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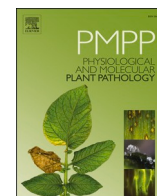
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Phylogeny and pathogenicity of *Lasiodiplodia* species associated with gummosis and dieback of cashew (*Anacardium occidentale* L.) in Burkina Faso

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ABSTRACT

Cashew (*Anacardium occidentale* L.) production in Burkina Faso is limited by dieback and gummosis disease, caused by *Botryosphaeriaceae* fungi. This study aimed to characterize *Lasiodiplodia* species associated with diseased cashew trees in Burkina Faso. Molecular analysis and pathogenicity tests of 37 fungal isolates revealed *Lasiodiplodia euphorbicola*, *Lasiodiplodia brasiliense*, *Lasiodiplodia theobromae*, *Lasiodiplodia crassispora*, *Lasiodiplodia pseudotheobromae*, and *Lasiodiplodia caatinguensis*, pathogenic to cashew leaves *in vitro* and capable of inducing dieback symptoms on cashew and mango (*Mangifera indica* L.) seedlings. The diversity of pathogenic species and their virulence on multiple hosts underscore the urgency of implementing effective management strategies in Burkina Faso orchards.

1. Introduction

Cashew (*Anacardium occidentale* L.) is a nut crop of considerable economic importance, cultivated in various tropical regions and exported to Europe and the USA for consumption. The cashew fruit consists of an edible hypocarp (cashew apple) topped by a drupe, from which the seed (nut) develops. In Burkina Faso, fruit crops, including cashew, play a critical role in the agricultural sector [1]. Cashew cultivation was promoted as part of governmental efforts to combat desertification, now covering 70 % of the area dedicated to fruit cultivation. This represents approximately 255,000 ha and engages nearly 60,000 producers. In 2022, Burkina Faso produced 145,000 tons of cashew nuts [2], making it the third most significant agricultural export after cotton and sesame. The cashew industry substantially contributes to the country's socio-economic development, generating over 117.11 billion FCFA annually. However, the sector faces critical phytosanitary challenges, including gummosis and dieback of cashew plants, which have been reported since 2018. Cashew gummosis is a severe phytosanitary issue that leads to significant yield losses and tree mortality in several

countries. Symptoms include cankers forming along trunks or branches, which exude a resin-like gum over time (Fig. 1).

In Brazil's North-Eastern region, this disease has caused up to 30 % stand losses [3]. In Nigeria, cashew dieback results in up to 70 % yield reductions and tree mortality rates exceeding 50 % [4].

Fungal pathogens from the Botryosphaeriales order, such as *Lasiodiplodia*, *Neofusicoccum*, and *Pseudofusicoccum* species, have been associated with cashew gummosis in Brazil and Guinea Bissau [5,6]. Botryosphaeriaceae are widespread latent endophytes that cause dieback, canker, and fruit rot on stressed plant hosts. For example, in Burkina Faso, mango trees (*Mangifera indica* L.) also suffers dieback caused by *Lasiodiplodia* spp. and the incidence and severity of the disease were positively correlated with lower rainfall and higher temperatures [7]. Using a specific polyclonal serum and microscopic analyses, Muniz et al. [8] showed that the fungus *Lasiodiplodia theobromae* primarily colonize cashew xylem vessels. Extensive hyphal development associated to cell wall degradation was recorded in the secondary xylem parenchyma. Importantly, *L. theobromae* hyphae were detected in the xylem vessels of asymptomatic samples [8]. These fungi may therefore

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spread via asymptomatic contaminated transplants, pruning tools, and grafting. Additionally, in Cameroon, cashew gummosis was found to spread rapidly during the rainy season, likely through waterborne transmission [9].

Managing cashew gummosis in commercial orchards relies on prophylactic measures, fungicide applications, and genetic resistance. In Brazil, the center of origin for cashew, efforts to identify resistant clones have yielded promising results, particularly through screening dwarf and wild-common cashew varieties [10,11]. Despite these advances, the disease remains a significant global threat, reducing the economic viability of cashew orchards and affecting both small-scale and large-scale producers.

This study aimed to identify the causal agents of cashew gummosis and dieback in the Western regions of Burkina Faso, focusing on the prevalence and pathogenicity of *Lasiodiplodia* species. Previous studies have highlighted the dominance of *Lasiodiplodia* spp. among Botryosphaeriaceae associated with cashew gummosis [5]. Identification of *Lasiodiplodia* species was conducted using micro- and macro-morphological features, pathogenicity tests and molecular sequence data, particularly the translation elongation factor 1-alpha gene (*tef1-a*) and the rDNA internal transcribed spacer region (ITS). In addition, because mango dieback is prevalent in the same geographical areas, fungi isolated from cashew were tested for pathogenicity against mango plantlets. This work provides insights into the diversity and pathogenic potential of *Lasiodiplodia* species affecting cashew and mango cultivation in Burkina Faso.

2. Material and methods

2.1. Surveys and sample collection

Samples from diseased cashew trees were collected during field surveys carried out between March and May 2021 and in May 2022 in cashew-producing localities of the Western provinces of Burkina Faso: Houet, Kénédougou, and Komoé (Fig. 2). When possible, the disease incidence and severity were recorded in localities sampled as described in Dianda et al. [12].

Within each orchard, samples were taken from 10 diseased cashew

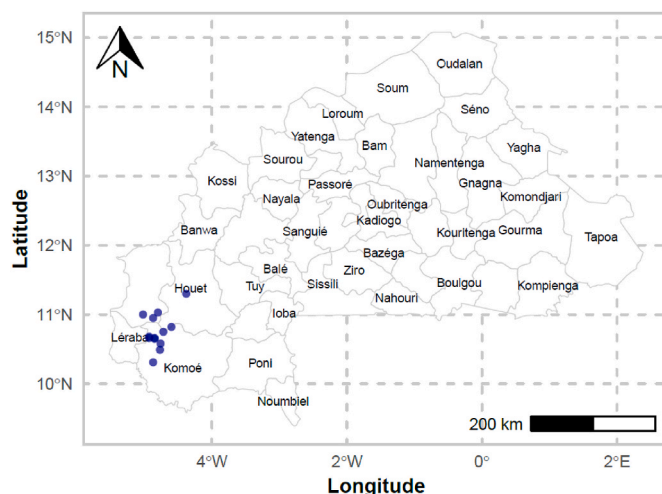


Fig. 2. Map of Burkina Faso indicating the localities (blue dots) and provinces where field surveys and sampling were conducted.

trees chosen at random along diagonals. The samples consisted of leaves, twigs, or roots collected from randomly selected trees showing the characteristic symptoms of dieback. They were placed in paper envelopes bearing the collection number, the date, the name and the geographical coordinates of the locality. Samples were transported and stored at 4 °C in the phytopathology laboratory of INERA (Institute of the Environment and Agricultural Research) at Farako-bâ for the inventory of associated fungal species.

