

# First Report on Colletotrichum sansevieriae Causing Anthracnose of Sansevieria trifasciata in Germany

Thomas Brand<sup>1</sup> · Alexandra Wichura<sup>2,3</sup>

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#### Abstract

*Sansevieria* species are valued in Europe as potted houseplants because of their modest maintenance requirements and low susceptibility to diseases and pests. Water-soaked leaf spots that eventually coalesced into large, drying lesions were observed on *Sansevieria trifasciata* freshly imported from Costa Rica. A *Colletotrichum* was isolated from the fungal fruiting bodies that developed on these necroses. It was confidently determined to be *C. sansevieriae* based on the host plant and microbiological as well as molecular biology test results. This is the first detection of *C. sansevieriae* in Germany.

Keywords Dracaena · Leaf spots · Mother-in-law's tongue · Ornamental plant

# Introduction

Species of *Sansevieria* occur naturally in Africa, the Arabian Peninsula (Yemen), India, Sri Lanka, Myanmar and the Comoros. They are xerophytic, perennial, herbaceous, caulescent or acaulescent plants, sometimes branching at the base and spreading by underground rhizomes or above-ground stolons. The leaves, flat or cylindrical, arranged in rosettes, are leathery to succulent (Newton 2020). Currently about 85 species are accepted by Newton (2020), whom we follow in this work in terms of retaining the genus name *Sansevieria*, although recent phylogenetic studies suggest that *Sansevieria* might represent a clade of herbaceous plants within the genus *Dracaena* only (Takawira-Nyenya et al. 2018; van Kleinwee et al. 2022).

Used as fiber crop (Brink and Achigan-Dako 2012; Fiscal and Dandan 2016; Adeniyi et al. 2020) or for ethno–botanical purposes (Mohana et al. 2008; Takawira-Nyenya and Stedje 2011; Takawira-Nyenya et al. 2014) *Sansevieria* is of regional economic significance. More-

- <sup>2</sup> Plant Protection Office, Chamber of Agriculture Lower Saxony, Wunstorfer Landstr. 9, 30453 Hannover, Germany
- <sup>3</sup> Organic Farming, Chamber of Agriculture Lower Saxony, Wunstorfer Landstr. 9, 30453 Hannover, Germany

over, especially varieties of *S. trifasciata* are worldwide cultivated as ornamentals. They are used outdoors in subtropical and tropical regions as ground cover or elements of landscaping, while in cooler regions indoors as potted houseplant and for decorative purposes (Henley 1982; Nakamura et al. 2006). Their advantages are modest maintenance, especially in water and nutrient supply, and low susceptibility to pests and diseases.

However, several fungal pathogens are recognized causing leaf spots on Sansevieria spp., such as Chaetomella sp. (Li et al. 2013), Stemphylium lycopersici (Kee et al. 2017a), S. vesicarium (Ahmadpour and Poursafar 2018), Neoscytalidium dimidiatum (Kee et al. 2017b; Monteles et al. 2020), Lasiodiplodia spp. (Kee et al. 2019), Curvularia spp. (Kee et al. 2020a), Fusarium spp. (Kee et al. 2020b) as well as Colletotrichum neosansevieriae and Stachybotrys sansevieriicola (Crous et al. 2015). However, based on the number of reports, another fungal leaf spot pathogen, appears to be of greater importance: After observations of leaf spot (anthracnose) symptoms on S. trifasciata 'Laurentii' occurring since 1996 in subtropical regions of Japan, a Colletotrichum species was found as causal agent and introduced as new species, C. sansevieriae (Nakamura et al. 2006). Subsequently, this pathogen was reported from other subtropical and tropical regions like Victoria/Australia, Florida/USA, Madhya Pradesh/India, Costa Rica, Korea, Iran, Thailand, and Malaysia (Aldaoud et al. 2011; Campoverde and Palmateer 2012; Gautam et al. 2012; Palmateer et al. 2012; Karimi et al. 2017; Kee et al. 2020c; Li et al. 2020; Park et al. 2013; Pérez-León et al.

