First report of *Kuehneola uredinis* (Link) Arthur infecting blackberry in Brazil

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**Abstract**

Blackberry (*Rubus* spp.) is a shrub plant of the Rosaceae, which fruits are used for fresh markets or processing. Blackberry cultivation has increased in Brazil and has turned into an alternative for family farmers. Among the significant diseases that can occur in plantations, there is rust, caused by phytopathogenic fungus species: *Gymnoconia nitens* (orange rust), *Kuehneola uredinis* (cane and leaf rust) and *Phragmidium violaceum* (blackberry rust). This research project was conducted to identify the fungi specie causative of rust occurred on experimental blackberry fields, in Diamantina, state of Minas Gerais, Brazil. Infected blackberry leaves with symptoms of rust fungi and full spores yellow were collected. Infection was limited to undersurfaces of the leaves and reddening occurred on corresponding upper areas. No stem or fruit infection occurred on the infected plants. Morphological analyzes of the microstructures (sorus and spores) were carried out under optical microscopy and scanning electron microscopy (SEM). It was observed uredinia with urediniospores uniformly echinulate golden-yellow and telia obtain with teliospores smooth hyaline, mostly four celled and with a short rounded apical papilla. The rust was identified as *Kuehneola uredinis* (Link) Arthur. This is the first record for Brazil. Morphological descriptions, illustrations of the microstructures, examined material, geographic distribution and taxonomic comments are provided for this species.

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1. Introduction

Blackberries were once considered only a wild fruit, but cultivation of this domesticated plant has become important in several countries in the world, including Brazil. The blackberry is a description used for the blend of a number of *Rubus* species (Rosaceae), subgenus *Rubus* Watson (Clark and Finn, 2014). Although there are native *Rubus* species in Brazil, such like red mulberry (*Rubus rosifolius*) (Lorenzi et al., 2006), it was only in the 1970s that, studies with blackberry were started on Embrapa Clima Temperado, in the state of Rio Grande do Sul, with a small cultivars collection: Brazos, Cherokee, Comanche and a no identified clone, original from Uruguai (Raseira, 2004). The most significant producers in Brazil are the states of Rio Grande do Sul, Santa Caterina, Paraná and Minas Gerais (Tullio, and Ayub, 2013).

Some diseases are important in blackberry crop production, rust among them. Three rust fungus species have been described as pathogenic on blackberry: orange rust, caused by *Gymnoconia nitens* (Schwein.) F. Kern and Thust; cane and leaf rust caused by *Kuehneola uredinis* (Link) Arthur; and blackberry rust caused by *Phragmidium violaceum* (Schultz) G. Winter.

Historically, the species *P. violaceum* is native to Europe and was first reported in North America on 2005 (Osterbauer et al., 2005). The rust by *K. uredinis* comes from North America. The first report was in 1925 on Hawaii (Gardner and Hodges, 1983). This agent was also registered in 2011, causing damage to *Rubus fruticosus* L. in Korea (Cheon et al., 2013). Subsequently, registered as occurring in Central and South America (Yun, 2016). It is, currently,
K. uredinis was identified attacking several wild mulberry plants in the state of California (Shands et al., 2018). G. nitens is present in Asia, Europe, the United States and Canada (Arthur, 1917; Morris et al., 1999).

In Brazil, occurrences of blackberry rust in Rio Grande do Sul were reported by Antunes (2002) and Pagot et al., (2007). In 2014, Vigolo (2014) reported rust on Tupy cultivar plants in the municipality of Laranjeiras do Sul, Paraná. None of this cases were any information about the etiologic agent for the diseases. Currently, Marchioretto et al. (2017), reported rust on blackberry genotypes on an assay about protected crop.

In 2012, rust was observed in experimental blackberry fields at an altitude region of 1387 m, situated at 18°14’56”S, 43°36’00”, Diamantina, State of Minas Gerais, Brazil. The present study aimed to identify the rust fungi species that is occurring in this plantation.

2. Material and methods

Leaf samples were collected in an experimental field at the Fruit Culture Laboratory of the Department of Agronomy of Federal University of the Jequitinhonha and Mucuri Valleys, Brazil. In the experimental cultivation in Diamantina, there were clear implications due to the predominance of rust with chemical management being carried out annually as reported by the study group of the Fruit Growing sector (unpublished results). Infected blackberry plants of ‘Brazos’, ‘Tupy’, ‘Guarany’ and ‘Xavante’ cultivars, with rich yellow sporulation, were photographed in the field. Symptomatic leaves were collected for symptoms and signs description, as morphological characterization of spores in stereo and light microscopes in the Plant Pathology Laboratory.

