

# *Colletotrichum siamense* causing anthracnose in *Bauhinia forficata* subsp. *pruinosa* in Argentina

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**Abstract** *Bauhinia forficata* subsp. *pruinosa* is a common perennial tree species native to South America that belongs to the Fabaceae. In December 2010 and 2011, plants from a nursery located in the province of Buenos Aires, Argentina presented typical symptoms of anthracnose. We isolated from the lesions of diseased leaves a fungus that based on morphological as well as the ITS (KC132841) and  $\beta$ -tubulin sequences (KJ829534) was identified as *Colletotrichum siamense*. After 12 days leaves inoculated with the fungal spore suspension developed lesions similar to those observed in natural infections. The fungus was then re-isolated fulfilling Koch's Postulates. To our knowledge, this is the first report of *Colletotrichum siamense* causing anthracnose in *Bauhinia forficata* subsp. *pruinosa* in Argentina.

**Keywords** *Colletotrichum siamense* · *Bauhinia forficata* · Anthracnose · *Colletotrichum gloeosporioides* complex · Pezuña de vaca · Tree native · Fabaceae

*Bauhinia forficata* subsp. *pruinosa* is a common perennial tree species native to South America that belongs to the

family Fabaceae. The tree is widely distributed mainly in tropical (Southern Brazil) and subtropical areas (Argentina), that are either free or have low risk of frosts (Burkart 1943). The tree has bilobed leaves and because of this is known as “pata de vaca”. This, and its large white flowers, are distinctive of the genus *Bauhinia* (Fig. 1). The plant is a source of medical compounds and is also used as a shrubby border plant (Burkart 1943; Cechimel Filho 2009). In December 2010 and 2011, within the area of La Plata, province of Buenos Aires, Argentina, nursery plants of *B. forficata* subsp. *pruinosa* that were routinely screened for diseases, presented circular or irregular, light brown necrotic lesions surrounded by a dark brown border, both in the center as well as the edges of the leaves (Fig. 1). Surface-sterilized disease leaf tissue was placed on Petri plates filled with potato dextrose agar (PDA) and incubated at 27 °C. We obtained monosporic cultures of a fungus that produced gray to black mycelium, whitish at the top, with peach to orange concentric rings of acervuli. Conidia were single-celled, hyaline, cylindrical-straight, with rounded ends and measured 9.6–14.4 long x 3–4.3  $\mu$ m wide.

Genomic DNA of the fungus was isolated as described by Meinhardt et al. (1993). By means of a PCR with two primers ITS5 and ITS4 (White et al. 1990), a DNA fragment of the isolate, containing the 3'terminal of the 18S rDNA, ITS1, the 5.8S rDNA, ITS2 and the 5'terminal of the 28S rDNA, was amplified in a M & J Research Minithermocycler. Also the  $\beta$ -tubulin partial sequences were amplified by means of two pairs of primers (Glass and Donaldson 1995). Both the ITS and  $\beta$ -tubulin amplicons generated were sequenced by means of the dideoxy termination methods (Sanger et al. 1977)

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**Fig. 1** *Bauhinia forficata* subsp. *pruinosa*: white flowers and bilobed leaves (left) and symptoms of anthracnose caused by *Colletotrichum siamense* (right)



at Macrogen (Korea). The ITS (KC132841) and the partial  $\beta$ -tubulin sequences (KJ829534) were used to run a query against the QBank Database of Quarantine Plant Pests and Diseases ([www.q-bank.eu](http://www.q-bank.eu)). The results confirmed that the fungus belonged to the *Colletotrichum gloeosporioides* complex, within the Musae clade of Weir et al. 2012. Several species within the clade are known to form perithecia in agar culture. However, the morphological characteristics as well as the ITS and  $\beta$ -tubulin sequences, confirmed the identity of the isolate as *Colletotrichum siamense* (Hyde et al. 2009; Weir et al. 2012). Both sequences were 99.8 and 99.3 % homologous to those of *Colletotrichum siamense*.