2.2. Fungal isolation

Symptomatic samples were washed with tap water. Pieces from the edges of the symptomatic and symptomless tissues were cut and surface-sterilized with 70 % ethanol for 30 s followed by 1 % NaOCl for 1 min, rinsed in sterile distilled water for 30 s and incubated at room temperature (25–28 °C) on a moistened blotting paper into Petri dishes.

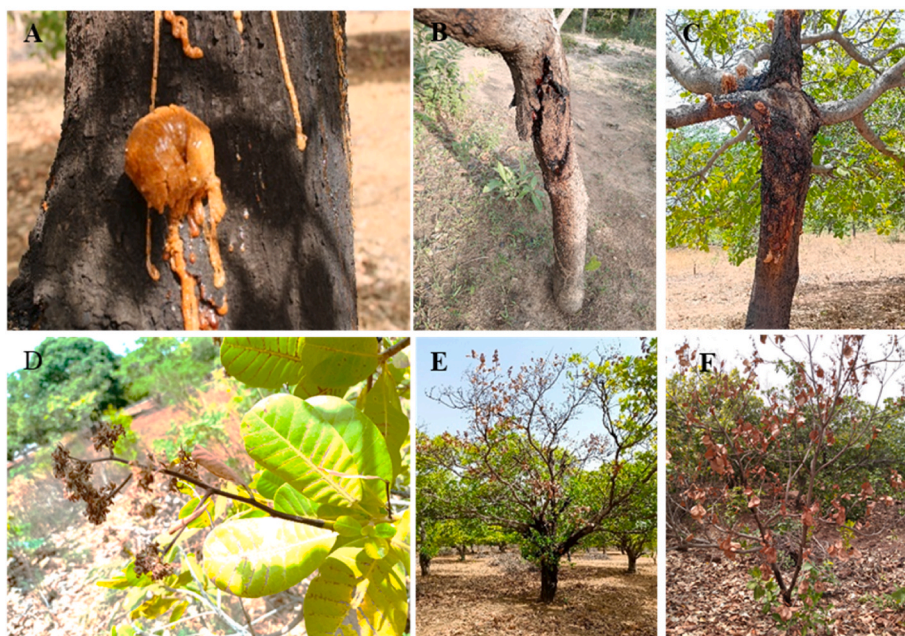


Fig. 1. Symptoms of dieback on cashew trees in orchards of Burkina Faso. A: exudation of gum along the trunk; B: bark cracking of trunk; C: canker along the trunk and branches; D: scorch-like appearance developed on infected leaf, dying twig and inflorescence blight; E: gradual drying out of branches; F: drying of whole canopy.

Eventually, hyphal tips of the fungal isolates were transferred onto fresh Potato Dextrose Agar (PDA) to obtain pure cultures.

2.3. Growth and morphology of *Lasiodiplodia* spp. isolates

Colony features and pigmentation were observed after seven days incubation on PDA, while the growth rate of each isolate was measured in triplicate after 72 h of incubation on PDA plates at 25 °C. Production of conidia was obtained by transferring a plug of 7-days mycelia culture on disinfested (as described above) cashew leaves maintained on 1 % water agar. The characteristics of conidia (shape, size, color, longitudinal striations and presence or absence of septum) were observed after three weeks of incubation in a 12^h photoperiod. Fifty conidia were measured for each isolate using a light microscope (Euromex, The Netherlands) with a micrometer under 400× magnification.

2.4. DNA extraction and PCR

DNA analyses were performed at the International molecular biology platform INERA/IRD PathoBios (Bobo Dioulasso, Burkina Faso) and at IRD (Montpellier, France). The total genomic DNA was extracted using a rapid extraction protocol modified from Groppe and Boller [13] and suitable for amplification of genomic sequences by Polymerase Chain Reaction (PCR) [14]. A small amount of fresh mycelium was scraped from the PDA surface of a 5-days fungal culture, placed in a sterile 1.5 mL microcentrifuge tube containing 50 µl of freshly prepared 0.5 M sodium hydroxide (NaOH) and grinded with a disposable pestle mounted on an electric homogenizer (Dutscher, France). The suspension was centrifuged for 5 min at 12,000 rpm. 5 µl of the supernatant was transferred to a fresh tube containing 45 µl of Tris-HCl 100 mM pH 8.0 and immediately used for PCR or stored at −20 °C. The ITS and *tef1-α* partial gene sequences of fungal isolates were amplified by PCR using universal fungal primers ITS4/ITS5 [15] and EF1-688-F/EF1-1251-R [16], respectively. Each PCR reaction was performed in a total reaction volume of 25 µl including 5 µl of 5× Green GoTaq buffer, 2 µl of deoxynucleotide triphosphate polymerase (dNTPs) (5 mM), 1 µl of each primers (10 µM), 0.125 µl of GoTaq G2 polymerase (Promega, France), 14.875 µl of sterile Milli-Q water, and 2 µl of genomic DNA. PCR was realized with a MyCycler™ Thermal Cycler following these different steps: initial denaturation at 94 °C for 2 min, then 30 cycles of denaturation at 94 °C for 30 s (ITS) and 95 °C for 30 s (*tef1-α*), annealing at 58 °C for 30 s (ITS)/55 °C for 45 s (*tef1-α*), elongation at 72 °C for 30 s (ITS)/90 s (*tef1-α*), and final elongation at 72 °C for 8 min (ITS)/10 min (*tef1-α*). The PCR products were separated by electrophoresis for 20 min at 100 V in a 1 % agarose gel stained with ethidium bromide. The gel was viewed and photographed using the Bio-Rad Molecular Imager® Gel Doc™ XR System and Bio-Rad Quantity One® Software. The size of the amplified PCR products was ascertained by utilizing a 100 bp GeneRulers™ DNA ladder (Promega). PCR products were sent to Genewiz/Azenta company for DNA sequencing in both directions.

