

First Report of *Stemphylium vesicarium* Causing Onion Stemphylium Leaf Blight in Mauritius

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In August 2021, severe leaf blight symptoms were observed on onion (*Allium cepa* L. cvs Francia and Askari F1 hybrid) in commercial fields located in Mauritius, namely La Forêt (20°19'56.1"S57°30'04.9"E), St Aubin (20°29'47.0"S57°32'29.4"E) and Chapiron (20°20'46.8"S 57°29'12.8"E). Infected leaves displayed small circular to oblong yellow-pale-brown and spindle shaped lesions which later coalesced and formed necrotic areas with black sporulation. Three fields were selected from each region, and along a W-pattern across the fields a disease incidence ranging 53-93% and a severity of 9-28% were recorded. Ten symptomatic leaves were collected in each region and small pieces of infected tissue were surface disinfected using 1% NaOCl for 2 min, rinsed with sterile distilled water, air-dried, transferred to potato dextrose agar (PDA) and incubated for 7 days at 20°C under a 12-h light/dark cycle. Fungal cultures with uniform appearance forming multi-septated conidia typical of the genus *Stemphylium* (Simmons 1969) were consistently isolated. Monosporic colony of isolates SVCWLF24/3, SVSSA23/1 and SVCWLMC26/1 developed similar olivaceous green to light and dark grey mycelium with an average daily growth rate of 6.5 mm at 25°C in the dark. Conidiophores were straight, light brown with a distinct swollen apex on which olive brown to dark brown, oblong to ovoid, septate conidia formed with dimensions 16.2–44.7 × 8.0–22.9 μm (av. 29.5 x 14.7 μm; n = 50) typical of *Stemphylium vesicarium* (Wallr) E.G. Simmons 1969 (Woudenberg et al. 2017). Genomic DNA of the three isolates

was extracted from fungal mycelium (Ranghoo and Hyde 2000). The ITS, cmdA and gapdh genes of the isolates were amplified with primers ITS4/ITS5 (White et al. 1990), CALDF1/CALDR1 (Lawrence et al. 2013) and Gpd1/Gpd2 (Beerbee et al. 1999) and sequenced. Sequences were submitted to GenBank under accession numbers OR131271, ON620213, OR188702 (ITS), OR350623, OR350622, OR166368 (cmdA) and OR684516, OR684517, OR684518 (Gapdh). The BLAST search of the sequences showed 100% similarity with *S. vesicarium* strain CBS 155.24 under accession numbers KU850555 (ITS), KU850702 (Gapdh) and KU850845 (cmdA) (Woudenberg et al. 2017). Phylogenetic trees inferred from the ITS, cmdA and Gapdh concatenated datasets with the maximum-likelihood algorithm allowed clustering of the isolates within *S. vesicarium* clade, confirming the morphological identification. Pathogenicity tests were performed using all three isolates, cultured on PDA at 25°C in a 12-h dark/light cycle. Ten 60-day-old onion plants (cv. Francia) were spray inoculated each with 10 ml of conidial suspension (1×10^4 /ml) of each isolate while 10 healthy plants sprayed with sterile distilled water served as control. They were incubated in a greenhouse at 25°C with a 12-h photoperiod and > 80% humidity. Necrotic circular lesions appeared on leaves after 7-10 days while control plants remained symptomless. Re-isolations made from symptomatic leaf tissues on PDA consistently yielded cultures with similar morphology as the original isolates, thus fulfilling Koch's postulates. This is the first report of *S. vesicarium* as the causal agent of leaf blight of onion in Mauritius. It is a re-emerging fungal disease (Hay et al. 2021) with a wide host range threatening local onion production. This finding will contribute to early detection of leaf blight, implementation of surveillance and integrated disease management in affected regions.

Keywords: Morphology, pathogenicity testing, PCR amplification, sequencing, phylogeny

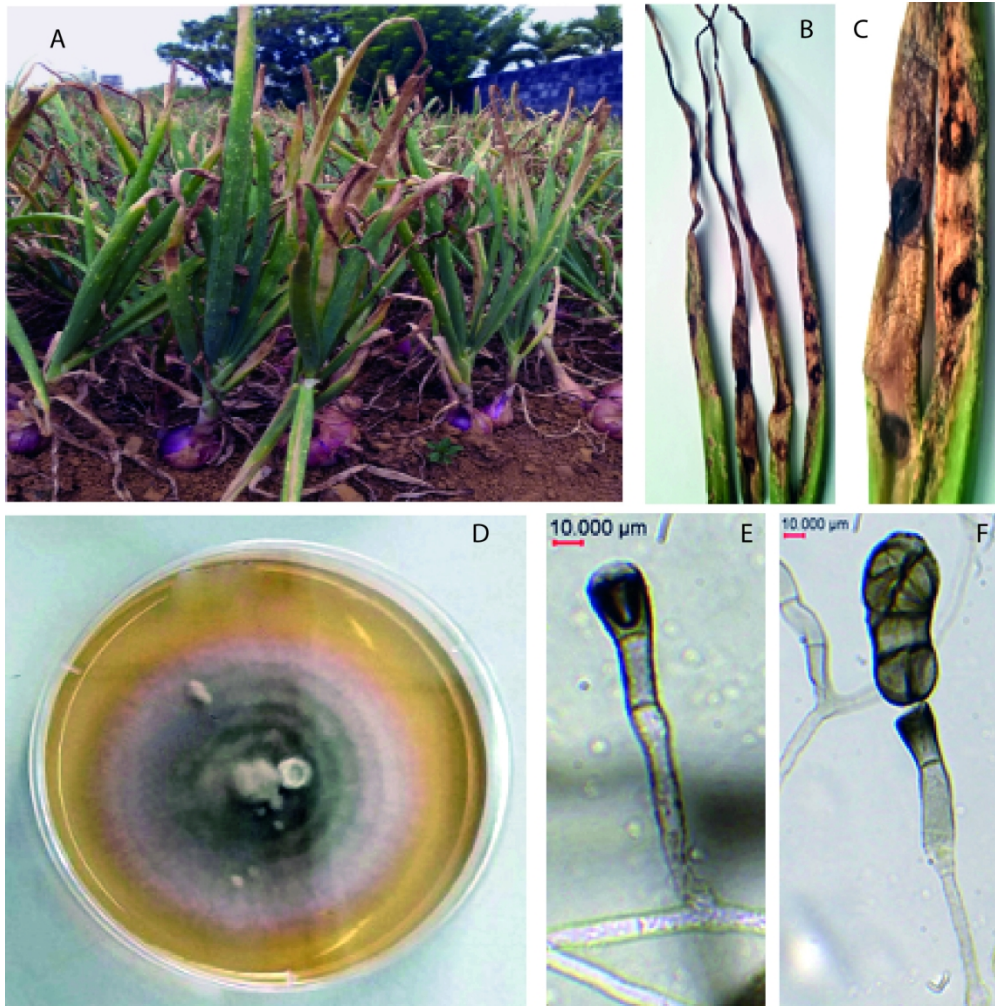
The authors declare no conflict of interest.

