Tropical Plant Pathology (2025) 50:18
https://doi.org/10.1007/s40858-025-00700-9

SHORT COMMUNICATION

Lasiodiplodia theobromae disease symptom development in young avocado (*Persea americana* L.) plants depends on the inoculation method

Edgar Rodríguez-Gálvez[1] · Cesar Haro-Diaz[2] · Samir Maza-Aguirre[3] · Fátima Canahuire-Castillo[1] · Julio Sullón-Saucedo[1] · Holger B. Deising[4]

Received: 9 June 2024 / Accepted: 4 November 2024

Abstract

Lasiodiplodia theobromae is a pathogenic fungus of more than 500 plant species, including avocado (*Persea americana* Mill.). The global production volume of avocado exceeded 911,000 metric tons in 2023. Although detailed quantitative surveys of yield losses caused by *L. theobromae* are not available for most avocado-producing regions, estimates indicate that this pathogen is associated with stem-end rot disease in 30-35% of plots located in the Department of Antioquia in Colombia (Ramírez-Gil et al. Heliyon 7: e05905, 2021), suggesting an enormous economic impact. As yield losses strongly depend on the virulence of isolates, a reliable method for determining their aggressiveness is indispensable for initiating disease control measures. In this study, we compared progression of external and internal necrosis caused by the aggressive *L. theobromae* isolate LA-VLCA3 inoculated into wounded middle parts of the stem and onto excised apices. Irrespective of the inoculation method, internal progression of necrosis preceded that of external necrosis. Spreading of external and internal necrosis was significantly more severe in plants inoculated at the apex than in mid-stem inoculated plants. We conclude that apex inoculation causes stronger symptoms and is therefore better suited to compare virulence of isolates than mid-stem inoculation.

Keywords Avocado · External and internal necrosis · *Lasiodiplodia theobromae* · *Persea americana* · Symptom development · Virulence

The polyphagous pathogen *Lasiodiplodia theobromae* (Pat.) belongs to the family *Botryosphaeriaceae* (Dissanayake et al. 2016; Zhang et al. 2021) and causes multiple disease symptoms on approx. 500 plant species (Punithalingam 1976). In Peru, this pathogen has been described as a causal agent of dieback and wood necrosis in blueberry (Rodríguez-Gálvez et al. 2020), mango (Rodríguez-Gálvez et al. 2017), avocado (Rodríguez-Gálvez et al. 2021) and table grapes (Rodríguez-Gálvez et al. 2015). As symptom severity and yield losses strongly depend on the virulence of isolates (Gunamalai et al. 2023), a reliable method for determining their aggressiveness is indispensable for performing efficient disease control measures. Virulence tests with *L. theobromae* isolates have hitherto been carried out using two methods, i.e. inoculation into the middle part of the stem after wounding (Bautista-Cruz et al. 2019; Berraf-Tebbal et al. 2020; Dong et al. 2020; Hernández et al. 2023; Úrbez-Torres et al. 2008; El-Ganainy et al. 2022; Coutinho et al. 2017; Rangel-Montoya et al. 2021; Briste et al. 2022; Kong et al. 2023), or onto excised apices of the stem (Alama et al. 2006; Billones-Baaijens et al. 2013; Hernández et al. 2023; Kwon et al. 2017; Polashock and Kramer 2006; Rodríguez-Gálvez et al. 2015, 2017, 2020, 2021). In spite of the fact that distinct inoculation methods have been employed, rigorous comparisons of these methods have not been carried

✉ Edgar Rodríguez-Gálvez
erg@unp.edu.pe

✉ Holger B. Deising
holger.deising@landw.uni-halle.de

1 Faculty of Agronomy, Department of Plant Health, National University of Piura, Piura, Peru

2 Faculty of Science, Department of Statistics, National University of Piura, Piura, Peru

3 Human Health Center of Colaya, Salas, Lambayeque, Perú

4 Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Chair of Phytopathology and Plant Protection, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Published online: 10 February 2025

out. The present study was performed to determine which of the two inoculation methods yields more severe dieback and wood necroses symptoms and is therefore more suitable to determine virulence of isolates.

