



Colletotrichum gloeosporioides causing leaf anthracnose on *Oxalis corniculata* in Brazil

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Abstract

Anthracnose leaf spot of creeping woodsorrel (*Oxalis corniculata*), caused by *Colletotrichum gloeosporioides*, is reported for the first time in Brazil. The identity of the etiological agent was confirmed by a combination of morphological and molecular information. Koch's postulates were fulfilled with a selected isolate of this fungus obtained at Santa Maria, state of Rio Grande do Sul, Brazil.

Keywords Fungal pathogen · Occurrence · Creeping woodsorrel · Leaf spots

Creeping woodsorrel (*Oxalis corniculata*) is a cosmopolitan species found in tropical and temperate areas, occurring as a weed in fields of beans, coffee, corn, potato and rice, where it can cause significant losses in yield. It is also present in grasslands, greenhouses, nurseries, and garden areas causing losses of plants (Hodi et al. 2014; CABI 2019). In addition, it may be an alternative host of pests and pathogens of crops (Guerra et al. 2016; Bellé et al. 2016).

Colletotrichum includes a large number of plant pathogens of major importance, causing diseases and affecting production of horticultural crops, such as avocado, banana, citrus, mango, strawberry, but can cause devastating disease with as well as crops such as coffee, maize, sorghum and sugarcane (Cannon et al. 2012). Anthracnose symptoms include limited, often sunken, necrotic lesions on leaves, stems, flowers and

fruit, as well as crown and stem rots, and seedling blight (Agrios 2005; Cannon et al. 2012).

Creeping woodsorrel leaf with anthracnose symptoms, circular to oval brown spots (Fig. 1), were collected in Santa Maria (GPS coordinates: 29°42'56"S; 53°42'21"W), Rio Grande do Sul state, Brazil, in September 2018. Leaf lesion samples were surface sterilized (5 × 3 mm) and placed on potato dextrose agar (PDA) at 25 °C with a 12-h photoperiod for 5 days. Colonies on PDA are circular, white to gray, with white margins and aerial hyphae, and the reverse of the colonies were gray to brown. Acervuli were dark brown without the presence of setae. Conidia were one-celled, cylindrical, hyaline, rounded at both ends, measuring ($n = 100$) 15.4 (14.4–17.5) ± 1.5 × 4.3 (3.5–5.0) ± 0.5 μm were observed similar to the description of *Colletotrichum* sp. (Weir et al. 2012).

Genomic DNA was extracted from a 7-day-old colony grown on PDA (isolate LPS031). Extraction was performed with a Wizard® Genomic DNA purification kit (Promega, USA) according to the manufacturer's instructions. The actin (ACT), calmodulin (CAL), chitin synthase (CHS-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-tubulin (TUB2) genes were amplified and sequenced (Weir et al. 2012). A Maximum Likelihood tree was constructed using representative isolates of *Colletotrichum* (Weir et al. 2012) to determine the phylogenetic relationship. Each gene sequence was aligned using CLUSTAL X (Thompson et al. 1997) for

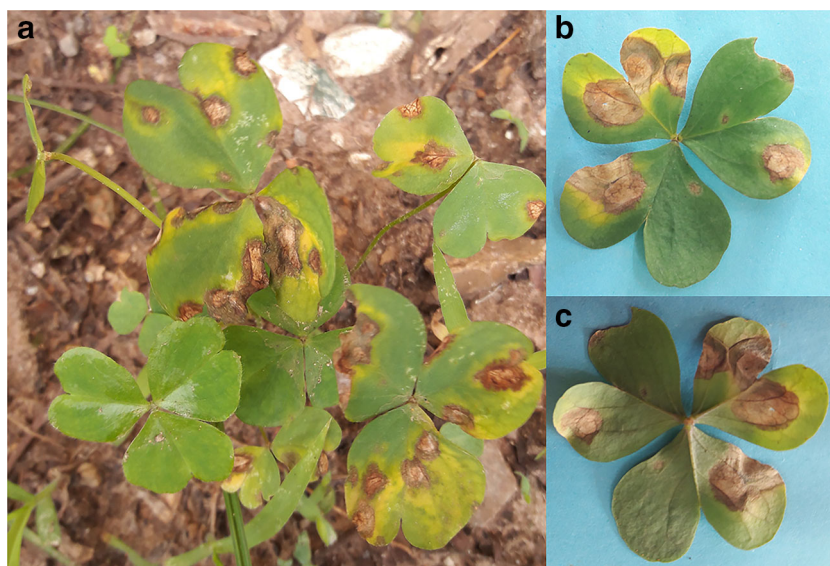
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Fig. 1 (A-C) Symptoms of leaf anthracnose caused by *Colletotrichum gloeosporioides* on *Oxalis corniculata*



a combined analysis using MEGA v7.0 (Kumar et al. 2016). The maximum likelihood analysis with 1000 bootstrap replicates was performed in MEGA v7.0 (Kumar et al. 2016) using the Jukes-Cantor model (Jukes and Cantor 1969).