2.5. Sequence alignment and phylogenetic analyses

Raw ITS and *tef1-α* sequences were trimmed, aligned, and manually adjusted, and consensus sequences were generated in Geneious (version 8.1.8 created by Biomatters, available from <http://www.geneious.com>) (Kearse et al., 2012). The genetic relatedness of the ITS and *tef1-α* of isolates sequences with other fungal sequences in the GenBank database was determined using the nucleotide Basic Local Alignment Search Tool (BLASTN) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) with default parameters. Unique ITS rDNA and *tef1-α* sequences obtained for representative isolates were deposited at GenBank under accession numbers PQ843375-843380 (ITS) and PV016865-016870 (*tef1-α*) (Suppl. Table 1). ITS and *tef1-α* datasets from well characterized isolates (type collection) of *Lasiodiplodia* spp. retrieved in GenBank [14,17] were used to assess the phylogenetic positions of cashew isolates. ITS rDNA and

tef1-α sequences of *Diplodia mutila* (strain CMW7060) were used as outgroup. Prior to phylogenetic analysis, ambiguous sequences at the start and end were deleted in order to optimize the alignments. Multiple sequence alignments of cashew isolates and reference type collection sequences were generated using the default settings of the ClustalW algorithm [18] in MEGA 11 software [19]. Alignment parameters were applied to find the best nucleotide substitution model in MEGA 11 with the Bayesian Information Criteria (BIC) and Akaike Information Criteria (AIC). Individual data sets (ITS rDNA and *tef1-α*) were analysed with the topological congruency test using Concaterpillar with $P = 0.05$ as cut off. Phylogenetic analysis was conducted using the maximum likelihood (ML) method. The Nearest Neighbor Interchange (NNI) heuristic method was used for trees inference. The initial tree was automatically generated using the Maximum Parsimony method. No branch swap filter was selected. Bootstrap values for branch support were determined using 1,000 replications. All sites were included in the analysis.

2.6. Pathogenicity tests of *Lasiodiplodia* spp. isolates on detached leaves

Lasiodiplodia isolates were tested for pathogenicity on detached cashew leaves under controlled laboratory conditions. Healthy cashew leaves of the same size and age were selected for the inoculation, surface disinfested by 10 % house bleach solution (3–5 % sodium hypochlorite) for 2 min then washed in sterile distilled water and left to dry in the air. Five leaves were used for each isolate. Leaves were wounded in one point on the upper surface of leave midrib by pressing slightly using a sterile needle [20]. For inoculation, a mycelium-colonized PDA plug (5 mm) collected from the actively growing margins of 7 days-old PDA cultures was placed on the wound point. Non-colonized PDA plugs served as control. Two perpendicular lines following the length and width of the leaves were drawn on the lid of the dish containing the detached leaves to measure the size of the lesions. Petri dishes were incubated at 28 °C in the laboratory an observations were made after 3, 5 and 7 days to monitor the appearance of necrosis. The average diameter (D) of the leaf lesions in each dish was evaluated through the measurement of the lesion lengths d1 and d2 at the two perpendicular lines and using the formula: $D = \frac{(d1+d2)}{2}$

The final average and standard deviation (SD) were further calculated for each isolate from the average diameters of lesions in the five boxes tested.

2.7. Pathogenicity tests of *Lasiodiplodia* spp. isolates on cashew and mango plantlets

The apical stem region of one year-old cashew or mango (cv. Amélie) plantlets were surface-sterilized with 70 % ethanol, and mechanical wounding was applied with sterilized scalpels. Plugs (5 mm in diameter) of *Lasiodiplodia* spp. mycelia were collected from the actively growing margins of 7 days-old PDA cultures and applied on the plant wounded area. The inoculation area was then wrapped with cotton and parafilm as described in Rodríguez-Galvez et al. [21]. Control plantlets were treated with sterile PDA plugs. Four plantlets were tested for each fungal isolate. All cashew and mango plantlets were further maintained in a greenhouse with a photoperiod extended to 15 h under fluorescent lights at 28 °C, and were examined for disease symptoms for 42 days. To satisfy Koch's postulates, pieces of inoculated organs were removed from sites showing disease symptoms at 5 dpi, surface sterilized as mentioned above and plated on PDA. An isolate was considered pathogenic when it was able to cause disease symptoms and further isolated from the inoculated plant.

2.8. Statistical analysis

Pathogenicity data of *Lasiodiplodia* isolates were checked for normal distribution and homogeneity, running Shapiro-Wilk and Levene tests,

respectively. A series of analysis of variance (ANOVA) tests were performed, and in case of significant differences, means were separated using Tukey's test ($P < 0.05$). All analysis were performed in R (R Core Team, 2021).

3. Results

3.1. Surveys and isolate collection

Surveys of diseased orchards were carried out in the main cashew-producing localities of Burkina Faso (Fig. 2). Characteristic disease symptoms included gummosis and bark cracking, canker on trunk, dying of twigs from tips to back, gradual drying out of branches, scorch-like appearance of leaves, inflorescence blight, drying of entire canopy (Fig. 1). Depending on locality, incidence varied between 52 % and 62 % and severity between 38.4 % and 49.6 % (Suppl. Table 2). Based on spore and mycelial characteristics, eight genera of fungi (*Lasiodiplodia* sp. *Pestalotiopsis* sp. *Colletotrichum* sp. *Alternaria* sp. *Curvularia* sp. *Fusarium* sp. *Botrytis* sp and *Aspergillus* sp) were inventoried from samples retrieved in orchards. *Lasiodiplodia* isolates were prevalent in samples, with 37 isolates of *Lasiodiplodia* spp. obtained from cashew exhibiting gummosis and dieback symptoms (Table 1).

3.2. Morphology and cultural characteristics

All isolates were characterized based on mycelial and conidial

Table 1

List of 37 *Lasiodiplodia* isolates obtained from symptomatic cashew trees in different Burkina Faso localities and molecular classification based on ITS and *tef1-α* sequences obtained in this study. Isolates in bold letters were chosen as representative isolate for each species.

