Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 44(3):307-311. 2017
www.rcia.uc.cl

PLANT PATHOLOGY

DOI: 10.7764/rcia.v44i3.1787

RESEARCH NOTE

Presence of false smut (*Graphiola phoenicis* (Moug. ex Fr.) Poit.) on Canary date palm (*Phoenix canariensis*) on Easter Island, Chile

Germán Sepúlveda[1], Mabel Arismendi[1], Wilson Huanca-Mamani[1], Steffany Cárdenas-Ninasivincha[1], Ricardo Salvatierra[2], and Bernardo Latorre[3]

[1]Universidad de Tarapacá, Facultad de Ciencias Agronómicas, Avda. General Velasquez 1775, Arica, Chile
[2]CEAZA,Universidad de La Serena, Campus Andrés Bello, La Serena, Chile.
[3]Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Departamento de Fruticultura y Enología. Casilla 306-22, Santiago, Chile

Abstract

G. Sepúlveda, M. Arismendi, W. Huanca-Mamani, S. Cárdenas-Ninasivincha, R. Salvatierra, and B. Latorre. 2017. Presence of false smut (Graphiola phoenicis (Moug. ex Fr.) Poit.) on Canary date palm (Phoenix canariensis) on Easter Island, Chile. Cien. Inv. Agr. 44(3): 307-311. *Graphiola phoenicis* was found on leaves of *Phoenix canariensis* in Hanga Roa, Easter Island, Chile. Amphigenous black pulvinate basidiomata were abundant on the leaf blades and rachides, causing extensive foliar damage. The samples were examined by a light microscope after three days in a humid chamber. In addition, the molecular tools scanning electron microscopy and histological sectioning were used to study the fungal/host relationship, complementing the identification. Morphometric and molecular characteristics led to the identification of the fungus as *Graphiola phoenicis* causing false smut on the Canary date palms (*Phoenix canariensis*). This is the first report of this plant pathogen in Chilean territory.

Keywords: Exobasidiales, false smut, Graphiola leaf spot

Introduction

Easter Island, part of Chilean territory, is located in the South Pacific (27° 08'S; 109° 26'W) 3,747 km west of the South American coast. The landscape of the island is dominated by grasses, shrubs and isolated tree species such as *Eucalyptus globulus* and other exotic trees. Palynological, phytolithic and paleontological studies showed that the endemic vegetation was extinct – for example, *Paschalococos disperta* (Dransfield *et al.* 1984), congeneric with Central Chile's *Jubaea chilensis,* (Grau, 2005). Among valued ornamental and introduced Arecaceae species, the Canary Island date palm *Phoenix canariensis* Hort. ex Chaub., native to the Canary Islands, populates urban and rural places, importing a tropical look to the landscape of Easter Island. Until now, there have been no reports of foliar diseases affecting *P. canariensis* on Easter Island. Here, we report the presence of a false smut fungus attacking *P. canariensis*.

Received May 25, 2017. Accepted December 01, 2017.
Corresponding author: gsepulve@uta.cl

Materials and methods

Plant Sample and morphologic identification

In January 2015, a random sample of diseased leaves from palms of *P. canariensis* was collected in Hanga Roa, Avenue Atanu Tekena (27° 09'08.48"S; 109° 25'53.52"W; 28 masl). Symptoms were characterized initially by very small yellow lesions that turned dark brown in the center with fuzzy edges, affecting primarily the oldest leaves. Lesions appeared isolated or else grouped on both side of the leaves. Morphometric studies were conducted on additional herbarium samples of diseased leaves, and micrometric leaf sections were obtained for optical microscope observations. Sections of approximately 0.25 cm^2 were obtained for environmental scanning electron microscope (SEM) observations in an EVO LS 10 microscope (Carl Zeiss, Germany), placed in aluminum sample holders with carbon-contact-bearing adhesives, and analyzed under vacuum with variable pressure mode (VP) (chamber pressure 150 Pa (under vacuum) and column 2×10^{-5} Torr (high vacuum)). The working distance (WD) varied depending on the sample type. The acceleration voltage was 15 KV, the tilt was 0° to 90°, and the images were taken with a resolution of 3.024 × 2.304 pixels at a scanning speed of 12 min 54 s.