<sup>☑</sup> Thomas Brand thomas.brand@lwk-niedersachsen.de

<sup>&</sup>lt;sup>1</sup> Plant Protection Office, Chamber of Agriculture Lower Saxony, Sedanstr. 4, 26121 Oldenburg, Germany

2013). In all reported cases, the infected host species was *S. trifasciata*.

Phylogenetical studies revealed that *C. sansevieriae* belongs to the clade 'Sansevieriae' which comprises species that infect succulent plants originating from Africa (Nakamura et al. 2018).

In spring 2022, leaf spots were observed in several cultivars of *Sansevieria trifasciata* ('Robusta', 'Zeylanica', 'Aubrytiana Sayuri', 'Futura', 'Laurentii', and 'Moonshine') at an ornamental plant nursery in Lower Saxony, Germany. The plants were imported from Costa Rica in February 2022. In order to be able to take appropriate countermeasures, inhibit further spread and prevent repeated occurrence, investigations were conducted to determine the cause of this disease.

# **Material and Methods**

#### **Source of Samples**

In April 2022, several symptomatic specimens of the cultivars 'Laurentii' and 'Moonshine' were brought from the above-mentioned nursery to the laboratory of the Plant Protection Office of the Chamber of Agriculture of Lower Saxony, Oldenburg.

#### Microbiological Identification

After visual inspection of the plants, detached symptomatic leaves were incubated in humid chambers on wet filter paper at room temperature.

Under axenic conditions, without surface disinfection, small sections of leave tissue taken from the border of leaf spots were laid out on half strength potato dextrose agar (PDA<sub>50%</sub>; 19.5 g l<sup>-1</sup> potato extract glucose agar (Carl Roth, Karlsruhe, Germany), 7.5 g l<sup>-1</sup> agar) and incubated at room temperature. Single spore isolates were produced on PDA<sub>50%</sub> by spreading a conidial suspension with a Drigalski spatula successively onto several plates, and finally separating solitary microcolonies.

Mycelia plugs (5 mm diameter) were transferred to PDA<sub>50%</sub> and the irregularly roundish grown colonies were measured along the axis of widest expansion after 14 days of growth at 25 °C in the dark (n = 10).

Conidia were harvested from mature fruiting bodies on diseased plant tissue, suspended in water and examined microscopically (Eclipse Ni-U; Nikon Europe B.V., Amstelveen, NL). Appressoria were obtained using the slide culture method described by Smith and Black (1990), and the length was measured.

Microscopic images and measurements of characteristic structures such as conidia (n=200) and appressoria (n=50)

were made using the digital camera DS-Fi3, equipped with the corresponding software NIS-Elements D 5.20.01 (Nikon Europe B.V., Amstelveen, NL). All measurements were made at 400 × magnification and are reported as (minimum–) mean $\pm$  standard deviation (–maximum) and as median.

### **Phylogenetic Identification**

For DNA isolation freshly grown mycelium was scraped off in a 2ml reaction tube and slightly homogenized with a pestle prior to further processing. DNA was extracted using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) following the manufacturer's instructions given in protocol 1.

Internal transcribed spacer (ITS) gene regions were amplified by using primer pairs ITS1F/ITS4 (Gardes and Bruns 1993; White et al. 1990) were used. PCR was performed in a reaction volume of 50 $\mu$ l containing final concentrations of 1 × ready-to-use MyTaq Plant-PCR Mix (Bioline Meridian Bioscience, London, UK), 5 $\mu$ l genomic DNA (1:10 diluted) and 0.4 $\mu$ M of each primer. Amplification conditions were 5 min at 95 °C, followed by 30 cycles at 94 °C for 30s, 55 °C for 45 s, 72 °C for 90 s, followed by a final extension at 72 °C for 10 min.

PCR products were purified using innuPREP PCRpure Kit (Analytik Jena AG, Jena, Germany). For bidirectional Sanger sequencing the same primer pairs were used as well. A consensus sequences was prepared by assembling and analyzing electropherograms in DNA Sequence Assembler v5 (Heracle BioSoft S.R.L, Arges, Romania). For preliminary molecular identification GenBank was searched for similar ITS sequences using blastn (Altschul et al. 1990).