Isolate code	Phylogenetically closest species	Province	Locality	Organ
LA17	<i>L. caatinguensis</i>	Komoé	Diarabakoko	Leaf
LA32	<i>L. crassispora</i>	Komoé	Lonassi	Leaf
LA4	<i>L. crassispora</i>	Komoé	Lafermé	Leaf
LA34	<i>L. euphorbicola</i>	Komoé	Diarabakoko	Twig
LA29	<i>L. euphorbicola</i>	Komoé	Lafermé	Twig
LA26	<i>L. euphorbicola</i>	Komoé	Lonassi	Leaf
LA28	<i>L. euphorbicola</i>	Komoé	Bakorani	Leaf
LA22	<i>L. euphorbicola</i>	Komoé	Diarabakoko	Leaf
LA13	<i>L. euphorbicola</i>	Komoé	Djolena	Twig
LA3	<i>L. euphorbicola</i>	Komoé	Lafermé	Twig
LA14	<i>L. euphorbicola</i>	Komoé	Lonassi	Leaf
LA24	<i>L. euphorbicola</i>	Komoé	Toumousseni	Twig
LA25	<i>L. euphorbicola</i>	Komoé	Toumousseni	Leaf
LA52	<i>L. euphorbicola</i>	Houet	Bama	Leaf
LA66	<i>L. euphorbicola</i>	Houet	Bama	Root
LA69	<i>L. euphorbicola</i>	Houet	Bama	Root
LA73	<i>L. euphorbicola</i>	Houet	Bama	Twig
LA44	<i>L. euphorbicola</i>	KénéDougou	Diéri	Leaf
LA42	<i>L. euphorbicola</i>	KénéDougou	Orodara	Leaf
LA23	<i>L. pseudotheobromae</i>	Komoé	Diarabakoko	Twig
LA35	<i>L. pseudotheobromae</i>	Komoé	Toumousseni	Twig
LA5	<i>L. theobromae</i>	Komoé	Lafermé	Twig
LA27	<i>L. theobromae</i>	Komoé	Lafermé	Twig
LA1	<i>L. theobromae</i>	Komoé	Djolena	Leaf
LA2	<i>L. theobromae</i>	Komoé	Djolena	Twig
LA40	<i>L. theobromae</i>	Houet	Kaka	Twig
LA56	<i>L. theobromae</i>	Houet	Bama	Twig
LA9	<i>L. brasiliense</i>	Komoé	Bakorani	Twig
LA31	<i>L. brasiliense</i>	Komoé	Djolena	Twig
LA30	<i>L. brasiliense</i>	Komoé	Kossara	Twig
LA36	<i>L. brasiliense</i>	Komoé	Kossara	Twig
LA33	<i>L. brasiliense</i>	Komoé	Toussiana	Leaf
LA18	<i>L. brasiliense</i>	Komoé	Diarabakoko	Leaf
LA37	<i>L. brasiliense</i>	Komoé	Diarabakoko	Leaf
LA8	<i>L. brasiliense</i>	Komoé	Takeledougou	Twig
LA16	<i>L. brasiliense</i>	Komoé	Toumousseni	Leaf
LA7	<i>L. brasiliense</i>	Komoé	Toussiana	Twig

characteristics. Growth was rapid for all isolates on PDA, with the maximum radial growth of 8.5 cm obtained at 5 days after incubation (DAI). Aerial mycelium was initially white, then turning dark greenish grey or greyish 14 DAI at 25 °C in the dark (Fig. 3).

Conidia exhibited the typical features of the genus *Lasiodiplodia*, such as hyaline conidia when immature, and brown with 1-septum, thick walls and longitudinal striations when mature (Fig. 4). Conidia size varied depending on isolates (Suppl. Table 3). The lowest length ($20.73 \pm 2.31 \mu\text{m}$) was recorded for isolate LA25. The highest length ($25.75 \pm 2.77 \mu\text{m}$) was noted in isolate LA32. Concerning mean widths, the lowest values were recorded in LA14 ($12.55 \pm 1.12 \mu\text{m}$) and the highest in LA32 ($16.17 \pm 1.44 \mu\text{m}$). All these morphological characteristics were similar to *Lasiodiplodia* species [5]. The minimum (min) and maximum (max) of the conidial measurement (min-max length and min-max width) and the ratio of average length to average width (L/W) are presented in Table 2 for each species further determined with molecular analyses.

3.3. DNA sequencing and molecular characterization

ITS rDNA is commonly employed for taxonomic characterization in fungi, including the genus *Lasiodiplodia*. However, ITS data alone are insufficient for distinguishing among *Lasiodiplodia* species. Integrating ITS with other nuclear loci enhances species discrimination within this genus [16,22]. We utilized the ITS and the partial *tef1-α* gene sequences to characterize the 37 fungal isolates (Table 1). High-quality consensus sequences were obtained for both the ITS region (average length of 528 bp, ranging from 429 to 549 bp) and the *tef1-α* region (average length of 485 bp, ranging from 418 to 548 bp). Alignment of ITS sequences separated the isolates into five groups, while the *tef1-α* sequences enabled further separation into six groups. Sequences in each group had 100 % identity. A phylogenetic analysis was inferred from the concatenated ITS + *tef1-α* sequences of six representatives isolates (LA5, LA9, LA17, LA23, LA32 and LA34; Suppl. Table 1) and 37 *Lasiodiplodia* species representatives by using the Maximum Likelihood method and Kimura 2-parameter model [23] in MEGA11 [19]. ITS rDNA and *tef1-α* sequences of *Diplodia mutila* (strain CMW7060) were used as outgroup. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1732)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 38.00 % sites). There were a total of 821 positions in the final dataset, with 197 variables and 97 sites that were parsimony informative. The resulting phylogenetic tree showed that cashew isolates clustered with relatively high bootstrap (60–100 %) with six *Lasiodiplodia* species: *Lasiodiplodia euphorbicola* (16 isolates), *Lasiodiplodia brasiliense* (10 isolates), *L. theobromae* (6 isolates), *Lasiodiplodia crassispora* (2 isolates), *Lasiodiplodia pseudotheobromae* (2 isolates), and *Lasiodiplodia caatinguensis* (1 isolate) (Fig. 5 and Table 1). This result shows that *Lasiodiplodia* isolates from diseased cashew trees in Burkina Faso are genetically diversified and belong to different species. Fungal genetic diversity was observed at a small geographical scale, with up to four species identified in Diarabakoko (six isolates analysed), and three species (four isolates analysed) in Djolena, Lafermé, and Toumousseni localities (Table 1).

3.4. Pathogenicity of *Lasiodiplodia* isolates on cashew detached leaves

Pathogenicity tests were successfully conducted for 34 *Lasiodiplodia* isolates on cashew detached leaves maintained *in vitro*. Inoculation of *Lasiodiplodia* isolates caused symptoms on detached *A. occidentale* leaves. Symptoms induced were brown necrotic lesions at the site of inoculation that appeared within 3 days after inoculation (DAI) (Fig. 6). Induced lesions progressed rapidly and may cover the entire leaf by 7 days, giving the appearance of rot. In addition, a whitish deposit with occasional blackish dots corresponding respectively to the mycelium

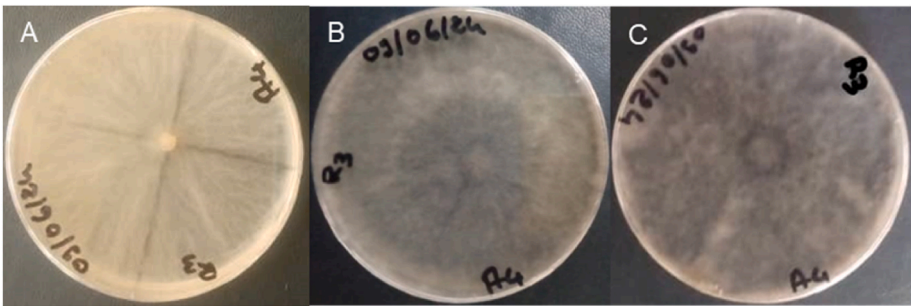


Fig. 3. Photographs of a *Lasiodiplodia* culture (isolate LA28) on PDA plates after 5 days (A), 7 days (B); and 14 days (C).

