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Report of *Paramyrothecium roridum* Causing Circular Leaf Spots on *Impatiens walleriana* in Brazil.


Garden impatiens (*Impatiens walleriana* Hook. f. – Balsaminaceae) has been largely employed as a flowering (ornamental) potted species in Brazil, being propagated by either cuttings or seeds. In August 2016, commercial container-grown *I. walleriana* plants exhibiting dark brown, circular to semicircular leaf spots (0.5 to 10 mm diameter) were found in commercial nurseries in Atibaia (São Paulo State, Brazil). Under high humidity, the abaxial surface of the lower leaves displayed black sporodochia. Leaf fall was observed a few days after the beginning of the symptoms. Approximately 30% of the plants were unsuitable for sale. Symptomatic tissues were disinfested using 70% alcohol for 1 min, 2% sodium hypochlorite for 1 min, followed by a double rinse in sterilized distilled water for 1 min. The tissues were transferred to Petri dishes with potato dextrose agar (Merck®) medium and incubated at 25°C (12 hours light/dark) for five days. Young fungal colonies displayed white mycelium with concentric rings varying from dark green to blackish shades. Hyaline, rod-shaped conidiogenic cells (9 to 21 × 1.9 to 3 µm), hyaline and cylindrical conidia (5.2 to 6.5 × 1.6 to 2.5) with rounded tips were observed microscopically. These characteristics were used to identify the fungus as *Paramyrothecium roridum* Tode ex Fr. (Tode: Fr.) L. (Lombard et al. 2016). Genomic DNA was extracted from two isolates with a modified CTAB method (Boiteux et al., 1999), followed by PCR and sequencing of the rDNA-ITS-derived amplicons obtained with the primer pair ITS1 and ITS4 (White et al., 1990). The fungal isolates were deposited in the public collection “Maria Menezes” at UFRPE (Recife–PE, Brazil) under the accession numbers CMM4727 and CMM4728. The two *I. walleriana* isolates (MF967280 and MF967281) displayed 100% identity to each other and 98.5% with the type *P. roridum* isolate ‘CBS 357.89’ (KU846301). Healthy *I. walleriana* plants were sprayed with a suspension of conidia (10⁴ spores/mL). Inoculated seedlings were incubated in a moist chamber at 25°C for 48 hours. Mock-inoculated plants (controls) remained free of symptoms. Leaf symptoms identical to those seen under nursery conditions were observed ten days after
inoculation. *Paramyrothecium roridum* isolates with similar morphological characteristics were recovered from these lesions, fulfilling Koch’s postulates. This fungus has been previously reported infecting *I. balsamina* in Asia (Farr & Rossman, 2017). However, to our knowledge, this is the first report of this pathogen in *I. walleriana* in Brazil or anywhere in the world. The occurrence of this broad host range fungal species in *I. walleriana* will require specific disease management approaches in order to avoid potential commercial losses.

References


Figure 1. Symptoms of spots on leaves of *Impatiens walleriana* (A), aspect of the colony of *Paramyrothecium roridum* on PDA (B) and morphological aspects of conidiophores, phialides and conidia of the fungi (C, D, E and F).
**Figura 2.** Phylogenetic tree constructed with sequences of the ITS region of the isolates Paralmpa01 and Paralmpa02 (CMM4727 and CMM4728) and other *P. roridum* reference isolates.