Molecular identification

For molecular identification, dark lesions on foliar pinnae were collected, and DNA extraction was successful using an E.Z.N.A.® Insect DNA Kit (Omega Bio-Tek, Georgia, EEUU) according to the manufacturer's instructions. Subsequently, the internal transcribed spacer region (ITS) and the D1/D2 domain of the large subunit ribosomal DNA 28S (LSU rDNA) were amplified using primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3) and ITS1 (5'-TGAACCTGCAGAAGGATCATTA-3') (White *et al*., 1990; Barnes and Szabo, 2007) as well as NL1m (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4m (5'-GGTCCGTGTTTCAAGACG-3') (O'Donnell, 1993). PCR amplifications of the LSU and ITS rDNA were performed in a final volume of 20 µL. The reactions contained 1 µL of DNA extract; 5 p moles of each primers; 2.5 mM each dNTP; 2 mM MgCl2; 1X PCR buffer (KCl); 1 unit of Taq DNA polymerase (Thermo Scientific) and sterile distilled water. Cycling conditions were 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C; and a final elongation step of 2 min at 72 °C. PCR blank reaction controls were incorporated. Each PCR product (3 µL) was visualized on a 1.5% agarose gel stained with gelred (Biotium). The amplified products were sent to Macrogen (South Korea) for purification and direct sequencing.

The nucleotide sequences were visualized and edited using 4Peaks software (http://nucleobytes.com/4peaks/) and checked manually; nucleotides with ambiguous positions were clarified.

The sequences obtained were compared with rDNA D1/D2 and ITS data sequences from strains available in GenBank (www.ncbi.nem.nih.gov) by using BLASTn, and sequences with ≥98% similarity were downloaded in FASTA format. The sequence alignment and phylogenetic analysis were conducted using MEGA version 6.0 (Tamura *et al.,* 2013). Alignments were checked and manually adjusted when necessary. The Kimura 2-Parameter model (Kimura, 1980) was used to estimate evolutionary distance, and the gaps were treated as missing data. Phylogenetic reconstruction was performed using the maximum likelihood algorithm, and the robustness of the branches was assessed by bootstrap analysis (Felsenstein, 1985) of 1,000 replicates.

Results and Discussion

Foliar pinnae showed small yellow to dark lesions on both sides of the leaf blade, with brown to black globular, cylindrical or irregular sori