However, according to Weir et al. (2012) Colletotrichum species could not reliably distinguished by using ITS sequence alone. Following their suggestions, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-sequence was additionally used for species identification in this study. GAPDH was amplified using primers GDF/GDR (Templeton et al. 1992). The PCR-mix was set up in a reaction volume of 25 µl containing 1 u BioTherm Taq DNA polymerase (GeneCraft UK Products Semiramis Genetics Ltd., Manchester, UK), the corresponding 1×buffer, 0.4µM of each primer, 0.4 µM dNTP-mix, 1 µl Bovine Serum Albumin (BSA; 50 mg/ml), 1 µl genomic DNA (1:10 diluted). Thermocycling conditions were as follows: 4 min at 95 °C, 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s and a final extension at 72 °C for 7 min. The product was purified, Sanger sequenced and edited as written above. Both consensus sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/Genbank/) under the accession numbers OP316868 (ITS) and OP345225 (GAPDH).

Detailed species identification was performed by creating a multi locus dataset of ITS and GAPDH reference sequences available in GenBank according to Liu et al. (2014), Crous et al. (2015) and Kee et al. (2020c). Phylogenetic analysis was performed by using MEGA X (Kumar et al. 2018). The sequences were aligned by using the incorporated software MUSCLE (Edgar 2004). Maximum likelihood (ML) interference was done using the Kimura 2-parameter model (Kimura 1980) for substitution and 1000 bootstrap replicates. The tree was rooted against *C. cliviae* as an outgroup species.

## Results

# **Microbiological Identification**

Visual inspection revealed on both sides of the leaves randomly distributed, initially small, roundish, water-soaked lesions, which rapidly enlarged and coalesced, eventually leading to severe leaf blight, sharply demarcated from healthy tissue. Concentric rings of dark acervuli sunken



Fig. 3 Conidia of Colletotrichum sansevieriae. Scale bar: 20 µm

into the epidermis appeared on the dried lesions (Figs. 1 and 2).

Under humid conditions, the fruiting bodies produced a bright orange mass of elongated, straight to slightly irregular, cylindrical to slightly club-shaped conidia, obtuse to rounded at apex, truncated at base with acuminate at-



Fig. 1 Appearance of leaf spots on *Sansevieria trifasciata* 'Laurentii'. **a** small water-soaked leaf spots coalescing, **b** the same leaf in backlight, **c** large coalesced leaf spots sharply demarcated from healthy tissue

Fig. 2 Acervuli arranged in concentric rings on necrotic leaf tissue of *Sansevieria trifasciata* 'Laurentii'. a Immature fruiting bodies, b Mature acervuli with bright orange spore mass (*arrow*)



Fig. 4 Colony of Colletotrichum sansevieriae after 14 days at 25  $^{\circ}\mathrm{C}$  on PDA50%

tachment point (Fig. 3). The microscopic measurements of conidia length showed (12.3–)  $18.7 \pm 2.3$  (–26.7)µm and a median of 18.4µm. The width of the conidia was (3.3–)  $6.1 \pm 1.0$  (–8.7)µm, the median 6.1µm.

Measurements of appressoria (n=50) revealed (6.57–) 9.00±1.14 (-12.21)µm, median 8.84µm.

After 14 days at 25 °C on PDA<sub>50%</sub> colonies measured (53.0–)  $60.8 \pm 4.6$  (–68.0) mm, median 59.5 mm in diameter. They appeared white at the edge, grayish-white to partly cream to greyish purple and dark grey in the center, felted with aerial mycelium (Fig. 4).

#### **Molecular Phylogeny**

Based on BLAST search the isolate showed 98.8–99.8% identity with available *C. sansevieriae* sequences for ITS gene. In the Maximum Likelihood analysis of the combined dataset containing 460 sites, the isolate clustered within *C. sansevieriae* with high bootstrap support (99%) (Fig. 5).

## Discussion

Both the microbiological and the phylogenetic examination resulted in the conclusive detection of *Colletotrichum sansevieriae* as causal agent of the observed leaf spots. Macroscopic as well as morphological characteristics are in good accordance with data in literature (Nakamura et al. 2006; Park et al. 2013; Kee et al. 2020c). However, the appressoria measurements of this study are slightly larger than documented in the first description of the species (Nakamura et al. 2006), but agree well with those of Park et al. (2013) and Kee et al. (2020c). The differences may be due to different cultivation methods for appressoria production. This is the first report of the occurrence of this pathogen on *Sansevieria trifasciata* in Germany and more widely in Europe.