Pathogenicity of the fungus was tested by inoculating healthy leaves of three plants of *Bauhinia forficata* subsp. *pruinosa* with a conidial suspension ( $1 \times 10^6$  spores/mL) until run-off according to a technique used by several authors (Wolcan and Larran 2000; Wright et al. 2006; Okryun et al. 2012). After inoculation leaves were covered with plastic bags for 48 h and kept at room temperature. Controls were the leaves of healthy plants that were wounded and sprayed with distilled water. After 12 days leaves inoculated with the fungal spore suspension developed lesions similar to those observed in natural infections. No symptoms were observed on non-inoculated control plants. Furthermore, we readily re-isolated the fungus from lesions on inoculated leaves, fulfilling Koch's Postulates. A culture of the isolate used in the pathogenicity test and taxonomic studies was deposited in the Culture Collection of the Centro de Investigaciones de Fitopatología (CIDEFI), Facultad de Ciencias Agrarias y Forestales, University of La Plata as CG2 (Email: [cidefi@gmail.com](mailto:cidefi@gmail.com)).

*Colletotrichum siamense* has been cited as a pathogen of coffee berries, citrus, chilli and loquat in Thailand, China, India and Australia, respectively (Prihastuti and Hyde 2009; Cheng et al. 2013; James et al. 2014; Sharma and Shenoy 2013). To our knowledge this is the first report of *C. siamense* causing anthracnose in *B. forficata* subsp. *pruinosa* in Argentina.

## References

- Burkart AE (1943) Las leguminosas argentinas silvestres y cultivadas. Buenos Aires Acme Agency, SRL. Legumes, Pp. 590
- Cechimel Filho V (2009) Chemical composition and biological potential of plants from the genus *Bauhinia*. *Phytother Res* 23:1347–1354
- Cheng BP, Huang YH, Song XB, Peng AT, Ling JF, Chen X (2013) First report of *Colletotrichum siamense* causing leaf drop and fruit spot of *Citrus reticulata* Blanco cv. Shiyue Ju in China. *Plant Dis Notes* 11: 1508
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 61:1323–1330
- Hyde KD, Cai L, Cannon PF, Crouch JA, Crous PW, Damm U, Goodwin PH, Chen H, Johnston PR, Jones EBG, Liu ZY, McKenzie EHC, Moriwaki J, Noireung P, Pennycook SR, Pfenning LH, Prihastuti H, Sato T, Shivas RG, Tan YP, Taylor PWJ, Weir BS, Yang YL, Zhang JZ (2009) *Colletotrichum* – names in current use. *Fungal Divers* 39:147–182
- James RS, Ray J, Tan YP, Shivas RG (2014) *Colletotrichum siamense*, *C. theobromicola* and *C. queenslandicum* from several plant species and the identification of *C. asianamin* the Northern Territory, Australia. *Aust Plant Dis Notes*. doi:10.1007/s13314-014-0138-x
- Meinhardt LW, Krishnan HB, Balatti PA, Pueppke SG (1993) Molecular cloning and characterization of a sym plasmid locus that regulates cultivar-specific nodulation of soybean by *Rhizobium fredii* USDA257. *Mol Microbiol* 9:17–29
- Okryun C, Okhee C, Youn-Sig K, Jinwoo K, Jin-Hyeuk K (2012) Spot anthracnose disease caused by *Colletotrichum gloeosporioides* on tulip tree in Korea. *Microbiologia* 40:82–84
- Prihastuti LC, Hyde KD (2009) Characterization of *Colletotrichum* species associated with coffee berries in Chiang Mai, Thailand. *Fungal Divers* 39:98
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977 Dec; 74(12): 5463–5467
- Sharma G, Shenoy BD (2013) *Colletotrichum fructicola* and *C. siamense* are involved in chilli anthracnose in India. *Arch Phytopathol Plant Protect* 47:1179–1194. doi:10.1080/03235408.2013.833749
- Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. *Stud Mycol* 73:115–180
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal rDNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*. Academic Press Inc. Pp. 315–322
- Wolcan S, Larran S (2000) First report of anthracnose caused by *Glomerella cingulata* on Passion fruit in Argentina. *Plant Dis Notes* 84:706. doi:10.1094/PDIS.2000.84.6.706A
- Wright ER, Rivera MC, Mascarini A, Núñez LS, Gentile CM (2006) Florist's *Cyclamen* anthracnose caused by *Colletotrichum gloeosporioides* in Argentina. *Australas Plant Dis Notes*. doi:10.1071/DN06001