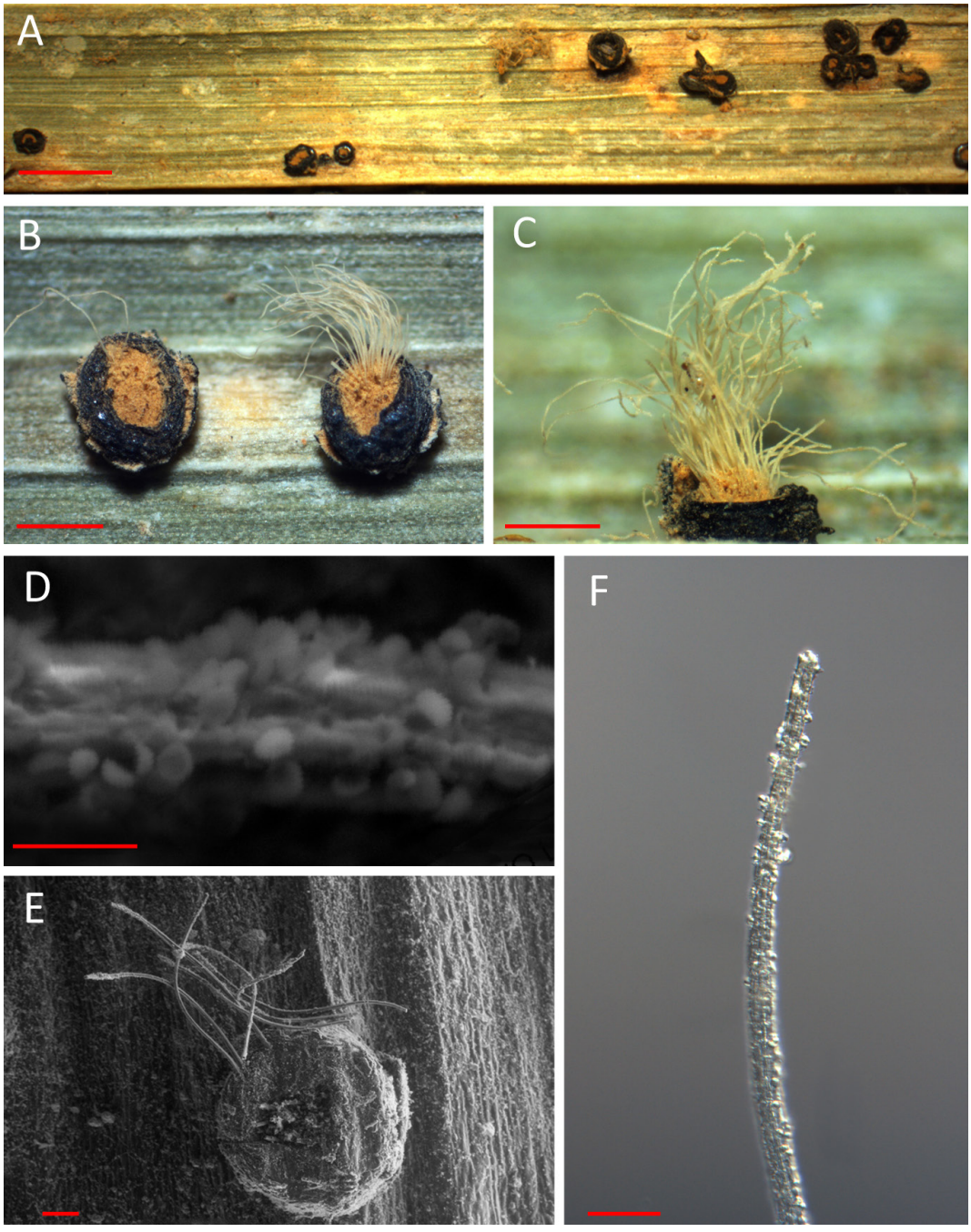

Figure 1. Reproductive structures of the false smut fungus (*Graphiola phoenicis*) affecting Canary date palms (*Phoenix canariensis*) on Easter Island, Chile. A: Sori distributed on the surface of *P. canariensis* leaflets. Bar=2 mm. B and C: Sorus profile view, with abundant thread-like filaments containing spermacia. Bars=0.5 mm. D: A thread-like filament with spermacia. Bar=10 µm. E: SEM image of sorus. Bar=100 µm. F: Detail of a thread-like filament with spermacia. Bar=10 µm.

(Figure 1A-C). Sori are fruiting bodies of 0.5 to 1.2 mm in diameter with a subepidermal origin, with dark and hard outer walls (Figure 1A-C). As sori mature, white to creamy thread-like filaments (Figure 1B-C) emerge through the ostiole of each sorus. Spherical to elliptical spermacia 2.5–3.0 µm

diameter, with thick hyaline walls, were produced (Figure 1D). In SEM images (Figure 1D-F), it was possible to observed abundant spermacia attached to the filament, suggesting that filaments help with dispersal. The morphometric characterization was coincident with previous descriptions of *G. phoenicis* (Cole, 1983; Tubaki and Yokoyama, 1971).

To confirm the morphological identification of *G. phoenicis*, a PCR fragment of ITS and LSU rDNA of isolates from Easter Island were successfully amplified and sequenced, obtaining 517 and 560 bp fragments for ITS and LSU, respectively, which were deposited in GenBank (Accession numbers KX344499 and KX344500, respectively).

Blast analysis performed with ITS sequences showed a 98% similarity of the Easter Island isolate with *G. phoenicis* from South Africa (KP730059), and LSU showed a 99% with *G. phoenicis* from Japan (AF009862). Phylogenetic analysis revealed a *Graphiola* cluster with nodal support of 86%. Between *Graphiola* species, the Easter strain has the highest similarity with *G. phoenicis* (Figure 2). This also supports the assignation of the Easter strain as *G. phoenicis.*

The results obtained confirm the identification of *G. phoenicis* as a basidiomycete fungus, the cause of false smut on Canary date palm trees on Easter Island. *G. phoenicis* is a plant pathogen affecting numerous species of palm trees in the world (Piepenbring, 2012). For instance, it has been reported on *P. roebelenii* in Argentina (Cúndom, 2009) and Florida, USA (Martinez, 1966) and on *P. dactylifera* in Brazil, Egypt, India, Kenya, Libya (Edongali, 1996), and Qatar (Abbas and Abdulla, 2004). The infection is favored by high humidity and high foliage density. This is the first record of *G. phoenicis* on *Phoenix canariensis* on Easter Island, Chile.

We used ITS and LSU sequences of this basidiomycete for molecular analysis and selected LSU for further analysis because this marker is widely recommended for genus- and species-level identification of all rust fungi (Hyde *et al.* 2014). LSU sequencing and phylogenetic analysis placed the *Pascua* strain isolated from Easter Island within the cluster composed by *G. phoenicis* and two other unidentified species. A similar cluster was also reported by Piepenbring *et al.* (2012).

These results lead us to assert that the "*Pascua"* strain isolated from Easter Island that is attacking Canary date palms in Rapa Nui belongs to *G. phoenicis.*

Acknowledgments

We thank the lab of Professor Bernardo Arriaza for the SEM image and the Dirección de Investigación y Posgrado de la U. Tarapacá (9711-15 Mayor Project) and Convenio de Desempeño en Educación Superior Regional UTA-1401 for financial support.

