INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 8, No. 1, p. 59-63, 2016

RESEARCH PAPER OPEN ACCESS

First report of lasiodiplodia theobromae causing shoot blight of ricinodendron heudelotii seedlings in Cameroon

Joseph Fovo Djeugap[1*], Louis Bernier[2], Daniel Dostaler[3], Gabin Régis Dongmo Zena[1]

[1]*Laboratory of Plant Pathology, Department of Plant Protection, Faculty of Agronomy and Agricultural Science, University of Dschang, Cameroon*

[2]*Laboratory of Forest Pathology, Department of Wood and Forest Sciences, Faculty of Forestry, Geography and Geomatic, Laval University, Quebec, Canada*

[3]*Department of Phytology, Faculty of Agriculture and Food Science, Laval University, Quebec, Canada*

Article published on January 25, 2016

Key words: *Lasiodiplodia theobromae*, Shoot blight, *Ricinodendron heudelotii*, Cameroon.

Abstract

The aim of this study was to identify the pathogen responsible to shoot blight, a new disease observed on *Ricinodendron heudelotii* seedlings in Cameroon. Isolation of the fungus from infected tissues was made on potato dextrose agar (PDA) milieu. After identification based on morphological characteristics (mycelium structure and conidia sizes), DNA was extracted and submitted to molecular analysis. The sequences obtained from rDNA were identified as *Lasiodiplodia theobromae*. Inoculums were then prepared and Koch´s postulate was verified both on detached leaves and seedlings by inoculating healthy detached leaves and seedlings with 5 mm diameter mycelia plugs and a solution of 10^6 conidial/ml respectively. Shoot blight symptoms were observed both on detached leaves and on seedlings. This was the first report of *L. theobromae* causing shoot blight on *R. heudelotii* seedlings.

* **Corresponding Author:** Joseph Fovo Djeugap ✉ joseph.djeugap@univ-dschang.org

Introduction

Ricinodendron heudelotii (Baill.) Pierre ex Heckel is a tropical forest species whose grains (non timber forest product) are used by local people living in the Congo basin forest for food and trade. In Cameroon, *R. heudelotii* is in a domestication process and is considered as an important tree species for agroforestry, food and income source for local people (Tchiegang *et al.,* 1997; Tchatat and Ndoye, 2006; Djeugap *et al*., 2013). However, in 2008, unusual, severe shoots blight was observed on seedlings both in forest and in nurseries of the World Agroforestery Center in Cameroon (Fig. 1). The disease is more severe in the forest and start on leaves by appearance of brown spots on the leaves which later become wilted and the dieback process start until the seedlings died when the environment is favorable to the pathogen. Then, the aim of the study is to identify the pathogen responsible to the shoot blight; later, a sustainable control measure will be developing to control the disease.

Fig. 1. Shoot blight on *Ricinodendron heudelotii* seedlings caused by *Lasiodiplodia theobromae* in the forest (A) and in nursery (B).Materials and methods.

Samples collection and microscopic identification of the fungus

Infected leaves and stems were collected from infected seedlings in the forests and nurseries. Isolations were made by plating small pieces (2mm²) of plant leaf or stem on potato dextrose agar (PDA) amended with chloramphenicol (500 mg/l). The Petri dishes were incubated at 23°C for 7 days (Djeugap *et al*., 2015). Fungal colonies were purified and pre identify based on their morphological characteristics (mycelium and spore) using a microscope (Alexopoulos and Mims, 1996; Agrios, 2005).