In this study, 10 months old avocado plants (*Persea americana* cv. Hass) plants grafted onto West Indian rootstock were inoculated with the virulent *L. theobromae* isolate LA-VLCA3 obtained from avocado branches with dieback symptoms (Rodríguez-Gálvez et al. 2021). This isolate was plated onto 2% (w/v) potato dextrose agar (PDA) (HiMedia Laboratories Pvt.Ltd., Dindhori, Nashik, India) and incubated at 30°C for 72 h.

Plants were inoculated using *L. theobromae*-covered agar blocks taken from the edge of a colony. This inoculation method allowed comparison of the data with those obtained from previous experiments (e.g. Rodríguez-Gálvez, et al. 2015, 2017, 2020, 2021; Úrbez-Torres and Gubler 2009; Úrbez-Torres at al. 2008). Moreover, we decided to use agar block inoculation because inoculation of conidial suspensions into wounds in vertical stems may result in major loss of conidia due to the outflow of an unknown volume of the inoculum. Mid-stem inoculation was done after wounding with a sterile corkborer (5 mm diameter) immediately above the grafting zone. An agar disc with mycelium (4 mm diameter) of the pathogen obtained from the edge of a colony at 72 hours post inoculation (hpi) was deposited into the wound and the inoculation site was covered with Parafilm (Bemis Company, Inc., Neenah, Wisconsin, USA) (Úrbez-Torres et al. 2008). For apex inoculation, the apical bud was cut off with a sterile scalpel and a 4 mm agar disc with fungal mycelium (see above) was placed onto the wound. The inoculated apex was covered with sterile cotton moistened with sterile distilled water and sealed with Parafilm (Bemis Company, Inc., Neenah, Wisconsin, USA) (Rodríguez-Gálvez et al. 2015). Five plants per treatment were inoculated and incubated for 28 days in a greenhouse at an average temperature of 26°C. The experiment was repeated four times, yielding a total of 20 tested plants per treatment.

Symptom development was observed daily and the expansion of external and internal necrosis caused by the pathogen was assessed at 28 days post inoculation (dpi) by measuring the length of necroses from the point of inoculation to the border of the visible infection using a digital vernier. In mid stem-inoculated plants, acropetal and basipetal external and internal necrosis were measured, and in plants inoculated at the excised apex, plausibly, only external and internal basipetal necrosis were quantified.

To confirm statistical normality of necrosis progression, the SPSS-V.25 software (IBM, New York, USA) was used, applying the Shapiro-Wilk test for data smaller than 50. For comparison of means in samples that did not show normal data distribution, the non-parametric Kruskal-Wallis test was used. For comparison of means in samples from normal populations, the parametric ANOVA test was used, followed by Tukey's test ($p \leq 0.05$). Statgraphics Centurion software version VII (Statgraphics Technologies Inc., The Plains, VA, USA) was employed for both comparisons.

In plants inoculated at the mid-stem, an irregular externally visible black necrotic spot was observed around the inoculation site. Developing necroses expanded acro- and basipetally from the inoculation site (Fig. 1a, arrowhead). After dissecting the cortical zone longitudinally, internal tissue necrosis was observed. Importantly, internal necrosis had a greater acropetal and basipetal extension than the externally visible necrosis (Fig. 1b, arrows). Thus, cross-sections and longitudinal sections revealed that internal necrosis first developed acro- and basipetally underneath the bark (Fig. 1c, arrows), established bark-associated necroses and subsequently colonized the xylem and grew towards the pith, resulting in the sectorial necrosis typically observed in *L. theobromae*-infected stems (Fig. 1d, arrows). Thus, development of necroses in avocado resembles those observed in *L. theobromae*-infected grapes.