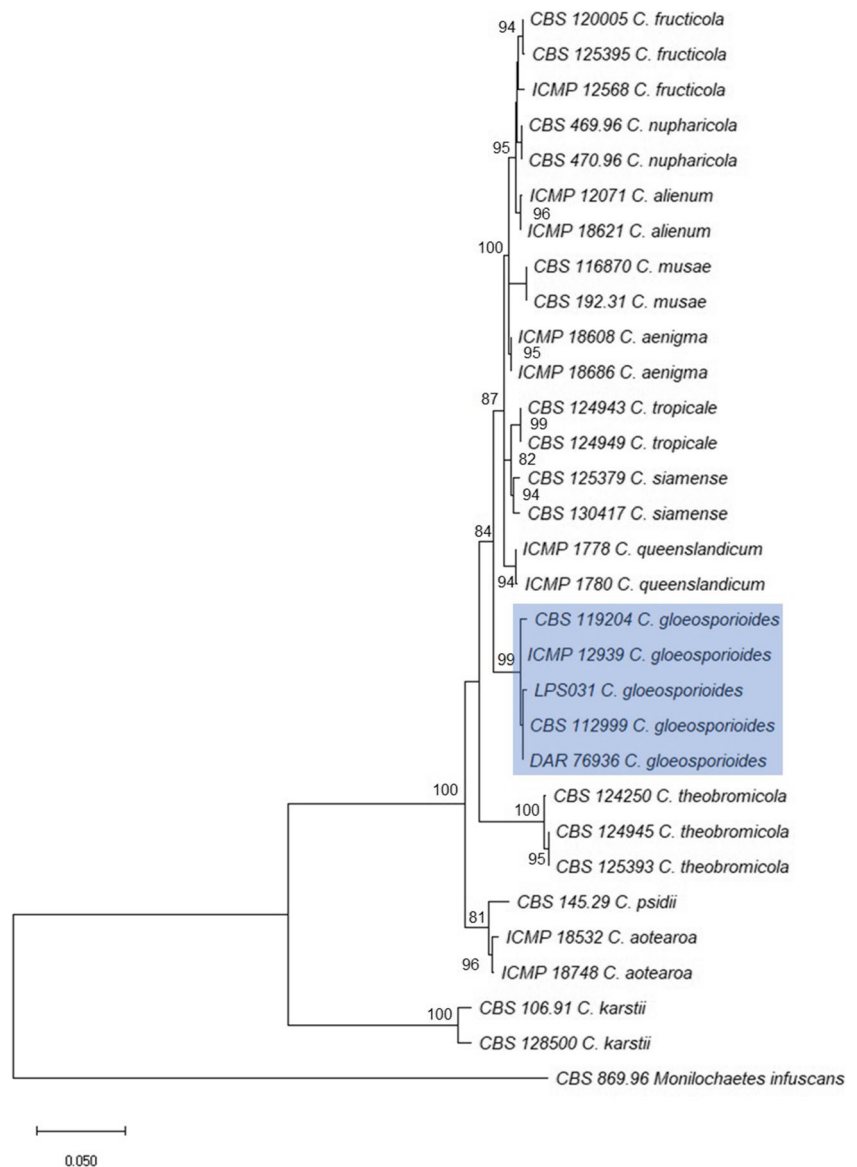
Sequences of the obtained DNA regions were submitted to GenBank (Accession Nos. ACT: MK639182, CAL: MK639180, CHS-1: MK639183, GAPDH: MK639184 and TUB2: MK639181.). MegaBLAST searches showed 99% to 100% identity with sequences of *Colletotrichum gloeosporioides* ex-type strain CBS 112999 (ACT: JQ005500, CAL: JQ005673, CHS-1: JQ005326, GAPDH: JQ005239 and TUB2: JQ005587). The phylogram constructed using combined datasets showed that isolates clustered into distinct clades with high bootstrap replicates; the isolate from this study clustered with *C. gloeosporioides* with 99% bootstrap (Fig. 2). Pure cultures were obtained and deposited in the culture collection of the Universidade Federal de Pelotas under the accession number LPS031.

To confirm Koch's postulates, six plants of *O. corniculata* were inoculated with a spore suspension (10^5 conidia/ml) of *C. gloeosporioides* from a 7-day-old culture. The conidial suspension was sprayed with an atomizer on all leaf surfaces of *O. corniculata* plants 20-days-old. After inoculation, the plants were covered with plastic bags to maintain high relative humidity, maintained at 25 °C with a 12-h photoperiod and mon-

itored daily for symptom development. Six plants inoculated with sterile water served as controls. Four days after inoculation, leaves displayed symptoms similar to those observed in the field, while controls remained symptomless. The pathogen was consistently re-isolated from the infected leaves and had the same morphological and molecular traits as the initial isolates.

Our findings highlight the relevance of *O. corniculata*, which may serve as a reservoir for *Colletotrichum gloeosporioides*. *Colletotrichum gloeosporioides* has been reported as a fungus causing various diseases mainly leaf anthracnose on a wide range of plant hosts in Brazil. It was, for example, reported as the cause of leaf spot of *Capsicum* sp., *Carica papaya*, *Citrullus lanatus*, *Citrus* sp., *Cucumis melo*, *Cucurbita pepo*, *Fragaria* × *ananassa*, *Glycine max*, *Gossypium* sp., *Malus domestica*, *Mangifera indica*, *Manihot esculenta*, *Olea europaea*, *Prunus persica*, *Solanum lycopersicum* and *Vitis* sp. (ANDEF 2019; Farr and Rossman 2019). From the literature consulted this is the first report in the presence of *C. gloeosporioides* affecting *O. corniculata* plants in Brazil. Therefore, control of this weed is important to manage the occurrence and impact of *C. gloeosporioides* in commercial crops.

Fig. 2 Consensus tree based on the Maximum Likelihood method based on the Jukes-Cantor model illustrating the phylogenetic relationships between *Colletotrichum gloeosporioides* and selected species. The tree was built using concatenated sequences of the TUB2, CHS-1, ACT, GAPDH, and CAL sequences genes. *Monilochaetes infuscans* was used as outgroup



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