Australasian Plant Dis. Notes (2017) 12: 23
DOI 10.1007/s13314-017-0246-5

Leaf spot of loquat (*Eriobotrya japonica*) caused by *Pseudocercospora eriobotryae* in Brazil

M. C. H. Rodriguez[1] · **R. W. Barreto**[1]

Received: 23 February 2017 / Accepted: 10 April 2017 / Published online: 23 April 2017
© Australasian Plant Pathology Society Inc. 2017

Abstract Loquat trees (*Eriobotrya japonica*) bearing leaf spot symptoms were found in Viçosa (State of Minas Gerais, Brazil). A cercosporoid fungus was associated to the disease and identified as *Pseudocercospora eriobotryae* based on morphological and molecular characters. Pathogenicity was demonstrated by inoculation of healthy leaves. This is the first report of *P. eriobotryae* in Brazil and the first time its pathogenicity to loquat is demonstrated.

Keywords Cercosporoid · First report · Fruit tree · Pathogenicity · Rosaceae

Loquat (*Eriobotrya japonica*, Rosaceae) is a subtropical, evergreen fruit tree, native to China. It is grown commercially for its edible fruits (Tziros 2013), which are eaten fresh or processed as jam and other products. Its leaves have been used as a source of medicine for the treatment of skin diseases and diabetes (De Tommasi et al. 1992). Loquat has been commercially grown in Brazil since the mid 1900s, mostly in the state of São Paulo (Caballero and Fernández 2003). It is also grown in cooler parts of south and southeastern Brazil. Although Brazil is one of the largest producers of loquat (Caballero and Fernández 2003), little has been investigated and published about the pathogens attacking loquat in Brazil and the crop is not mentioned in the main Brazilian plant disease compendium (Amorim et al. 2016). Only four fungal species are listed as occurring in Brazil on this host by Mendes and Urben (2017). Nevertheless, there are around 250 records of fungi associated with *E. japonica* worldwide (Farr and Rossman 2017).

In 2016 a leaf spot disease, known to occur on loquat plants at several localities in the Brazilian states of Minas Gerais and Rio de Janeiro, was examined in detail for the first time. A sample was taken from plants grown in a private garden in Viçosa (state of Minas Gerais), dried in a plant press and taken to the laboratory for further analysis. A dematiaceous cercosporoid fungus was consistently found sporulating on infected leaves. A pure culture was obtained by direct transfer of spores obtained from sporulating lesions onto PDA with the help of a sterile fine pointed needle. Fungal structures were obtained by scraping the colonized plant tissue with a scalpel or by sectioning the lesions. These were mounted with lactic acid and examined under a light microscope (Olympus BX 51) equipped with a Olympus® E-volt E-330 camera. A representative sample was deposited in the local herbarium (Herbário da Universidade Federal de Viçosa) under the accession number VIC 44135. The pure culture was deposited in the culture collection under the accession number COAD 2099. Two weeks-old colonies grown at 25 °C under continuous light (light provided by two white and one near-UV lamps placed 35 cm above the plates) in PDA and PCA were described following the terminology of Crous et al. (2009). For color terminology Rayner (1970) was followed.

Symptoms started as small irregular to angular leaf spot becoming semicircular, partly concentric, pale brown to grayish centrally with dark brown periphery, 2–16 × 2–11 mm diam, coalescing to cover large parts of leaves in time. (Fig. 1a). The fungus had the following morphology: Internal mycelium, 2–3 μm diam, branched, septate, subhyaline to brown. Stromata, erumpent, pulvinate, 30–105 × 30–90 μm, dark brown. Conidiophores sporodochial, 0–1-septate, 15–21 × 2.5–3 μm, conidiogenous cell integrated, with unthickened loci, 1–1.5 μm diam. Conidia solitary,

The original version of this article was revised: The provisional plate (Fig. 1) was published instead of the improved version.

✉ R. W. Barreto
rbarreto@ufv.br

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil

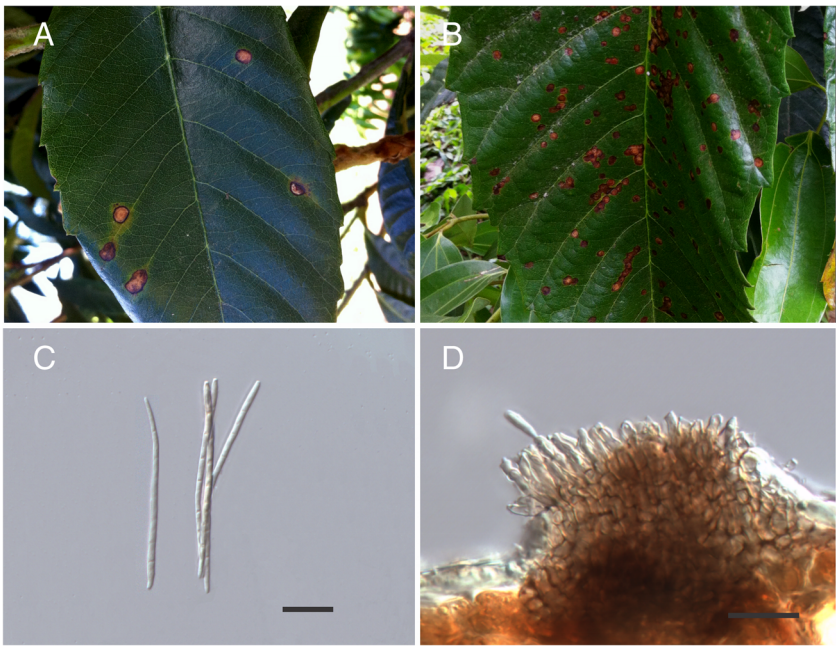

Fig. 1 *Pseudocercospora eriobotryae* on *Eriobotrya japonica*. **a** Leaf spot symptoms as observed in the field. **b** Infected loquat leaf six weeks after inoculation with *P. eriobotryae* for demonstration of pathogenicity. **c** Conidia. **d** Sporodochium. Bars: 10 μm

holoblastic, cylindrical to obclavate, straight to mildly curved, 30–66 × 2–3 μm, apex rounded to subacute, base obconic, 3–7 septate, smooth, hilum 1–1.5 μm unthickened and not darkened (Fig. 1c, d).