Following apex inoculation, necroses developed dramatically and expanded basipetally, affecting all stem tissues from the point of inoculation, and resulted in necrotizing leafs and branches at 28 dpi (Fig. 2a). Formation of enormous numbers of conidia covering a large part of the infected stem was visible as whitish coating (Fig. 2a and, at larger magnification, Fig. 2b, co). Internal necrosis extending basipetally beyond the edge of the externally visible

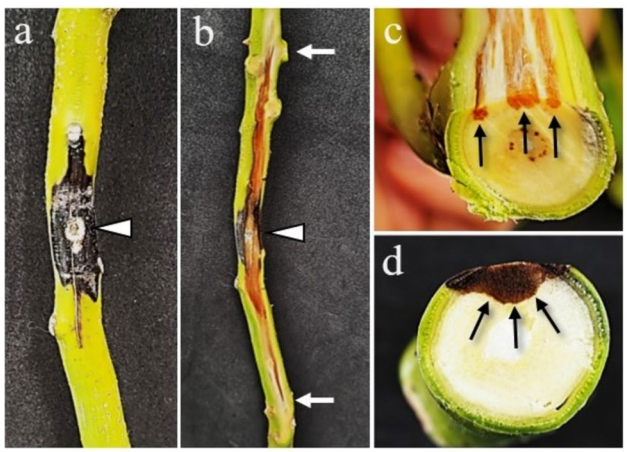

Fig. 1 Necrosis symptoms in stems of avocado (*Persea americana*) cv. Hass inoculated with *L. theobromae* in the mid-part of the stem. **a**, external necrosis. **b**, internal necrosis of the same inoculation site after longitudinal sectioning of the bark. Arrowheads in a and b indicate the inoculation point. Arrows in b mark ends of internal necrosis. **c**, longitudinal extension of surface-associated necroses (arrows). **d**, cross section showing horizontal spread of necrosis (arrows) from the bark towards the pith

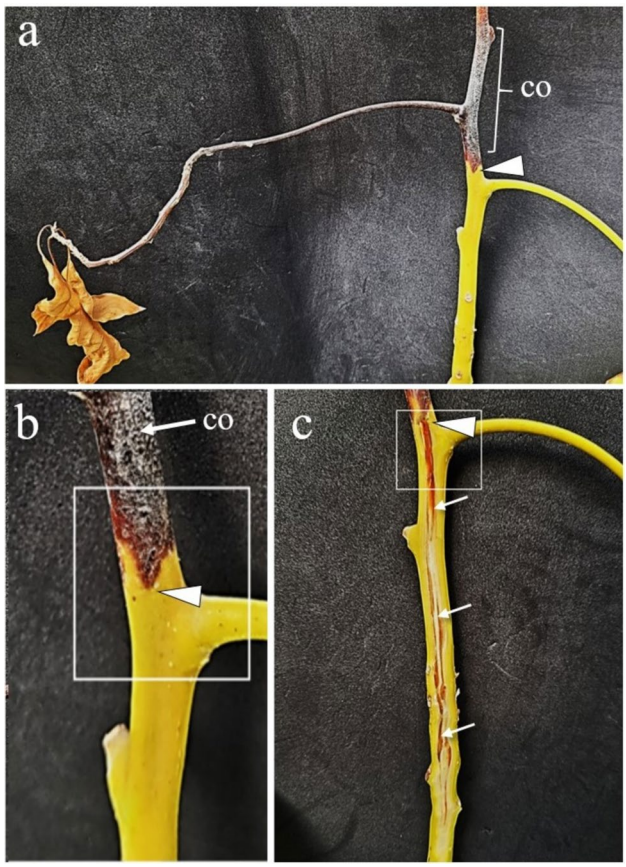

Fig. 2 Necrosis symptom on avocado (*Persea americana*) cv. Hass plant after apex inoculation with *L. theobromae*. **a**, external apical necrosis. The arrowhead marks the edge of external necrosis, the bracket marks the area of conidiation (Co). **b**, Enlargement of a, showing a whitish conidiation area (Co) of the pathogen and arrowhead pointing at the edge of necrosis. **c**, Internal necrosis of the same infection site (compare squares in b and c), observed after longitudinal sectioning of the bark. The arrowhead indicates the edge of the external necrotic area; note the downward extension of internal necrosis as indicated by arrows below the arrowhead

necrosis (Fig. 2c, arrowhead) was detected after cutting the bark longitudinally (Fig. 2c, arrows).

