated cashew tree.

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class were Bg-KO690, Bn-NO7702, W-MO556, M-GO25, and P-LA686. The pathogenic groups shown in Figure 4 are not associated with a specific agroecological zone. However, class 2, which contains the fewest isolates (6), was observed only in agroecological zones II, V, and VI. Classes 1 and 3 were distributed similarly within each agroecological zone. In some cases, two or all three pathotypes co-occured within the same plantation. These results are similar to those of Dianda et al. (2025), who identified six species of *Lasiodiplodia*, namely *L. euphorbicola*, *L. brasiliense*, *L. theobromae*, *L. crassispora*, *L. pseudotheobromae*, and *L. caatinguensis*, as the

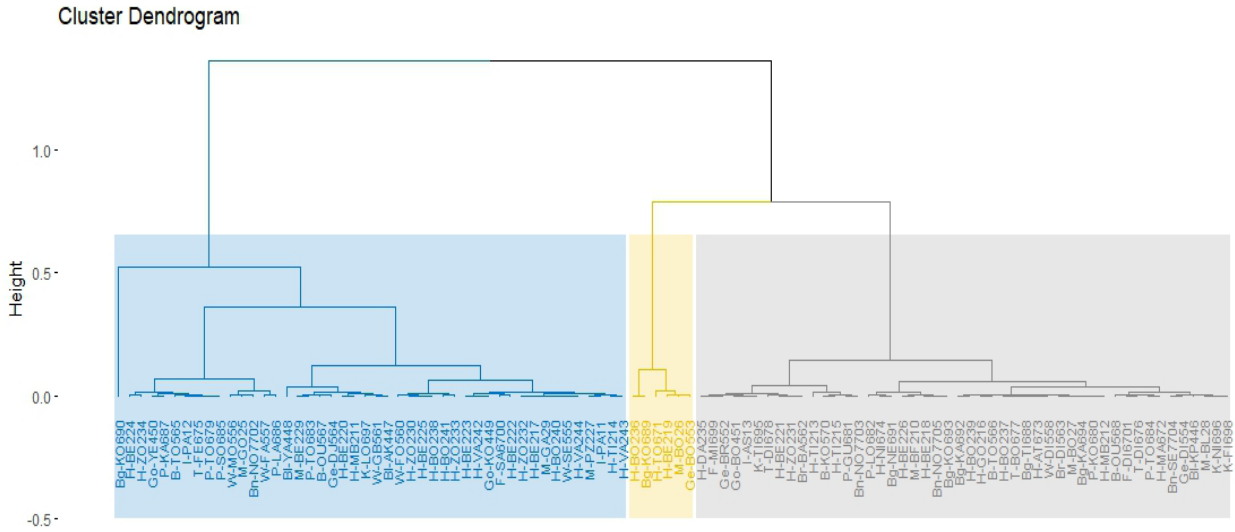
Table 1

Variation in Virulence Parameters of Lasiodiplodia Isolates by Agroecological Zone of Origin

Agroecological zone (AEZ)	Number of isolates	Lesion length (cm)	Lesion width (cm)	Lesion area (cm2)	Incubation period (days)
AEZ I	3	18.99 ± 4.69	1.42 ± 0.14	28.49 ± 8.45	3.67 ± 0.33
AEZ II	39	18.42 ± 0.90	1.40 ± 0.03	26.89 ± 1.77	4.31 ± 0.65
AEZ IV	6	21.77 ± 2.91	1.31 ± 0.10	28.73 ± 3.86	5.17 ± 0.83
AEZ V	18	19.99 ± 1.77	1.37 ± 0.05	28.98 ± 3.25	4.88 ± 0.82
AEZ VI	30	17.70 ± 1.21	1.36 ± 0.09	24.24 ± 2.18	4.86 ± 0.63
AEZ VII	4	19.06 ± 5.07	1.29 ± 0.17	28.93 ± 12.42	4.50 ± 0.96
Average		18.73 ± 0.66	1.37 ± 0.03	26.71 ± 1.23	4.62 ± 0.35
F value		0.5473	0.1654	0.4218	0.1782
P value		0.74	0.9747	0.8325	0.9702