Culture characteristics: Colonies slow-growing (2.2 mm diam after 14 days), umbonate, of felty aerial mycelium, pale olivaceous grey to pale mouse grey centrally followed by smoke grey to mouse grey periphery; not sporulating.

Pathogenicity was tested on selected healthy leaves of a plant in the field through inoculation with a 10^5 conidia/ml suspension. Conidia were obtained by growing the fungus for 3 weeks in a potato-dextrose broth in constant agitation and then triturating the mycelium and pouring it on oatmeal-agar plates and incubating for two days. After that period the surface of plates was flooded with sterile tap water and scraped with a rubber spatula. The conidial suspension was sprayed on each of three healthy branch apices of a tree growing in the campus of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil). The leaves of each branch included mature leaves and pale-green immature leaves. One branch was sprayed with sterile distilled water and served as control. Each group of leaves on a branch was wrapped with an internally wet plastic bag immediately after spraying to maintain a high level of humidity and the plastic bag was removed after 48 hs. Leaves were observed weekly for the emergence of disease symptoms. Ten weeks after inoculation, spots equivalent to the original symptom observed in the field were found only on the inoculated leaves. Control leaves remained symptomless. A fungus with the same morphology as described above was found growing on the necrotic tissues and was isolated in pure culture generating colonies identical to those described above, hence fulfilling Koch's postulates (Fig. 1b).

Genomic DNA was extracted from 3 weeks-old colonies formed on Potato Dextrose (PD). A mycelium sample was placed into 1.5 mL sterile Eppendorf tubes and macerated with mechanical cell disruptor L-BEADER-3 using microspheres (beads), DNA was extracted with a Wizard Genomic DNA Purification kit by following the manufacturer's instructions. A portion of the actin (ACT) gene was amplified using the primers ACT-512F and ACT-783R (Carbone and Kohn 1999). The amplicons were directly sequenced by Macrogen Korea (http://dna.macrogen.com/eng/) and deposited in GenBank (Accession No. KY514352). A BLAST search of the GenBank database revealed that the sequence of the fungus from loquat in Brazil had a 100% homology with *Pseudocercospora eriobotryae* ex-epitype - isolate MUCC1007 (Nakashima et al. 2016). It also had a 100% homology with *Pseudocercospora rhapisicola* CBS:282.66 (Crous et al. 2013). Nevertheless, the morphology of the cercosporoid from loquat examined in this study matched well with that described for *P. eriobotryae* by Nakashima et al. (2016) but not with *Pseudocercospora rhapisicola* and the host of *P. rhapisicola* (*Rhapis flabelliformis*, Arecaceae) is not related to loquat. Therefore, the identification of the cercosporoid on loquat in Brazil was confirmed as *P. eriobotryae*.

This is the first report of *P. eriobotryae* on loquat in Brazil and the first time its status as a pathogen causing leaf spots on this host is experimentally demonstrated. It is likely that several other fungal pathogens of loquat also exist in Brazil but have, so far, remained unreported.

References

Amorim L, Rezende JAM, Bergamim Filho A, Camargo LEA (2016) Manual de Fitopatologia: Doenças de Plantas Cultivadas (volume 2). São Paulo, Brazil

Caballero P, Fernández MA (2003) Loquat, production and market. In: First international symposium on loquat. CIHEAM-IAMZ, Zaragoza, pp 11–20

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (2009) CBS: Fungal Biodiversity. The Netherlands.

Crous PW, Braun U, Hunter GC, Wingfield MJ, Verkley GJM, Shin HD, Nakashima C, Groenewald JZ (2013) Phylogenetic lineages in *Pseudocercospora*. Stud Mycol 75:37–114

De Tommasi N, Aquino R, De Simona F, Pizza C (1992) Plant metabolites. New sesquiterpene and ionona glycosides from *Eriobotrya japonica* Journal of Natural Products 55:1025–1032

Farr DF, Rossman AY (2017) Fungal Databases. Systematic Mycology and Microbiology Laboratory, ARS, USDA Retrieved (7 Feb 2017) from http://nt.ars-grin.gov/fungaldatabases/

Mendes MAS, Urben AF (2017) Fungos relatados em plantas no Brasil, Laboratório de Quarentena Vegetal. Brasília, DF: Embrapa Recursos Genéticos e Biotecnologia. Retrieved (23 Jan 2017) from: http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fgbanco01.asp

Nakashima C, Motohashi K, Chen C, Groenewald JZ, Crous PW (2016) Species diversity of *Pseudocercospora* from far East Asia. Mycol Progress

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew

Tziros TG (2013) *Alternaria alternata* causes leaf spot and fruit rot on loquat (*Eriobotrya japonica*) in Greece. Australasian Plant Dis Notes 8:123–124