Forest Pathology WILEY-BLACKWELL

For. Path. doi: 10.1111/efp.12223

SHORT COMMUNICATION

First report of *Puccinia psidii* on *Corymbia citriodora* and *Eucalyptus* in Colombia

By C. A. Rodas[1], J. Roux[2,4], W. Maier[3], G. M. Granados[1], M. D. Bolaños[1], A. R. McTaggart[2] and M. J. Wingfield[2]

[1]Forestry Protection Programme, Smurfit Kappa Cartón de Colombia, Yumbo, Valle, Colombia; [2]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; [3]Julius Kühn-Institut (JKI), Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany; [4]E-mail: jolanda.roux@fabi.up.ac.za (for correspondence)

Summary

A rust disease was observed during routine disease surveys of Eucalypt species in Colombia. The cause of the disease was identified, using morphology and DNA sequence data, as the myrtle rust pathogen, *Puccinia psidii*. We evaluated the susceptibility of *Eucalyptus grandis* and the hybrid *Eucalyptus urograndis* to *P. psidii.* This is the first report of this pathogen on *Corymbia citriodora* and *Eucalyptus* species in Colombia.

1 Introduction

Puccinia psidii Winter causes serious damage to *Eucalyptus* species where they are grown in plantations in South and Central America, with reduction in diameter and height of 25–35% recorded in Brazil (Junghans et al. 2003; de Silveira and Higashi 2003; Tommerup et al. 2003). The disease caused by this fungus was previously referred to as guava or Eucalyptus rust, but in recent years has been more commonly referred to as myrtle rust. It is found on many members of the Myrtaceae, occurring widely in Central and South American countries, the Caribbean, Australia, China, Japan, North America (including the Hawaiian Islands) and South Africa (Coutinho et al. 1998; Carnegie and Lidbetter 2012; Roux et al. 2013). The importance of this pathogen is enhanced by the fact that it has an extensive host range including multiple genera and species of Myrtaceae (Morin et al. 2012; Pegg et al. 2013).

Myrtle rust has been known in Colombia since the late 1930s (Kern and Toro 1935) where it was reported on non-native Myrtaceae, including *Syzygium jambos* (*Eugenia jambos*) and *Psidium guajava.* In 2010, typical symptoms were observed on ornamental *Corymbia citriodora* and a few months later, during routine plantation disease surveys, on *Eucalyptus* species and hybrids in the country. The aim of this study was to identify the rust pathogen on *C. citriodora* and *Eucalyptus* trees and obtain preliminary information on the relative susceptibility of two *Eucalyptus* genotypes to the pathogen.

2 Materials and methods

The identity of the rust pathogen on *C. citriodora* and *Eucalyptus grandis* in Colombia was determined from morphology of the urediniospores and DNA sequence analyses. The 5′ end of the nuclear large subunit (LSU) of the ribosomal DNA, using universal primers LR0R and LR6, and the full internally transcribed spacer regions 1 and 2 (ITS), including the 5.8S gene of the rRNA operon were amplified, using universal primers ITS1-F and ITS4, and then sequenced at the DNA Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. BLAST searches of the sequence data were performed with the NCBI database (www.ncbi.nlm.nih.gov) to compare the sequences obtained from Colombian isolates with confirmed rust species.

We tested whether *Eucalyptus* clones were susceptible to *P. psidii* present in Colombia. Five pure *E. grandis* clones and three hybrid *Eucalyptus urophylla* × *E. grandis* clones were inoculated with *P. psidii* taken with a fine brush from a natural infection on an *S. jambos* tree growing in a eucalypt plantation. The spores were suspended in deionized water with 0.035% Tween-20 at 2×10^5 urediniospores/ml. The spore suspension was sprayed on the abaxial leaf surfaces of 40 individuals for each evaluated species or hybrid of *Eucalyptus*. Inoculated trees were maintained under continuous darkness during the first 48 h. Plants were then kept under natural day/night light conditions in a greenhouse. During the assay, a constant temperature of 24°C and a humidity of 78% were maintained. The incidence and severity of infection was evaluated 14 days after inoculation according to Junghans et al. (2003), in which the plant is classified as tolerant with scores of S0 and S1 and susceptible with scores of S2 and S3.

3 Results and discussion

The identity of the rust pathogen on *C. citriodora* and *E. grandis* species in Colombia was confirmed using DNA sequences of the ITS and LSU regions of rDNA. The ITS and LSU sequences of rust on *C. citriodora* (ITS: KP863476, LSU: KP863473) and *E. grandis* (ITS: KP863478, LSU: KP863475) and *S. jambos* (ITS: KT231983, LSU: KT231982) from Colombia were near identical to each other. There were four single nucleotide polymorphisms present in the sequences from *C. citriodora* and

Received: 11.5.2015; accepted: 28.7.2015; editor: L. Belbahri

E. grandis, which were called as degenerate bases. BLAST searches of the ITS and LSU regions were 99% (529/530 base pair identities to KF318433, KF318434, KF318435) and 100% (1017/1017 base pair identities to KF318452, KF318453, KF318454) similar to sequences of *P. psidii,* respectively. This supported the identification based on symptomatology and light microscopy.

