



Leaf spot on *Clerodendrum x speciosus* in Brazil caused by *Pseudocercospora clerodendricola* sp. nov.

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ABSTRACT

Pseudocercospora clerodendricola sp. nov. is described herein. The fungus was found causing leaf spots in *Clerodendrum x speciosum* (bleeding heart) and its pathogenicity was demonstrated. Inoculation with culture discs placed on healthy leaves resulted in typical leaf spots appearing 30 days after inoculation.

Keywords: Cercosporoids, Mycosphaerellaceae, ornamental plant, plant pathology, taxonomy, Verbenaceae.

RESUMO

Mancha foliar em *Clerodendrum x speciosus* no Brasil causada por *Pseudocercospora clerodendricola* sp. nov.

Uma nova espécie de fungo, *Pseudocercospora clerodendricola* é descrita em associação com *Clerodendrum x speciosum* (coração sangrento). Ela teve sua patogenicidade demonstrada pela inoculação de folhas sadias com discos de micélio. Manchas foliares equivalentes às observadas no campo foram produzidas 30 dias após a inoculação das plantas com discos de cultura.

Keywords: Cercosporoids, Mycosphaerellaceae, ornamental plant, plant pathology, taxonomy, Verbenaceae.

Studies on fungal pathogens of ornamental plants in Brazil have yielded numerous new disease records for Brazil (e.g.: Silva et al, 2006; Pereira et al. 2006; Ribeiro et al. 2006; Macedo & Barreto, 2008; Soares et al., 2009) and also novel fungal species such as *Cordana versicolor* Soares & R.W. Barreto (Soares et al., 2005) and *Cercospora neomaricae* Macedo & R.W. Barreto (Macedo & Barreto, 2008). Damage caused by such fungi varies from minor to very significant leading to losses in the commercial production of ornamentals or in the loss of their ornamental value through rendering infected plants unsightly.

Clerodendrum x speciosum Teijsm. & Binn. (Verbenaceae), is a hybrid between *C. thomsoniae* Balf. and *C. splendens* G. Don, which are from Africa. Several species belonging to *Clerodendrum* are widely used as ornamentals in Brazil, mostly to cover fences, gates and walls, because of their shrubby/scandent habit and attractive inflorescences (Lorenzi & Souza, 2001). There is little published information on pathogens of *Clerodendrum* worldwide (Farr et al., 2009; Mendes et al., 1998). In Brazil, besides three old records (Batista et al., 1964, 1967a, b), there is only a recent note on the occurrence of a fungal disease on a *Clerodendrum*: namely that of gray mold, *Botrytis cinerea* Pers. ex Fries, on *Clerodendrum splendens* (Macedo & Barreto, 2009). In January 2008, *Clerodendrum x speciosum* individuals bearing abundant leaf spots were found in a private garden in Viçosa, Minas Gerais, Brazil (Figure 1). A search through the literature yielded no record

of that disease on *Clerodendrum* in Brazil or elsewhere. A study was then undertaken to clarify the etiology of this disease.

Specimens were collected, immediately brought to the lab and examined while still fresh. A fungus sporulating on diseased tissues was consistently associated with the leaf spots. Fungal structures were scraped with a scalpel from the infected tissues, mounted in lactophenol on glass slides and examined with a light microscope (Olympus BX 51) equipped with a digital camera (Olympus evolt E-330). The fungus was also isolated in VBA (vegetable broth-agar) (Pereira et al., 2003). A representative culture was deposited in the culture collection of the Universidade Federal de Viçosa (Coleção Micológica da Universidade Federal de Viçosa – CMUFV) under the provisional accession number DMM 75. Cultures were described based on observations of 19 day-old colonies on potato dextrose agar (PDA), malt extract agar (MEA) and vegetable broth agar (VBA) at 25°C, either under a 12-h light regime or under constant dark. Furthermore, the pathogenicity of the fungus was investigated. In this test two young *Clerodendrum x speciosum* plants around 20 cm tall were used. One plant was inoculated with culture disks obtained from the margin of an actively growing culture in VBA and placed on each side of three leaves. The other plant serving as control was treated the same way but only had sterile culture medium disks deposited on leaves. After inoculation, plants were covered with plastic bags with moist paper placed at the