Figure 3

Dendrogram Showing Similarity among Lasiodiplodia Isolates Based on Phytopathological Characteristics

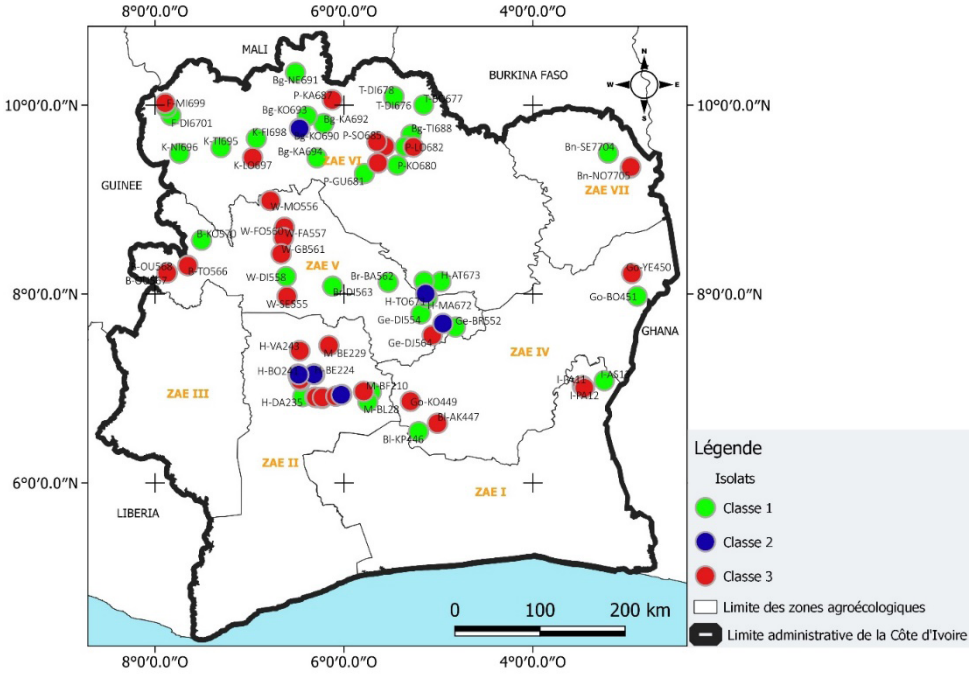


Note. Names of *Lasiodiplodia* isolates: the letters before the hyphen designate the letters or initials of the name of the region, the letters after the hyphen designate the initials of the name of the locality, the first number indicates the zone number and the other number(s) indicates the isolate number.

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Figure 4

Distribution of Lasiodiplodia Pathotypes Across Cashew-Production Agroecological Zones in Côte d'Ivoire



agents of gummosis and dieback. Thus, several *Lasiodiplodia* species may be responsible for cashew gummosis in Côte d'Ivoire, as illustrated by the dendrogram obtained on the basis of isolate virulence. However, molecular diagnostic studies could confirm or refute this.

Conclusions

This is the first study in Côte d'Ivoire to characterize and differentiate the fungal pathogens causing cashew gummosis. The findings substantiate the etiological role of *Lasiodiplodia* spp. in the development of gummosis lesions in *Anacardium occidentale* orchards across the region. Because symptom development requires physical tissue compromise, implementing strict phytosanitary measures specifically, mitigating mechanical wounding during routine grove maintenance is paramount if smallholders are to reduce fungal infection rates. Establishing localized epidemiological surveillance networks is critical for early disease intervention. The highly aggressive *Lasiodiplodia* strains identified in this phenotypic profiling provide valuable material for marker-assisted breeding of gummosis-resistant cultivars. These isolates also provide a standardized pathological baseline for screening sustainable, low-impact fungicidal controls. While this work clarifies genus-level dynamics, high-resolution multi-locus sequence typing is warranted to resolve the precise delimitation among *Lasiodiplodia* species responsible for agricultural gummosis across Côte d'Ivoire.

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