Puccinia psidii was confirmed from *C. citriodora* (Fig. 1a) and *E. grandis* in commercial plantations in Valle del Cauca department. Records now include infections on *C. citriodora* trees in Restrepo (SKC04201008; 3°51′45″ N – 76°29′49″ W), 1-year-old *E. grandis* at La Suiza Farm (SKC01201303; 3°50′55″ N – 76°29′33″ W) and on cuttings and seedlings of *E. grandis* in Rancho Grande Nursery of SKCC (SKC04201204 3°51′43″ N – 76°30′48″ W).

Both urediniospores and teliospores were observed on the material examined from eucalypts in Colombia. Urediniospores from eucalypt species were ellipsoid to globose in shape with echinulate hyaline cell walls. They were an average of 21.1 (13.8–26.9) μm long and 16.4 (10.4–23.1) μm wide (n = 48). Teliospores were two-celled and orange-brown (Fig. 1 b) with an average 36.8 (32–43) μm long and 19.5 (17–22) μm wide (n = 30). Teliospores presented a shorter range than other descriptions from South America (Ferreira 1989; Pérez et al. 2011). Urediniospores of *P. psidii* were similar in shape but wider than reported from Brazil (Ferreira 1989) and Uruguay (Pérez et al. 2011).

In Colombia, we observed that the primary symptoms of rust on *C. citriodora* and *Eucalyptus* occur on young tissues, such as leaves and shoots. These include masses of bright yellow urediniospores, which commonly caused deformation of

Fig. 1. Rust infection associated with *Puccinia psidii*: (a) *C. citriodora*. (b) Urediniospores and teliospores from *E. grandis* (Bar 20 μm). (c) Uredinial pustules. (d) *E. grandis*. (e) *E. urograndis*.

the infected tissue, necrotic lesions and shoot death in the advanced stages of disease. In the inoculation studies, symptoms were observed after 10 days and sorus formation was visible after 14 days (Fig. 1c). Variation was observed, with *E. grandis* (Fig. 1d) clones being less susceptible than *Eucalyptus urograndis* (Fig. 1e). Studies are now being conducted to screen commercial clones of *E. grandis* and hybrids of *E. urograndis* for resistance. These will provide useful information for future planting strategies aimed to minimize damage to the commercial production of eucalypts by *P. psidii*.

References

Carnegie, A. J.; Lidbetter, J. R., 2012: Rapidly expanding host range for *Puccinia psidii* sensu lato in Australia. Australas. Plant Pathol. **41**, 13–29.

Coutinho, T. A.; Wingfield, M. J.; Alfenas, A. C.; Crous, P. W., 1998: Eucalyptus rust: a disease with the potential for serious international implications. Plant Dis. **82**, 819–825.

Ferreira, F. A., 1989: Ferrugem do eucalipto. In: Patologia Florestal – principais doenças florestais no Brasil, Viçosa, Sociedade de Investigações Florestais, p. 129-151. 570 pp.

Junghans, D. T.; Alfenas, A. C.; Maffia, L. A., 2003: Escala de notas para quantificação da ferrugem em *Eucalyptus*. Fitopatol. Bras. **28**, 184–188.

Kern, F. D.; Toro, R. A., 1935: Notes on some fungi from Colombia. Mycologia **27**, 615–617.

Morin, L.; Aveyard, R.; Lidbetter, J. R.; Wilson, P. G., 2012: Investigating the host-range of the rust fungus *Puccinia psidii sensu lato* across tribes of the family Myrtaceae present in Australia. PLoS ONE **7**, e35434.

Pegg, G. S.; Giblin, F. R.; McTaggart, A. R.; Guymer, G. P.; Taylor, H.; Ireland, K. B.; Shivas, R. G.; Perry, S., 2013: *Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. Plant Pathol. **63**, 1005–1021.

Pérez, C. A.; Wingfield, M. J.; Altier, N. A.; Simento, S.; Blanchette, R. A., 2011: *Puccinia psidii* infecting cultivated *Eucalyptus* and native Myrtaceae in Uruguay. Mycol. Prog. **10**, 832–859.

Roux, J.; Greyling, I.; Coutinho, T. A.; Verleur, M.; Wingfield, M. J., 2013: The Myrtle rust pathogen, *Puccinia psidii*, discovered in South Africa. IMA Fungus **1**, 155–159.

de Silveira, R. L. V. A.; Higashi, E. N., 2003: Aspectos nutricionais envolvidos na ocorrencia de doencas com enfase para o eucalipto. Circular Tecnica IPEF n. 200, pp. 01–13, dezembro de 2003.

Tommerup, I. C.; Alfenas, A. C.; Old, K. M., 2003: Guava rust in Brazil – a threat to *Eucalyptus* and other Myrtaceae. N. Z. J. For. Sci. **33**, 420–428.