For stereo microscopy, abaxial face of diseased leaves was directly captured for observation. For morphological analysis and measurement of the microstructures of rust (sori, urediniospores and teliospores) under an optical microscope (MO) were taken from diseased leaves and were mounted on semi-permanent slides with lactoglycerol (distilled water + lactic acid + glycerin) or lactophenol or cotton blue solution, were prepared by the methods described by Cummins and Hiratsuka (2003). They were examined under an Zeiss microscope Axiostar plus. Illustrations were prepared based on photomicrographs obtained from a digital camera coupled to an Olympus BX51. For spores observation in scanning electron microscope (SEM), the methodology adopted was from Franca and Sotão (2009) and the images were obtained in SEM brand LEO, model 1450VP and recorded in digital mode. Dry herbarium specimens have been deposited in Herbaria of Museu Paraense Emílio Goeldi in Belém, state of Pará, registered as MG227487.

3. Results

The symptomatic plant on the adaxial faces showed reddish areas (Fig. 1A). The signs were limited to undersurfaces of the leaves and reddening occurred on corresponding upper areas (showed Fig. 1B-C-D-E). No stem, petiole, branch, flower or fruit infection occurred on the symptomatic plants. It was observed uredinia and telia state. uredinia presented orange-yellow and telia in the whitish serum (Fig. 1D-E).

Urediniospores and teliospores morphological aspect on Fig. 2A-B, on lactophenol solution, uredinia subepidermal amarelo with parafises and urediniospores pedicelado, golden-yellow, globoid to obovoid, 20–25 × 15–20 μm, thick wall and uniformly echinulate (Fig. 2A-C-D). Telia subepidermal white to cream with urediniospores and teliospores hyaline, cylindric, 2–5 celled by horizontal septa, 40–70 × 7–10 μm, wall smooth; short rounded apical papilla; pedicel short (Fig. 2B).
4. Discussion

Our study provides evidence that the rust affecting blackberry cultivation in the Diamantina - MG region is caused by *K. uredinis* and not by *G. nitens* or *P. violaceum*. Our data are corroborated by the previous description for *K. uredinis*, which was reported infecting *Rubus ursinus* plants in California (Shands et al., 2018). The authors noted the occurrence of symptoms mainly on older leaves, with edges and the upper face of the leaves showing areas with necrotic spots and with the formation of bright yellow-orange pustules occurring on the abaxial face (Shands et al., 2018). In addition, they described uredinia as erumpent and measuring 100 to 400 µm in diameter, and obovoid, pale yellow-orange urediniospores (28–40 × 24–32 µm) and with equinulate spore walls 1.0–1.5 µm thick (Shands et al., 2018).

In contrast, *G. nitens* can be distinguished from *K. uredinis* based on the description performed on teliospores formed in chains of uninucleated sporogenous cells in large sori on the abaxial side of infected *Rubus argutus* leaves (Mims and Richardson, 2007). The authors described teliospores, in their two sporulated forms, as morphologically identical to aeciospores, and after the germination process, teliospores formed promycelia that subsequently gave rise to basidiospores in the form of two or four spores (Mims and Richardson, 2007).

On the other hand, *P. violaceum* has been morphologically characterized with the presence of yellow spots described with orange-yellow hypophyllous aecia and uredinia on the abaxial side of the leaf (Osterbauer et al., 2005). Furthermore, the authors reported the presence of globeose or ellipsoid eciospores, with hyaline and equinulate walls with spines. Regarding uredinia, they observed similarities to those described for aecia, while urediniospores were similar to aeciospores, except for the more pronounced ellipsoid shape and thicker walls and telia similar to uredinia, but with a black coloration. Teliospores were characterized as cylindrical and presenting between 3 and 5 cells, with slight contraction in the septa, rounded Apex, and rounded hyaline papilla (Osterbauer et al., 2005). In conclusion, based on the morphological parameters associated *K. uredinis*, the current data described will be useful for following studies involving *Rubus* sp. plants.

5. Conclusion

Based on morphological characteristics, the etiologic agent of blackberry rust studied was identified as *Kuehneola uredinis* (Link) Arthur, by mycologist Doctor Hellen M. P. Sotão.

Author contributions

F.R.V., H.C.M., and I.T.O., designed the experiments. F.R.V., and H.C.M., conducted the experiments. F.R.V., and C.M.T.F., performed the acquisition of samples and data. F.R.V., C.M.T.F., and I.T.O., analyzed the data. F.R.V., C.M.T.F., and H.C.M., wrote the manuscript with contributions of H.M.P.S., and I.T.O.

Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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