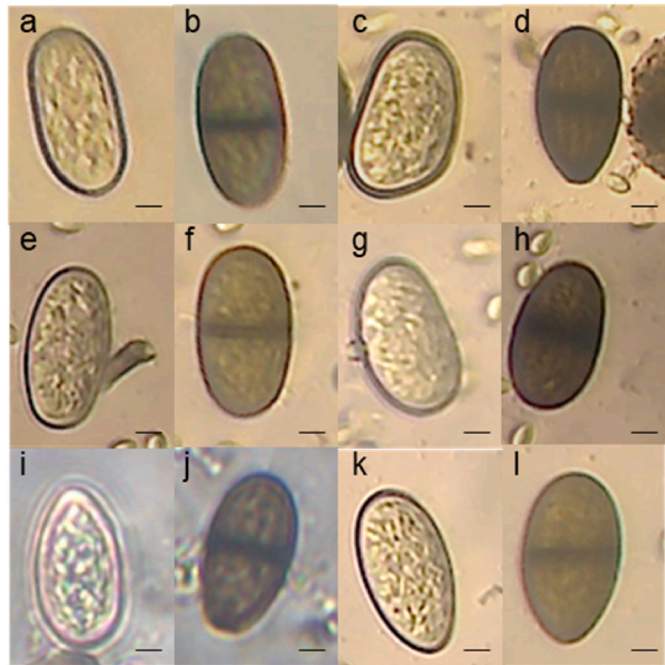


Fig. 4. Photographs of immature (left) and mature (right) conidia of *L. caatinguensis* (a, b), *L. crassisporea* (c, d), *L. brasiliense* (e, f), *L. euphorbicola* (g, h), *L. pseudotheobromae* (i, j), and *L. theobromae* (k, l) observed under a light microscope. Bars = 5 μ m.

Table 2
Conidia size of *Lasiodiplodia* spp. isolated from cashew in Burkina Faso.

Phylogenetic group ^a	Minimum - maximum conidia length (L) x width (W) (μ m)	L/W
<i>L. caatinguensis</i>	23.96 $\times$ 13.60	1.8
<i>L. crassisporea</i>	25.7–26.9 $\times$ 15.7–16.1	1.6
<i>L. brasiliense</i>	23.7–27.3 $\times$ 13.6–15.4	1.8
<i>L. euphorbicola</i>	20.7–24.3 $\times$ 12.5–15.0	1.7
<i>L. pseudotheobromae</i>	24.2–26.7 $\times$ 14.3–14.4	1.8
<i>L. theobromae</i>	23.8–25.3 $\times$ 14.1–14.6	1.7

^a As determined with ITS and *tef1*- α sequences.

and acervuli of the fungus were observed at 7 DAI. A necrotic spot (0.5 cm length) occurred in mock control leaves from 3 DAI and did not expand further (Fig. 6A).

Necrotic spots were measured at 3, 5 and 7 DAI and the last are presented in Table 3. Cashew fungal isolates induced necrotic lesions on leaves extending from (mean diameter $\pm$ SD) 1.8 $\pm$ 0.2 cm (LA23) to 6.2 $\pm$ 0.3 cm (LA69) at 7 DAI. The statistical analysis showed that 18 of 34 isolates displayed significant difference in lesion diameter as compared to the control ($F = 6.554$, $p = 5.34 \times 10^{-12}$) at 7 DAI. No difference was

found between *Lasiodiplodia* isolates of different species as determined by the molecular analysis (Table 1).

3.5. Pathogenicity of *Lasiodiplodia* isolates from Burkina Faso on cashew seedlings

Pathogenicity tests were successfully conducted for 35 *Lasiodiplodia* isolates on cashew seedlings. All *Lasiodiplodia* isolates tested induced various symptoms characteristic of dieback on inoculated cashew plants, including internal necrotic lesions, cankers, leaf necrosis and apical dieback (Fig. 7).

The incidence of diseased plants varied between 0 and 100 % depending on the strain of *Lasiodiplodia* inoculated and type of symptoms (Table 4). Only two isolates did not induce internal discoloration on inoculated cashew (LA14 and LA23) after seven weeks of inoculation. Control plants did not develop symptom.

No strict correspondence could be established between pathogenicity data obtained on detached leaves (Table 3) and plantlets (Table 4). All the isolates found non pathogenic on leaves induced symptoms on cashew plantlets.

3.6. Pathogenicity test of *Lasiodiplodia* spp. isolated from cashew on mango plantlets

In order to check if isolates of *Lasiodiplodia* spp. from cashew were pathogenic on mango, a subset of isolates from 5 *Lasiodiplodia* species was selected and inoculated on mango plantlets. Strains of all *Lasiodiplodia* species caused dieback symptoms, including internal lesions on mango plants (Fig. 8). The size of the internal lesion measured after seven weeks varied between 6.4 $\pm$ 1.6 cm to 13.0 $\pm$ 2.8 cm depending on the isolate and differed significantly from the control ($F = 8.76$, p -value = 1.47×10^{-6}) (Table 5).

4. Discussion

This is the first study of *Lasiodiplodia* species associated with cashew (*A. occidentale*) dieback in Burkina Faso supported by morphological, molecular and pathogenicity data. Based on phylogenetic analyses, the 37 *Lasiodiplodia* isolates from cashew displayed close relationships to six species, including *L. caatinguensis*, *L. crassisporea*, *L. brasiliense*, *L. euphorbicola*, *L. pseudotheobromae* and *L. theobromae* (Fig. 5 and Table 1). The species were named following the recent genus revision that identified hybrid species based to incongruences displayed between phylogenies inferred from different loci [22,24]. The species *L. brasiliense* is considered as hybrid that could have arisen from hybridisation between *L. theobromae* and another currently unknown species [24]. These authors recommend using several nuclear loci for new *Lasiodiplodia* species delimitation, including ITS, *tef1*- α , *rpb2* and *cmdA*. However, ITS and *tef1*- α are adequate to separate between *L. caatinguensis*, *L. crassisporea*, *L. brasiliense*, *L. euphorbicola*, *L. pseudotheobromae* and *L. theobromae*.

