First report of *Seimatosporium hypericinum* causing leaf spots on the ornamental shrub *Hypericum* × *inodorum* and in Brazil

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Abstract

Hypericum × *inodorum* is an ornamental plant that has become popular for the cut flower industry in Brazil, in recent years. No pathogen has been reported on this plant in Brazil. A coelomycete asexual morph was found associated with leaf spot and severe foliage blight on this host and is reported here for the first time. A combination of morphological and molecular features allowed it to be identified as *Seimatosporium hypericinum*. Koch's postulates were performed and demonstrated, for the first time, that *S. hypericinum* is pathogenic to *H.* × *inodorum*.

Keywords Coelomycete asexual morph · Cut flower · Etiology · Foliage blight · Ornamental plant · Taxonomy

There is little information published in the scientific literature about the pathogens that affect many of the ornamentals grown in Brazil, particularly for “minor” or novelty ornamental crops. A case in point is sweet amber or tutsan (hipérico in Brazil) *Hypericum* × *inodorum* (Hypericaceae), a hybrid of *H. androsaemum* and *H. hircinum*, two perennial shrubs from Eurasia. It is grown for its stems which bear yellow flowers and red and pink coloured fruits (de Assis 2015) (Fig. 1a), and has been growing in popularity in the cut flower market in Brazil (Lorenzi and Souza 2008).

In May 2015, *H. inodorum* plants cultivated in an experimental plot measuring the effect of light regimes on flowering, on the campus of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil), had severe foliar necrosis (Fig. 1b). Samples of leaves with necrotic symptoms were collected, dried in a plant press, and deposited in the herbarium at the Universidade Federal de Viçosa (Acc. No VIC 43000). All leaves were examined under a dissecting microscope and fungal structures were

consistently associated with the necrotic tissues. Sections of infected leaves bearing fungal structures were prepared and slides were mounted in lactophenol or lactofuchsin. Observations of the fungal morphology were made under an Olympus BX 51 light microscope equipped with DIC and an Olympus E 330 camera.

The fungus had the following morphology: Lesions beginning as numerous, small irregular or angular blackened areas on leaves 1–6 mm wide later surrounded by reddish brown poorly defined necrotic areas developing a greyish center, coalescing and leading to entire necrosis of leaves and defoliation of plants; *Conidiomata* acervular, sub-epidermal, 26–106 µm diam., dark brown; *Conidiogenous cells* cylindrical, holoblastic, phialidic, 4–12 × 2–3 µm, hyaline, smooth; *Conidia* fusiform, straight or curved, 12–24 × 3–5 µm, 3–septate, two median cells, medium brown, apical cells hyaline, each terminal cell bearing two unbranched, whip-like, hyaline appendages, 8–23 µm long (Fig. 1c–e). This morphology is equivalent to that of *Seimatosporium hypericinum*, as described in Sutton (1980) and Nag Raj (1993).

Isolation in pure culture was performed by direct transfer of spores or other fungal structures onto plates containing potato dextrose-agar (PDA), with a sterile finely pointed needle. Pure cultures were preserved in 10% glycerol and silica-gel. One sporulating single-spore culture was

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Fig. 1 **a.** *Hypericum* × *inodorum* flowering and fruiting; **b.** Symptoms of leaf blight caused by *Seimatosporium hypericinum* on *H. × inodorum* **c.** Section of an acervulus with conidia; **d.** Conidia and conidiogenous cells;

e. Mature conidia with pigmented median cells and hyaline apical cells ending in whip-like hyaline appendages; **f.** Colony on PCA after 15 days at 24 °C. Scale bars = 20 µm

deposited in the culture collection of the Universidade Federal de Viçosa (Acc. No COAD 2362b). Culture morphology was described based on observation of the colonies formed after 10 days in plates containing PDA and potato carrot-agar (PCA) and incubated at 25 °C with a 12 h daily light regime. Colour terminology followed Rayner (1970). Colonies were slow growing (reaching 3–3.5 cm diam. after 8 days), low convex to umbonate, margin entire, aerial mycelium wooly white to saffron; reverse centrally salmon or cinnamon, white to salmon at periphery; sporulation on PCA after 14 days, concentrated in black and globular masses (Fig. 1f).

Additionally, in order to better clarify the identity of the pathogen, a molecular characterisation of the isolate

was performed. COAD 2362b was grown in plates containing potato dextrose and incubated at 23 ± 2 °C for four days. DNA was extracted from the fungus mycelium with the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region was amplified with primers ITS1 and ITS4 (White et al. 1990). PCR amplification was performed as described by Tanaka et al. (2011). Sequences were generated by MACROGEN, Inc. (South Korea). Bayesian inference analyses were performed with the CIPRES web portal using MrBayes v. 3.2.6. (Miller et al. 2015). The jmodeltest (Darriba et al. 2012) was used for estimation of sequence evolution. Phylogenetic analysis was performed with COAD 2362b

and selected sequences from additional taxa withdrawn from GenBank (Table 1). Phylogenetic trees were visualised in FigTree (Rambaut 2009).