Macroscopic evaluation of infected plants (Figs. 1 and 2), and quantification of the length of external and internal necroses revealed that the fungus had massively spread in the stem (internal necrosis) before symptoms became externally visible (Table 1). In mid-stem-inoculated plants, internal necrosis extended significantly more rapidly than external necrosis, and no significant differences were observed between acropetal and basipetal expansion of necrosis ($p = 0.432774 > \alpha = 0.05$), indicating comparable fungal upward- and downward-directed dissemination and symptom development. Importantly, in stems inoculated at the excised apex, basipetal extension of both external and internal necrosis were significantly more pronounced, as compared with mid stem-inoculated plants ($\alpha < 0.05$). Also in apex-inoculated plants, internal spread of necroses was significantly more efficient than external spread (Table 1). Clearly, these data indicate that excised-stem-inoculation is an excellent method to evaluate virulence of field isolates of *L. theobromae*.

Our results show that development of necroses in stems of young avocado plants inoculated with *L. theobromae* is strongly affected by the inoculation method used. The advance of internal necrotization preceded that of external necrotization, suggesting that the fungus spreads vertically within the stem before expressing external disease symptoms. Interestingly, basipetal spread of the fungus as well as generation of internal and external necrosis occurred more efficiently after inoculation of the excised apex than after mid-stem inoculation. In comparison, Úrbez-Torres et al. (2008) reported that *L. theobromae* caused larger basipetal than acropetal lesions in rooted cuttings of grapevine cv. Chardonnay and cv. Thompson, and on green shoots of grapevine cvs.

Chardonnay and Thompson. Reports of these two types of colonization were also addressed in other research studies on wood colonization by various plant pathogenic fungi, including *L. theobromae* (Bautista-Cruz et al. 2019); *L.*

Table 1 Quantification and comparison of the length of external and internal necrosis in plants wound-inoculated at the mid-stem or at the excised apex. In mid-stem-inoculated plants, acropetal and basipetal extension of external and internal necrosis is shown. In apex-inoculated plants, only basipetal extension of external and internal necroses are given

Mode of inoculation		Necrosis length (mm)	Significance (Tukey 0.05)
Mid-stem	Acropetal External	12.71 ± 3.07	a
	Basipetal External	14.09 ± 4.41	a
	Acropetal Internal	110.93 ± 20.56	a
	Basipetal Internal	120.01 ± 23.22	a
Plant Apex	Basipetal External	97.29 ± 12.28	a
	Basipetal Internal	189.97 ± 18.57	b

crassispora, *L. euphorbicola* and *L. pseudotheobromae* (Dianda et al. 2023), *Neofusicoccum luteum* and *N. parvum* (Billones-Baaijens et al. 2013).

The differences between external and internal colonization addressed in this study have not been considered in other investigations yet. In most of the previous studies only internal colonization has been studied (Berraf-Tebbal et al. 2020; Biju et al. 2021; Briste et al. 2022; Dianda et al. 2023; Dong et al. 2020; El-Ganainy et al. 2022; Gunamalai et al. 2023; Hernandez et al. 2023). In avocado, we measured a sum of acropetal plus basipetal internal necrosis of 230 mm in only 28 days of incubation. In comparison, Úrbez-Torres et al. (2008) reported a lesion length of 338 mm when inoculating rooted cuttings of grapevine cv. Chardonnay and 183.1 mm in cv. Red Globe, with an incubation time as long as 140 days. The same authors obtained lower values in green shoots of grapevine cv. Red Globe and in rooted cuttings of this cultivar after an incubation time of 180 days.