These *Lasiodiplodia* species were previously reported to cause gummosis or/and dieback of *A. occidentale* in northeastern Brazil, Guinea

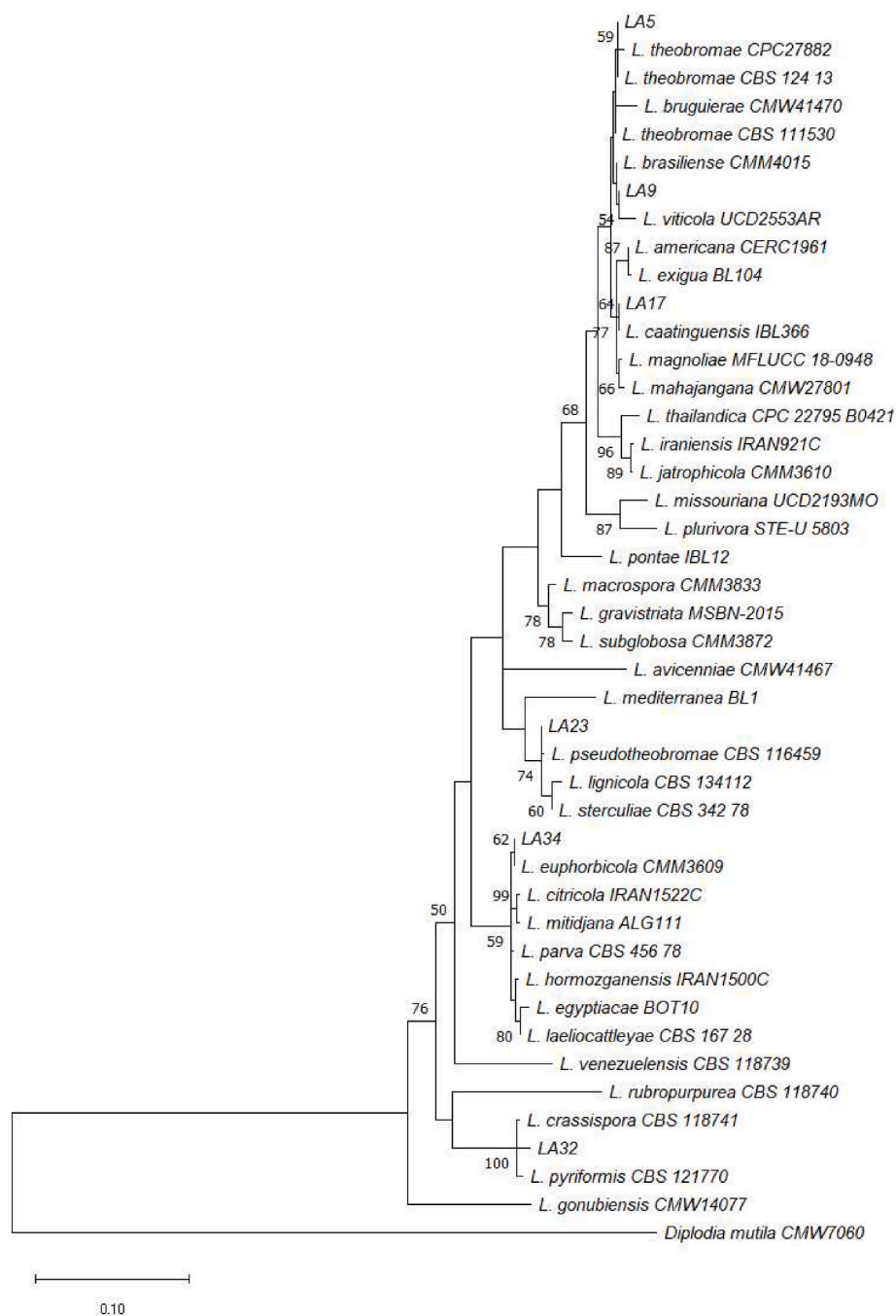


Fig. 5. Phylogenetic tree resulting from the combined analysis of ITS and *tef1- α* sequence data of six representatives cashew isolates (LA5, LA9, LA17, LA23, LA32 and LA34) and 37 *Lasiodiplodia* species representatives. The tree with the highest log likelihood (−3266.80) is shown. The percentage of trees (bootstrap values above 70 %) in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. ITS rDNA and *tef1- α* sequences of *Diplodia mutila* (strain CMW7060) were used as outgroup.

Bissau, and Panama [5,25–27]. Prior to this study, these *Lasiodiplodia* species were described on mango trees in Burkina Faso [7] and Ivory Coast [14]. The different *Lasiodiplodia* species were distributed in all regions sampled, with the predominance of *L. euphorbicola* (16 isolates) and *L. brasiliense* (10 isolates). Up to four distinct species could be found in a single locality (Table 1), indicating that dieback may be caused by different *Lasiodiplodia* isolates even at a small geographic scale.

The study highlighted a high incidence (up to 62 %) and severity of gummosis and dieback symptoms in surveyed cashew orchards, suggesting a possible severe impact on cashew productivity in Burkina Faso without targeted mitigation strategies. This aligns with global reports of *Lasiodiplodia*-induced diseases, demonstrating its adaptability to diverse

hosts and climatic conditions. In general, *Lasiodiplodia* species, such as *L. theobromae*, have a wide distribution and host range, and have been reported on woody species cultivated under temperate to tropical and subtropical climates [16,20,27–32,36]. The fungus is endophytic and able to adapt to and exploit stressed host plants under local environmental stressors such as prolonged droughts and elevated temperature. For instance, the higher prevalence of mango dieback in some regions of Burkina Faso could be attributed to changes in bioclimatic factors [7].

The identification of multiple *Lasiodiplodia* species reflects the genetic and phenotypic diversity of this fungal genus in cashew orchards, critical for designing effective control strategies. Pathogenicity tests confirmed the ability of *Lasiodiplodia* species to cause severe necrotic

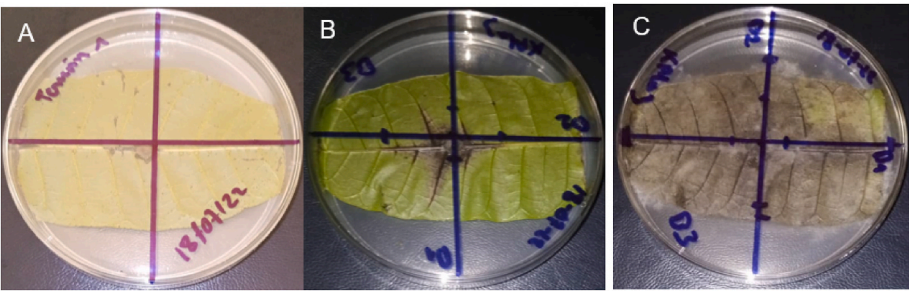


Fig. 6. Necrotic symptoms on cashew leaves inoculated with *Lasiodiplodia* isolates. A: control 3 days after inoculation (DAI); B: Brown necrosis at 3 DAI; C: Maximum evolution of necrotic lesions at 7 DAI.