A BLAST search of GenBank accessions resulted in 100% homology between COAD 2071b and a previously deposited sequence of *S. hypericinum* (isolate NBRC32647 -Accession No. AB594805). The sequence of COAD 2071b was deposited in GenBank under the Accession No, MG385139. The combination of morphological features and phylogenetic analyses (Fig. 2) placed the fungus unequivocally in *S. hypericinum*.

Conidia were produced on PDA and a suspension (1.0×10^6 conidia/ml) was used to inoculate four healthy plants. Two plants were sprayed with sterile water and served as

controls. The plants were covered with internally humidified plastic bags, containing a cotton plug soaked in water, and maintained in a greenhouse with a temperature at 22 and 26 °C for 48 h. After that period, the plastic bags were removed and the plants were observed daily for symptom development. All inoculated plants showed typical leaf spot symptoms 5 days after inoculation and the pathogen was re-isolated from diseased tissues. Controls remained healthy.

Seimatosporium was regarded by Tanaka et al. (2011) as a monophyletic genus of saprobic or plant pathogenic coelomycetous species of so-called ‘pestalotioid fungi’. Norphanphoun et al. (2015) stated that “*Seimatosporium* species are common as saprobes and pathogens on plants, but are

Fig. 2 Phylogenetic tree inferred from Bayesian analysis based on combined ITS sequences. Isolate from Brazil in bold face. Bayesian posterior probabilities are indicated at the nodes. The tree was rooted with *Discosia pini* (isolate MAFF 410149). Note that the name *Discostroma* has been generally used for the sexual morph of *Seimatosporium*

Table 1 GenBank Accession numbers of the sequences used in phylogenetic analysis

Fungal Species	Strain Number	GenBank Acc. No. ITS
<i>Discostroma fuscillum</i>	NBRC 32625	AB594794
	NBRC 32680	AB594806
	418-BB	GU244511
<i>Discostroma stoneae</i>	NBRC 32690	AB594797
<i>Discostroma tostum</i>	NBRC 32626	AB594795
<i>Pseudopestalotiopsis theae</i>	MFLUCC 12–0055	JQ683727
<i>Sarcostroma bisetulum</i>	CBS 122695	EU552155
<i>Sarcostroma restionis</i>	CBS 118153	DQ278923
	CBS 118154	DQ278922
<i>Seimatosporium biseptatum</i>	CPC 13584	JN871199
<i>Seimatosporium botan</i>	H4619	AB594799
<i>Seimatosporium botan</i>	H4619	AB594779
<i>Seimatosporium discosioides</i>	H4621	AB594800
	KACC 42490	EF600969
<i>Seimatosporium elegans</i>	NBRC 32674	AB594801
<i>Seimatosporium elegans</i>	NBRC 32674	AB594801
<i>Seimatosporium foliicola</i>	NBRC 32676	AB594802
<i>Seimatosporium glandigenum</i>	NBRC 32677	AB594803
<i>Seimatosporium grevilleae</i>	ICMP 10981	AF405304
<i>Seimatosporium hakeae</i>	NBRC 32678	AB594804
<i>Seimatosporium hypericinum</i>	NBRC 32647	AB594805
<i>Seimatosporium lichenicola</i>	MFLUCC 14–0623	KT198724
<i>Seimatosporium mariae</i>	NBRC 32681	AB594807
<i>Seimatosporium obtusum</i>	CPC 12935	JN871206
<i>Seimatosporium parasiticum</i>	NBRC 32682	AB594808
<i>Seimatosporium physocarp</i>	MFLUCC 14–0625	KT198722
<i>Seimatosporium pistaciae</i>	CPC 24455 / CBS 138865	KP004463
	CPC 24457	KP004464
<i>Seimatosporium rosae</i>	MFLUCC 14–0621	KT198726
<i>Seimatosporium walkeri</i>	CPC 17644	JN871207
<i>Seimatosporium hypericinum</i>	RWB 2362b	MG385139
<i>Discosia pini</i>	MAFF10149	AB594776

not considered as destructive foliar pathogens”. However, from observations of this fungus on *H. perforatum*, Zimowska (2004) stated that it is frequently isolated from plants showing symptoms of necrosis and cracks in the bark of stems as well as from leaves showing red-amber necrotic spots. Our observations of *S. hypericinum* on *H. × inodorum* showed it is a damaging pathogen to tutsan.

Seimatosporium hypericinum has been reported on *H. perforatum* in Italy (Nag Raj 1993; Sutton 1980) and Poland (Adamska 2007) on *H. quadrangulum* in Sweden (Sutton 1975) and on an undetermined species of *Hypericum* in Australia (Cunnington 2003). Additionally there is a record of this fungus on *Lonicera astandishii* (Caprifoliaceae) in Ukraine. This record, however, requires confirmation as it is the only existing record of this fungus on a non-*Hypericum*

host and on a genus belonging to a plant family different from Hypericaceae. It appears that *S. hypericinum* is a pathogenic fungus specialised on members of *Hypericum*. *Hypericum × inodorum* represents a new host for this fungus, which is newly reported from Brazil.

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