On many plant hosts, *Colletotrichum* belong to the most serious pathogens. Some *Colletotrichum* species have a very wide host range, while others are host-specific (Bhunjun et al. 2021; Talhinhas and Baroncelli 2021). On *Sansevieria* 



0.010

**Fig. 5** Maximum likelihood tree inferred from combined ITS and GAPDH sequences of *Colletotrichum* species. The species name is followed by the strain number and GenBank accession numbers. The tree is rooted to *C. cliviae*. Bootstrap values ( $\geq$  50%) are indicated at the nodes. The tree with the highest log likelihood (-1205.61) is shown

only two species are known to occur, C. sansevieriae and C. neosansevieriae. However, the latter being found only once in South Africa and seems to be extremely rare (Crous et al. 2015; Talhinhas and Baroncelli 2021). In contrast, C. sansevieriae is reported from several countries worldwide (Nakamura et al. 2006; Aldaoud et al. 2011; Campoverde and Palmateer 2012; Palmateer et al. 2012; Pérez-León et al. 2013; Gautam et al. 2012; Park et al. 2013; Karimi et al. 2017; Li et al. 2020; Kee et al. 2020b). So far known, the host plant range of C. sansevieriae is very narrow. All the above observations of natural infection were made on S. trifasciata or its cultivars. After artificial inoculation, besides S. trifasciata and its cultivars, only S. stuckyi (Nakamura et al. 2006) developed symptoms. Other Sansevieria species-S. cylindrica, S. masoniana-as well as other tested plants from various genera were not susceptible (Nakamura et al. 2006; Pérez-León et al. 2013; Kee et al. 2020c).

According to currently available data, the high host specificity of *C. sansevieriae* is outstanding compared to other *Colletotrichum* species (Nakamura et al. 2006; Kee et al. 2020c, Talhinhas and Baroncelli 2021). However, the number of tested potential host species remains very low and does not include close relatives of *S. trifasciata* (van Kleinwee et al. 2022). Further investigation with respect to the host range is needed to better understand the host specificity of *C. sansevieriae*.

Infection always required injury to the leaves; unwounded leaves could not be infected, although penetration into stomata was observed (Kee et al. 2020c). It appears that *C. sansevieriae* is a comparatively weak pathogen and the risks for *Sansevieria* producing nurseries are manageable if good professional practice and hygiene are observed. These include avoiding injury, keeping leaves dry, and eliminating diseased leaves or plants (Campoverde and Palmateer 2012). According to the same authors, treatments with modern fungicides (e.g., triazoles, strobilurins) are effective when applied prophylactically, but not curatively.

As the infected plants were imported from Costa Rica, where the occurrence of *C. sansevieriae* is known from farms producing *Sansevieria* for the markets in the USA and Europe (Pérez-León et al. 2013), the source of introduction is obvious. Apparently, the observation confirms the general risk of distributing plant pests and diseases by global plant trade (Hantula et al. 2014; Spence et al. 2020). However, at least for European horticulture, the occurrence of *C. sansevieriae* does not pose a significant risk: because of its high host specificity, low virulence, low economic importance of *Sansevieria*, its exclusive use as a houseplant, and easy-to-implement countermeasures. Serious economic as well as ecological damage is not to be expected.

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**Conflict of interest** T. Brand and A. Wichura declare that they have no competing interests.

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**Thomas Brand** After studying horticultural sciences at the TUM Munich-Weihenstephan, Thomas Brand obtained his doctorate under Prof. V. Zinkernagel, with the experimental work being carried out at the Geisenheim Research Station. This was followed by three years of research at Sveriges Lantbruksuniversitet, Alnarp, Sweden, before he moved to the Plant Protection Office of the Weser-Ems Chamber of Agriculture, now LWK Lower Saxony, as a specialist for plant protection in horticulture in 2003. He is currently head of the department for plant protection in ornamental horticulture, tree nurseries and public greenery.