Table 3
Average diameters of lesions induced by strains of *Lasiodiplodia* from Burkina Faso on detached cashew leaves at 7 days after inoculation.

Isolate	Phylogenetically closest species ^a	Mean diameter $\pm$ SD ^b (cm)	Significance letters ^c
LA7	<i>L. brasiliense</i>	5.7 $\pm$ 0.5	bd
LA8	<i>L. brasiliense</i>	3.3 $\pm$ 0.8	aefgh
LA16	<i>L. brasiliense</i>	5.4 $\pm$ 0.5	abcdefgh
LA18	<i>L. brasiliense</i>	2.4 $\pm$ 0.6	abcdeg
LA30	<i>L. brasiliense</i>	3.7 $\pm$ 0.6	acdefgh
LA31	<i>L. brasiliense</i>	4.6 $\pm$ 1.1	abcdefgh
LA33	<i>L. brasiliense</i>	3.1 $\pm$ 1.4	bcdeg
LA36	<i>L. brasiliense</i>	2.6 $\pm$ 0.4	abcdefgh
LA37	<i>L. brasiliense</i>	2.1 $\pm$ 0.2	acdefgh
LA17	<i>L. caatinguensis</i>	5.2 $\pm$ 0.2	abcdefg
LA4	<i>L. crassispota</i>	4.5 $\pm$ 1.6	bcde
LA32	<i>L. crassispota</i>	5.4 $\pm$ 0.9	acdefgh
LA13	<i>L. euphorbicola</i>	5.4 $\pm$ 0.8	bcde
LA14	<i>L. euphorbicola</i>	1.9 $\pm$ 0.1	afh
LA22	<i>L. euphorbicola</i>	3.0 $\pm$ 1.0	abcdefg
LA24	<i>L. euphorbicola</i>	5.8 $\pm$ 0.5	fh
LA25	<i>L. euphorbicola</i>	2.0 $\pm$ 0	abcdefgh
LA26	<i>L. euphorbicola</i>	1.9 $\pm$ 0.1	aefgh
LA28	<i>L. euphorbicola</i>	2.7 $\pm$ 0.4	afgh
LA29	<i>L. euphorbicola</i>	2.4 $\pm$ 0.6	abcdefg
LA34	<i>L. euphorbicola</i>	5.7 $\pm$ 0.1	c
LA42	<i>L. euphorbicola</i>	5.6 $\pm$ 0.4	aefgh
LA44	<i>L. euphorbicola</i>	5.9 $\pm$ 0.2	bcd
LA52	<i>L. euphorbicola</i>	3.8 $\pm$ 1.5	bcd
LA66	<i>L. euphorbicola</i>	2.0 $\pm$ 0.1	abcdefgh
LA69	<i>L. euphorbicola</i>	6.2 $\pm$ 0.3	b
LA73	<i>L. euphorbicola</i>	3.0 $\pm$ 0.2	abcdefgh
LA23	<i>L. pseudotheobromae</i>	1.8 $\pm$ 0.2	bcde
LA35	<i>L. pseudotheobromae</i>	5.4 $\pm$ 0.7	bcd
LA1	<i>L. theobromae</i>	5.5 $\pm$ 0.8	abcdefg
LA2	<i>L. theobromae</i>	2.6 $\pm$ 0.4	abcdefgh
LA5	<i>L. theobromae</i>	2.1 $\pm$ 0.1	abcdefg
LA27	<i>L. theobromae</i>	3.4 $\pm$ 1.8	bcd
LA40	<i>L. theobromae</i>	5.1 $\pm$ 0.8	abcdefgh
Control		0.5 $\pm$ 0.1	h

^a As determined with ITS and *tef1-α* sequences.
^b SD: standard deviation; n = 5 leaves.
^c Mean values that have the same significant letters are not different.

lesions, cankers, and dieback on cashew plants, validating their role as primary causal agents. These findings align with previous research demonstrating that inoculating host plants with *Lasiodiplodia* species can lead to a range of external and internal symptoms, including bark necrosis, vascular discoloration, defoliation, apical dieback, and gummosis [21,33–35]. Moreover, the ability of *Lasiodiplodia* spp. isolated from *A. occidentale* to induce similar symptoms on mango plants underscores the cross-host potential of these pathogens. This finding raises concerns about the broader agricultural implications, as *Lasiodiplodia* species could pose a threat to multiple economically important crops in Burkina Faso, exacerbating crop losses.

The pathogenicity data presented in this study do not allow for

definitive conclusions regarding the aggressiveness of certain *Lasiodiplodia* species on cashew or mango plantlets. This limitation arises from the methodology employed, as mycelial plugs from freshly grown isolates on PDA were used, whereas calibrated spore suspensions are generally preferred for an accurate assessment of fungal aggressiveness. The cultivation of the fungus for spore production necessitates the presence of plant material under specific light and temperature conditions, and the process can take several weeks to yield conidiomata and spores [28,36]. Consequently, conducting pathogenicity tests with multiple strains, as done in this study, was challenging. Some isolates exhibited low sporulation rates, substantial delays in sporulation, or produced an insufficient number of spores, further complicating the experimental procedures. For these reasons, studies dealing with *Lasiodiplodia* population analyses preferentially use mycelial plugs in pathogenicity tests [5,21,42,43]. Spore suspensions are applied to a limited number of isolates or when describing a new *Lasiodiplodia* species [37,38].