Molecular identification of the fungus

DNA extraction was performed using the protocol described in the DNeasy Plant Mini Kit (Qiagen Company) from lyophilized mycelium crushed in the presence of liquid nitrogen (Levy and Mavrodieva, 2004). Oligonucleotide primers ITS_4 and ITS_5 were used to amplify the ITS region of the nuclear ribosomal DNA of the fungus (White *et al*., 1990). A final volume of 25µl of a PCR reaction was prepared. For this purpose, a volume of 1.2µl ITS_4 + 1.2µl ITS_5 + 12.5µl polymerase (Premix ExTaq) + a corresponding volume of 10ng of DNA and the whole was completed to 25µl with sterilized distilled water (SDW). Ther DNA portions were sequenced at the Genomic Analysis Platform of the Institute of Integrative Biology and Systems of Laval University (Quebec, Canada) where the amplicons were purified using the kit Ultra clean PCR Clean-up (MOBIO, Solana Beach, CA) and ITS genes were sequenced using 1,5µM of the primers ITS_4 and ITS_5 (Prober *et al*., 1987). DNA sequences were compared with those of the Nucleotide bank collection from National Center for Biotechnology Information (NCBI) using the WU-BLAST (Washington University-blast) algorithm (Altschul *et al*., 1997).

Koch Postulates

After molecular identification, Koch's postulates were conducted on both detached leaves (in Lab conditions) and on ten 35-day- old potted *R. heudelotii* seedlings containing a mixture of sterile sand and soil (1/3, v/v) (Djeugap *et al.,* 2014). Mycelial plugs (5mm diameter) obtained from a 7-day-old pure culture of *L. theobromae* placed on detached leaves surface and 50 ml conidial suspension (10^6 conidial/ml) was sprayed on each seedling. Leaves were previously perforated with disinfected needle. Each inoculated detached leaf was

then place in a Petri dish containing wet blotting paper. Detached leaves inoculated with PDA plugs without mycelium and seedlings sprayed with sterile distilled water served as controls. Inoculated leaves were incubated in the Lab at 23°C for 5 days and seedlings were placed in an adapted screen house at 22 ± 2°C, 12-h photoperiod and 70% relative humidity for 14 days.

Results and discussion

Fungal colonies developed from 85% of the plated plant tissues and they had white mycelia that turned grey to black with age and formed black pycnidia (Fig. 2A). Conidia were oval and brownish with size of 25.3-30.2 x 10.4-15.7 µm (Fig. 2B). The perfect stage (teleomorph) was not observed. The fungus was identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., the anamorph of *Botryosphaeria rhodina* Berk. & Curt. Arx., based on morphological features and confirmed by DNA sequencing of the internal transcribed spacer region of rDNA from pure fungal colony which revealed 100% homology with those of other *L. theobromae* isolates in the GenBank (e.g. Accession N°: EU938331.1 and EU918707.1). Two days after inoculation (DAI) and within 4 to 6 DAI, leaf blight and shoot blight symptoms develop on detached leaves and seedlings respectively (Fig. 3). *L. theobromae* was successfully re-isolated from all inoculated detached leaves and seedlings, thereby completing Koch's postulates. Genomic DNA isolated from the re-isolations and PCR amplification of the ITS region was performed with the same primers. There was 100% nucleotide identity with sequences of the original isolates. This pathogen is also known to cause dieback and gommosis on mango in Pakistan (Khanzada *et al.*, 2004), grapevine decline in Italy (Burruano *et al.*, 2008), dieback on cocoa tree in Cameroon (Mbenoun *et al.*, 2007), root rot and collar rot disease of *Jatropha curcas* in India (Latha *et al.*, 2004) and canker on tapped *Boswellia papyrifera* trees in Ethiopia (Gezahgne *et al.*, 2014). To our knowledge, this is the first report of *L. theobromae* causing shoot blight on *R. heudelotii*, which represents a new constraint to the domestication of *R. heudelotii* in Cameroon.

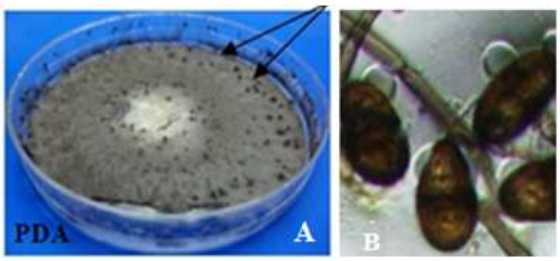

Fig. 2. Seven day-old culture of *L.theobromae* in PDA with mycelia turn grey to black with visible black pycnidia (A) (arrow); oval and brownish conidia of *Lasiodiplodia theobromae* under microscope, 400X (B).