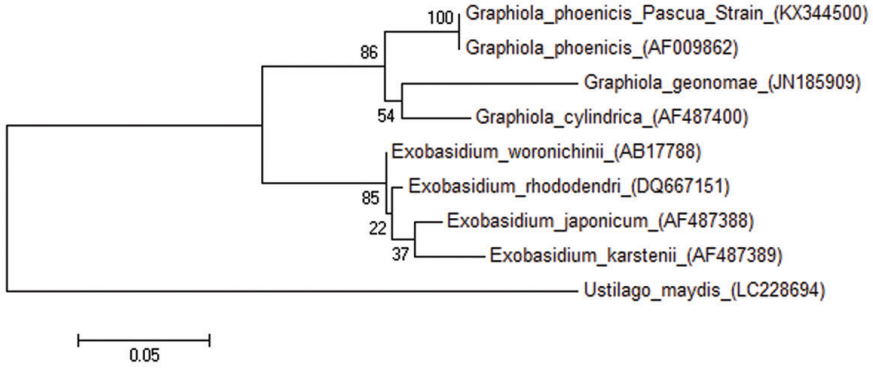

Figure 2. Phylogenetic tree based on LSU analysis of *Graphiola phoenicis Pascua strain* and closest species using the Kimura two-parameter model and maximum likelihood algorithm with 1,000 bootstrap replicates.

Resumen

G. Sepúlveda, M. Arismendi, W. Huanca-Mamani, S. Cárdenas-Ninasivincha, R. Salvatierra, y B. Latorre. 2017. Presencia del falso carbon (*Graphiola phoenicis* (Moug. ex Fr.) Poit.) sobre Palma de canarias (*Phoenix canariensis*) en Isla de Pascua, Chile. Cien. Inv. Agr. 44(3): 307-311. En hojas de *Phoenix canarienis* en Hanga Roa, Isla de Pascua, Chile, se encontró abundantes basidiomas pulvinados, negros, amfígenos negros sobre las pinnas y en el raquis de las hojas, causando daño foliar extensivo. Después de tres días en cámara húmeda se examinaron muestras con microscopio compuesto. Además, para estudiar la relación hongo/hospedero, se utilizó microscopía electrónica de barrido y cortes histológicos, complementariamente se aplicaron herramientas moleculares en la identificación. Las características morfométricas y moleculares permitieron la identificación del hongo como *Graphiola phoenicis*, agente causal del falso carbon en Palma de Canarias (*Phoenix canariensis*) en Isla de Pascua, Chile. Este es el primer reporte de este fitopatógeno en territorio chileno.

Palabras clave: Exobasidial, falso carbón, fitopatógenos foliar.

References

Abbas, E.H., and S.A. Abdulla. 2004. First report of false smut disease caused by *Graphiola phoenicis* on date palm trees in Qatar. Plant Pathology. 53:815–815.

Barnes, C.W., and L.J. Szabo. 2007. Detection and identification of four common rust pathogens of cereals and grasses using Real-time polymerase chain reaction. Phytopathology. 97:717–727.

Cole, G. 1983. *Graphiola phoenicis:* A taxonomic enigma. Mycologia. 75:93–116.

Cúndom, M.A. 2009. *Graphiola phoenicis* manifestation over *Roebelenii phoenicis* in Argentina. Summa Phytopathologica 35:239–239.

Dransfield, J., J.R. Flenley, S.M. King, D.D. Harkness, and S. Rapu. 1984. A recently extinct palm from Easter Island. Nature. 312:750–752.

Edongali, E.A. 1996. Diseases of date palm *(Phoenix dactylifera)* of Libya. Arab Journal of Plant Protection. 14:41–43.

Felsenstein, J. 1985. Confidence limits in phylogenies: an approach using the bootstrap. Evolution. 39:783–791.

Grau, J. 2005. Prehistoric presence of the Chilean palm in Easter Island. Proceedings of the VI International Conference on Rapa Nui and the Pacific, pp: 29–34. The Easter Island Foundation, Los Osos, California.

Hyde, K.D., R.H. Nilsson, A. Alias, A.H. Ariyawansa, E. Blair, *et al*. 2014. One stop shop: backbones trees for important phytopathogenic generates: I. Fungal Diversity. 67:21–125.

Kimura, M.A. 1980. Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16:111–120.

Martinez, A.P. 1966. False smut of palms. 43 Circular Plant Pathology, Florida Department of Agriculture. Division of Plant Industry.

O'Donnell, K. 1993. Fusarium and its near relatives. The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics (Reynolds DR & Taylor JW, eds); p. 225–233. CAB International, Wallingford, UK.

Piepenbring, M., F. Nold, T. Trampe, and R. Kirschner. 2012. Revision of the genus Graphiola. New Hedwigia. 94:67–96.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 30:2725–2729.

Tubaki, K., and T. Yokoyama. 1971. Cultural aspects of *Graphiola phoenicis*. Mycopathologia et Mycologia applicata. 43:49–60.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: MA Innis, DH Gelfand, JJ & White Sninsky TJ (eds). Academic Press, New York, USA. PCR Protocols: A guide to methods and applications; p 315–322.