Lasiodiplodia is a widespread fungus able to infect different plant and fruit tissues, with a wide host range [36], but the mechanisms by which it invades woody plants and establishes itself inside the vessels remain unclear. *Lasiodiplodia* spores can be dispersed at short distance by wind or rain splash [39]. Uncertainty also surrounds the roles of seed transmission, pruning wounds in disease epidemiology, and the potential for the fungus to be spread by bark-colonizing insects (beetles), as evidenced for mango and fig (*Ficus carica*) wilt diseases caused by different fungi [40,41]. Once entered in the plant, *Lasiodiplodia* hyphae colonizes the xylem vessels by extensive hyphal development and further distribution along the secondary xylem associated to parenchymatic cell wall disintegration [8]. Fighting an endophytic fungus that colonizes plant vessels is challenging because these fungi reside within the plant tissues, making them difficult to target through external treatments like fungicides. This survey in Burkina Faso suggests that existing management practices may be insufficient to mitigate the spread and impact of *Lasiodiplodia*-induced diseases. While resistant clones have been identified in other regions (e.g., Brazil) [11], their effectiveness in Burkina Faso agroecological conditions remains uncertain. This underscores the importance of localized breeding programs to develop resistant varieties tailored to regional climates. Furthermore, prevention of pathogen dissemination through contaminated plant material in nurseries highlights the need for stringent quarantine measures and best practices in orchard management. Expanding surveys to other South and Center Western Burkinabé regions and crops will help to map the distribution and economic impact of these pathogens more comprehensively.

5. Conclusion

This study contributed significantly to understanding the phytosanitary threats posed by *Lasiodiplodia* species in Burkina Faso cashew industry. The identification of diverse species and their demonstrated pathogenicity on multiple hosts underscores the urgency of implementing effective management strategies. Addressing these challenges is



Fig. 7. Dieback symptoms on cashew plants inoculated with *Lasiodiplodia* isolates from Burkina Faso seven weeks after inoculation. **A:** Cankers; **B:** vascular browning; **C:** Plants drying out after inoculation; **D:** vascular system of a control (mock) plant not inoculated.

Table 4
Symptoms induced on cashew (*A. occidentale*) plantlets after seven weeks of inoculation by *Lasiodiplodia* isolates from Burkina Faso.

Isolate	Phylogenetically closest species ^a	Percent plants with symptoms		
		Internal lesions	Cankers	Leaf drying
LA7	<i>L. x brasiliense</i>	25	75	0
LA8	<i>L. x brasiliense</i>	25	25	75
LA16	<i>L. x brasiliense</i>	50	100	0
LA18	<i>L. x brasiliense</i>	100	0	0
LA30	<i>L. x brasiliense</i>	25	100	0
LA31	<i>L. x brasiliense</i>	50	50	50
LA33	<i>L. x brasiliense</i>	50	50	50
LA36	<i>L. x brasiliense</i>	50	25	50
LA37	<i>L. x brasiliense</i>	25	100	0
LA17	<i>L. caatinguensis</i>	50	100	25
LA4	<i>L. crassispora</i>	75	75	25
LA32	<i>L. crassispora</i>	25	75	0
LA3	<i>L. euphorbicola</i>	25	100	0
LA13	<i>L. euphorbicola</i>	50	75	25
LA14	<i>L. euphorbicola</i>	0	100	0
LA22	<i>L. euphorbicola</i>	25	50	50
LA24	<i>L. euphorbicola</i>	50	0	0
LA25	<i>L. euphorbicola</i>	50	50	50
LA26	<i>L. euphorbicola</i>	50	50	25
LA28	<i>L. euphorbicola</i>	25	100	0
LA29	<i>L. euphorbicola</i>	50	50	0
LA34	<i>L. euphorbicola</i>	50	75	75
LA42	<i>L. euphorbicola</i>	25	100	0
LA44	<i>L. euphorbicola</i>	50	50	50
LA52	<i>L. euphorbicola</i>	50	50	50
LA66	<i>L. euphorbicola</i>	50	75	25
LA69	<i>L. euphorbicola</i>	25	75	100
LA73	<i>L. euphorbicola</i>	50	75	25
LA23	<i>L. pseudotheobromae</i>	100	50	0
LA35	<i>L. pseudotheobromae</i>	0	100	0
LA1	<i>L. theobromae</i>	100	75	25
LA2	<i>L. theobromae</i>	25	75	25
LA5	<i>L. theobromae</i>	75	75	25
LA27	<i>L. theobromae</i>	50	100	0
LA40	<i>L. theobromae</i>	25	100	0

^a As determined with ITS and *tef1-α* sequences.

vital for ensuring the sustainability and growth of cashew production, securing livelihoods, and maintaining Burkina Faso position as a key player in the global cashew market.

CRedit authorship contribution statement

Oumarou Zoéyandé Dianda: Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Yéri Jacqueline Kansie:** Methodology, Formal analysis. **Issa Wonnii:** Project administration, Funding acquisition. **Diana Fernandez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis,

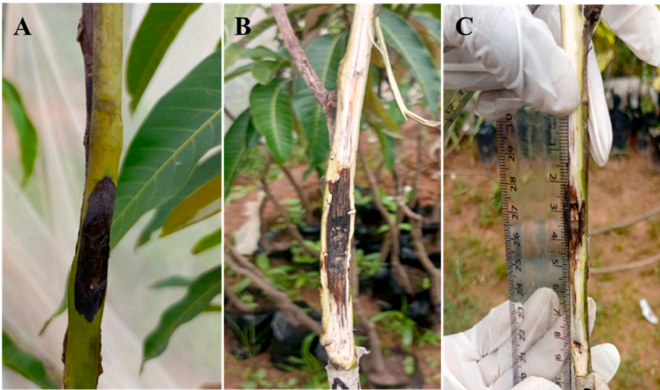


Fig. 8. Dieback and gummosis symptoms on mango plants inoculated with *Lasiodiplodia* isolated from cashew trees (**A:** Canker; **B:** Internal necrotic lesion; **C:** Canker lesion size measurement).

Table 5
Lesion size of *Lasiodiplodia* isolates from cashew on mango plantlets after seven weeks of inoculation.

Isolate	Phylogenetic group	Lesion length (mean ± SD ^a) (cm)
LA7	<i>L. brasiliense</i>	8.3 ± 1.3 b
LA37	<i>L. brasiliense</i>	13.0 ± 2.8 b
LA4	<i>L. crassispora</i>	6.4 ± 1.6 ab
LA29	<i>L. euphorbicola</i>	6.7 ± 1.8 ab
LA69	<i>L. euphorbicola</i>	8.3 ± 1.1 a
LA23	<i>L. pseudotheobromae</i>	8.8 ± 2.9 ab
LA2	<i>L. theobromae</i>	11.6 ± 3.3 b
Control		0.5 ± 0.1 c

^a SD: standard deviation; n = 5: values followed by the same letters are not different.

Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT/ OpenAI in order to improve English language of the text and format the references to APA style. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of competing interest

DF is an associate editor for this journal and the manuscript was independently handled by another member of the editorial board.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pmpp.2025.102698>.

Data availability

Data will be made available on request.

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