FIGURE 1 - *Pseudocercospora clerodendricola* on *Clerodendrum x speciosum*. **A.** *Clerodendrum x speciosum* plant bearing *Pseudocercospora clerodendricola* leaf spots; **B.** Leaf of *Clerodendrum x speciosum* with *Pseudocercospora clerodendricola* leaf spots at various stages of development; **C.** close-up of a fully developed leaf spot showing an irregular necrotic centre and a chlorotic halo.

base of the plants in order to maintain high humidity. After 48 hours the plastic bags were removed and the plants were left in a greenhouse at $25 \pm 2^\circ\text{C}$ and wetted twice a day. After a week plants were observed regularly for disease symptoms.

Pseudocercospora clerodendricola D.B. Almeida, D.M. Macedo & R.W. Barreto, sp. nov. MycoBank MB 516522 (Figure 2)

Etymology: based on the host genus *Clerodendrum*.

Differt a *P. clerodendrigena* laesio emmortus irregularis et halo luteos 0.4-1.2 mm longitudo et 0.3-2.7 mm latis; conidiophoris epigenous 15-23 longitudo et 3-6 μm latis; conidiis 74-103 x 3-4 μm , 5-11 septati. Differt a *P. kashotoensis* laesio; *stromatibus* 40-55 μm latis, conidiophoris numerosibus, etiam conidiophoriis epigenous et erumpens.

Holotype: BRAZIL, MINAS GERAIS: Viçosa. On living leaves of *Clerodendrum x speciosum*, 07 April 2008, D.M. Macedo (HOLOTYPE: VIC: 30734).

In folliis *Clerodendrum x speciosum* Teijsm. & Binn. (Verbenaceae).

Lesions on living leaves, starting as small chlorotic spots, becoming irregular, necrotic, dark brown to black with a yellow halo, 0.4-1.2 x 0.3-2.7 mm, coalescing and leading to blight of large areas of infected leaves; *internal mycelium* inter and intra-cellular, 3-5 μm diam, branched, septate, pale brown; *stromata* hypophyllous, erumpent, irregular to sub-globose, composed of pale to dark brown *textura angularis*, 15-50 x 40-55 μm diam.; *conidiophores* mostly reduced to short conidiogenous cells grouped over stromata in sporodochia, sub-cylindrical, 15-23 x 3-6 μm ,

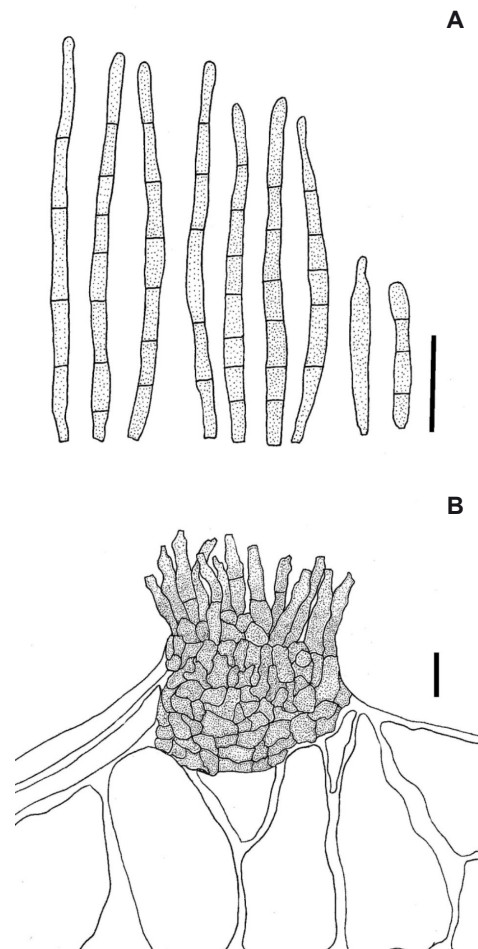


FIGURE 2 - *Pseudocercospora clerodendricola* on *Clerodendrum x speciosum*. **A.** conidia; **B.** fascicle of conidiophores (bars = 20 μm).