Rapid generation of significant external and internal necrosis caused by *L. theobromae* observed after inoculation of the excised apex indicates that this method is not only suitable for quantitative determination of virulence of field isolates of this fungus, but could also be an excellent and time-saving alternative in testing virulence of isolates of other members of the *Botryosphaeriaceae*.

Acknowledgements The authors would like to thank Technician Angela Peña Gonzales for her excellent technical assistance and the "Los Viñedos" nursery for the donation of the avocado seedlings. This work was financed by the Central Research Office (OCIN) of the National University of Piura through the University Research Fund (FEDU).

Authors' contribution The conceptualisation of this research was developed by E. Rodríguez-Gálvez (Principal Investigator) and Holger B. Deising. The experimental design and statistical analyses were developed by C. Haro-Díaz, the maintenance of the plants and the preparation of the virulent isolate LA-VLCA3 of *L. theobromae* for inoculation was performed by S. Maza-Aguirre. Inoculation of plants and evaluation of symptoms was performed by J. Sullón-Saucedo and F. Canahuire-Castillo. E. Rodríguez-Gálvez analyzed the data, and E. Rodríguez-Gálvez and Holger B. Deising wrote the manuscript. Revision of the article was performed by all authors.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability All data generated and analyzed are available upon request to the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Alama I, Maldonado E, Rodríguez-Gálvez E (2006) *Lasiodiplodia theobromae* afectando el Cultivo de Palto (*Persea americana*) en las condiciones de Piura-Perú. Universalia 11:4–13

Bautista-Cruz MA, Almaguer-Vargas G, Leyva-Mir SG, Colinas-León MT, Correia KC, CamachoTapia M, Robles-Yerena L, Tovar-Pedraza JM (2019) Phylogeny, distribution, and pathogenicity of *Lasiodiplodia* species associated with cankers and dieback symptoms of Persian lime in Mexico. Plant Dis 103:1156–1165

Berraf-Tebbal A, Mahamedi AE, Aigoun-Mouhous W, Špetík M, Čechová J, Pokluda R, Baránek M, Eichmeier A, Alves A (2020) *Lasiodiplodia mitidjana* sp. nov. and other Botryosphaeriaceae species causing branch canker and dieback of Citrus sinensis in Algeria. PloS ONE 15:e0232448

Biju CN, Jeevalatha A, Peeran MF, Bhai RS, Basima F, Nissar VM, Srinivasan V, Thomas L (2021) Association of *Lasiodiplodia theobromae* with die-back and decline of nutmeg as revealed through phenotypic, pathogenicity and phylogenetic analyses. 3 Biotech 11:422

Billones-Baaijens R, Jones EE, Ridgway HJ, Jaspers MV (2013) Virulence affected by assay parameters during grapevine pathogenicity studies with *Botryosphaeriaceae* nursery isolates. Plant Pathol 62:1214–1225

Briste PS, Akanda AM, Bhuiyan MAB, Mahmud NU, Islam T (2022) Morphomolecular and cultural characteristics and host range of *Lasiodiplodia theobromae* causing stem canker disease in dragón fruit. J Basic Microbiol 62:689–700

Coutinho IBL, Freire FCO, Lima CS, Lima JS, Gonçalves FJT, Machado AR, Silva AMS, Cardoso JE (2017) Diversity of genus *Lasiodiplodia* associated with perennial tropical fruit plants in northeastern Brazil. Plant Pathol 66:90–104

Dianda OZ, Issa W, Ouédraogo L, Sankara P, Tollenaere C, Del Ponte EM, Fernandez D (2023) Lasiodiplodia species associated with mango (*Mangifera indica* L.) decline in Burkina Faso and influence of climatic factors on the disease distribution. Physiol Mol Plant Pathol 126(102041):2023–03

Dissanayake AJ, Phillips AJL, Li XH, Hyde KD (2016) *Botryosphaeriaceae*: current status of genera and species. Mycosphere 7:1001–1073