Fig. 3. Leaf blight on inoculated detached leaf (A), 4 days after inoculation and shoot blight on inoculated 35-day-old seedlings of *R. heudelotii* (B), 7 days after inoculation.

Acknowledgements

The authors express their gratitude to the Institute of Integrative Biology and Systems (IBIS) and the «Centre d'étude de la Forêt» of Université Laval (Quebec, Canada) for their collaboration in the molecular analysis of the pathogen. They are grateful to Professor TABI OBEN Fritz (Department of Soil Science, Faculty of Agronomy and Agricultural Science, University of Dschang) for the correction of the English language.

References

Agrios GN. 2005. Plant pathology. 5th Ed. Elsevier Academic Press, California, USA, 922p.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein data base search programs. Nucleic Acids Ressources **25**, 3389-3402.

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory Mycology. 4th Ed. Wiley, New York.

Burruano S, Mondello V, Conigliaro G, Alfonzo A, Spagnolo A, Mugnai L. 2008. Grapevine decline in Italy caused by *Lasiodiplodia theobromae*. Phytopathologica Mediterranea **47**, 132-136.

Djeugap FJ, Bernier L, Dostaler D, Khasa D, Fontem DA, Nwaga D. 2013. Opportunités et contraintes agroforestières de *Ricinodendron heudelotii* au Cameroun. International Journal of Biological and Chemical Science **7(1)**, 344-355.

Djeugap FJ, Bernier L, Dostaler D, Fontem DA, Avana ML. 2014. Germination Constraints of *Ricinodendron heudelotii* in Cameroon. Seed Technology **36 (1),** 25-36.

Djeugap FJ, Tsopmbeng NG, Keuete KE, Yaouba A, Serferbe S. 2015. Isolation and Identification of Fungi Associated with Avocado Fruits from Local Markets of the West Region of Cameroon. International Journal of Agricultural and Biosciences **4 (2),** 64-68.

Gezahgne A, Yirgu A, Kassa H. 2014. First report of *Lasiodiplodia theobromae* causing canker on tapped *Boswellia papyrifera* trees in Ethiopia. New Disease Reports **29**, 11.

Khanzada MA, Lodhi AM, Shahzad S. 2004. Mango dieback and gummosis in Sindh Pakistan caused by *Lasiodiplodia theobromae*. Plant Health Progress [http://www.plantmanagementnetwork.org /pub/php/diagnostic guide/2004/mango/].

Latha P, Prakasam V, Kamalakannan A, Gopalakrishnan C, Raguchander M, Paramathma M, Samiyappan R. 2009. First report of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. causing root rot and collar rot disease of physic nut (*Jatropha curcas* L.) in India. Australasian Plant Disease Notes **4**, 19-20.

Levy L, Mavrodieva V. 2004. PCR detection and DNA isolation methods for use in the *Phytophthora ramorum* national program. DNA and PCR Protocol elaborated at Oregon State University and the University of California at Berkeley for *P. ramorum,* 11p.

Mbenoun M, Zeutsa EHM, Samuels G, Amougou FN, Nyasse S. 2007. Dieback due to *Lasiodiplodia theobromae*, a new constraint to cocoa production in Cameroon. New Disease Reports **15**, 59.

Prober JM, Trainor GL, Dam RJ, Hobbs FW, Robertson CW, Zagursky RJ, Cocuzza AJ, Jensen MA, Baumeister K. 1987. A system for rapid DNA sequencing with fluorescent chain terminating dideoxynucleotides. Science 238, 336-341.

Tchatat M, Ndoye O. 2006. Étude des produits forestiers non ligneux d'Afrique centrale: réalités et perspectives. Bois et forêts des tropiques **288**, 27-39.

Tchiegang C, Kapseu C, Ndjouenkeu R, Ngassoum MB. 1997. Les amandes de *Ricinodendron heudelotii* (Baill.): matière première potentielle pour les huileries tropicales. Journal of Food Engineering **32**, 1-10.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. Innis MA, Gelfand DH, Sninsky JJ and White TJ eds. Academic Press, San Diego, CA.