0-1 septate, smooth, pale brown; *conidiogenous loci*, often at attenuate apex of conidiogenous cells, 2. μm diam, not thickened, not darkened; *conidia* isolate, cylindrical to sub-cylindrical, 74-103 x 3-4 μm , base attenuated to a 1-3 μm diam truncate hilum not thickened, not darkened, 5-11 septate, guttulate, pale brown smooth.

In culture: colonies slow-growing (2.8-3.1 cm diam in 19 days); aerial mycelium ranging from smoke-gray to olivaceous-gray; colonies usually elevated centrally, of feltrose aerial mycelium, in VBA with a black rim of immersed mycelium at periphery; with diurnal zonation. In MEA with radiating immersed mycelium or corrugations at periphery, in PDA reverse dark gray with a black diffusate, sporulation absent.

Six cercosporoid taxa have been described in association with the genus *Clerodendrum*, namely: *Cercospora apii* s. lat. (originally described as *C. volkameriae* Speg. (according to Crous & Braun, 2003), *Cercospora apii* f. sp. *clerodendri* Sorbes & Martínéz, *Cercospora bakeri* Syd., *Passalora clerodendri* (Goh & W.H. Hsieh) U. Braun & Crous, *Pseudocercospora clerodendrii* (J. Miyake) Deighton and *P. kashotoensis* (W. Yamam) Deighton (Crous & Braun, 2003; Farr et al., 2009). The new fungal species found on *C. x speciosum* clearly belongs to *Pseudocercospora* as it has the typical features delimiting this genus: specifically, conidiophores bearing unthickened and not darkened conidiogenous loci, and brown conidiophores and conidia (Crous & Braun, 2003). It is, therefore, clearly dissimilar to fungi in the *Cercospora apii* complex, as these have very long conidiophores (some being over 380 μm long) and long acicular conidia that are hyaline and have thickened and darkened hila (Crous & Braun, 2003). Although *C. bakeri* Syd. was regarded as having a doubtful taxonomic status and not belonging to *Cercospora* according to Crous & Braun (2003), it was described by Chupp (1953) as having significantly smaller conidia (30-60 x 4-6.5 μm) than those of *P. clerodendricola*. *Passalora clerodendri* was formerly placed in *Mycovellosiella* but recombined into *Passalora* by Braun & Crous (2003) for having the typical features for this genus, such as subhyaline to pigmented conidiophores and conidia and scars somewhat thickened and darkened. Features of conidial scars clearly separate the new species from members of *Passalora*. There are only two species of *Pseudocercospora* described in association with this plant genus: *P. clerodendri* [described as *P. clerodendrigena* by Braun (2002) and later treated as a synonym] and *P. kashotoensis* (W. Yamam) Deighton (Chupp, 1953; Yen et al., 1982). Conidiophores in *P. kashotoensis* are long-fasciculate (25-75 x 4-6 μm) and 2-7 septate instead of grouped in sporodochia and predominantly reduced to conidiogenous cells as in the new species. In *P. clerodendri*, the conidia are shorter and narrower (10.0-35.0 x 2.0-2.5 μm) (Braun, 2002). After 30 days of inoculation with *P. clerodendricola*, leaf spot symptoms

identical to those seen in the field were observed on inoculated leaves but not on controls. The fungus was reisolated and formed colonies having a morphology equivalent to that described for *P. clerodendricola*.

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