Dong W, Doilom M, Hyde KD, Phillips A, Yan K, To-anun C, Xu JC, Zhang H, Nalumpang S (2020) Pathogenicity of five *Botryosphaeriaceae* species isolated from *Tectona grandis* (teak): the pathogenic potential of *Lasiodiplodia* species. Asian J Mycol 3:399–407

El-Ganainy SM, Ismail AM, Iqbal Z, Elshewy ES, Alhudaib KA, Almaghasla MI, Magistà D (2022) Diversity among *Lasiodiplodia* species causing dieback root rot and leaf spot on fruit trees in Egypt, and a description of Lasiodiplodia newvalleyensis sp. nov. J Fungi 8:1203

Gunamalai L, Duanis-Assaf D, Maurer D, Sharir T, Feigenberg O, Sela N, Alkan N (2023) Comparative characterization of virulent and less virulent *Lasiodiplodia theobromae* isolates. Mol Plant-Microb Interact 36:502–515

Hernández D, García-Pérez O, Perera S, González-Carracedo MA, Rodríguez-Pérez A, Siverio F (2023) Fungal pathogens associated with aerial symptoms of avocado (*Persea americana* Mill.) in Tenerife (Canary Islands, Spain) focused on species of the family Botryosphaeriaceae. Microorganisms 11:585

Kong Q, Xing S, Xie K, Jia Z, Li Z, Liu Y, Xu C, Shi X, Wang C, Yuan S (2023) First report of *Lasiodiplodia theobromae* causing stem brown rot of loquat in China. Plant Dis 107:2234

Kwon JH, Choi O, Kang B, Lee Y, Park J, Kang DW, Han I, Kim J (2017) Identification of *Lasiodiplodia pseudotheobromae* causing mango dieback in Korea. Can J Plant Pathol 39:241–245

Polashock JJ, Kramer M (2006) Resistance of blueberry cultivars to *Botryosphaeria* stem blight and *Phomopsis* twig blight. Hort Sci 41:1457–1461

Punithalingam E (1976) *Botryodiplodia theobromae*. CMI Descriptions of Fungi and Bacteria: 52, Sheet-519. Commonwealt Mycological Institute, Kew, Surrey

Ramírez-Gil JG, Henao-Rojas JC, Morales-Osorio JG (2021) Postharvest diseases and disorders in avocado cv. Hass and their relationship to preharvest management practices. Heliyon 7:e05905

Rangel-Montoya EA, Paolinelli M, Rolshausen PE, Valenzuela-Solano C, Hernandez-Martinez R (2021) Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. Phytopathol Mediterr 60:237–251

Rodríguez-Gálvez E, Maldonado E, Alves A (2015) Identification and pathogenicity of *Lasiodiplodia theobromae* causing dieback of table grapes in Peru. Eur J Plant Pathol 141:477–489

Rodríguez-Gálvez E, Guerrero P, Barradas C, Crous PW, Alves A (2017) Phylogeny and pathogenicity of *Lasiodiplodia* species associated with dieback of mango in Peru. Fungal Biol 121:452–465

Rodríguez-Gálvez E, Hilário S, Lopes A, Alves A (2020) Diversity and pathogenicity of *Lasiodiplodia* and *Neopestalotiopsis* species associated with stem blight and dieback of blueberry plants in Peru. Eur J Plant Pathol 157:89–102

Rodríguez-Gálvez E, Hilário S, Batista E, Lopes A, Alves A (2021) *Lasiodiplodia* species associated with dieback of avocado in the coastal area of Peru. Eur J Plant Pathol 161:219–232

Úrbez-Torres JR, Gubler WD (2009) Pathogenicity of *Botryosphaeriaceae* species isolated from grapevine cankers in California. Plant Dis 93:584–592

Úrbez-Torres JR, Leavitt GM, Guerrero JC, Guevara J, Gubler WD (2008) Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. Plant Dis 92:519–529

Zhang W, Groenewald JZ, Lombard L, Schumacher RK, Phillips AJL, Crous PW (2021) Evaluating species in *Botryosphaeriales*. Pers: Mol Phylogeny Evol Fungi 46:63–115