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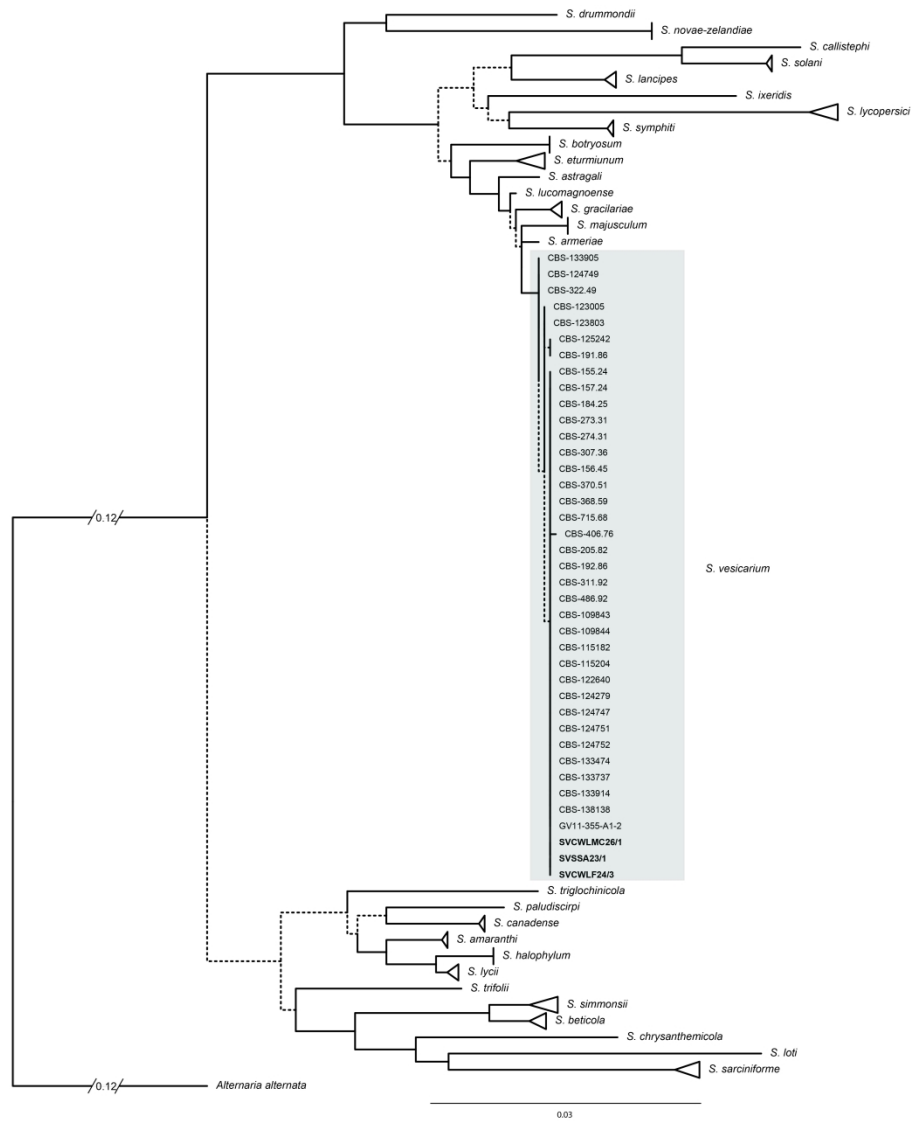
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eXtra 1. *Stemphylium* leaf blight symptoms and morphological characteristics of the causal pathogen *Stemphylium vesicarium* on onion in Mauritius. A, B and C) Blight symptoms on leaves; D) Colony morphology of *S. vesicarium* grown on PDA after 10 days under 12-h light/dark cycle; E) Conidiophore; F) Conidiophores and conidium

157x158mm (300 x 300 DPI)



eXtra 2. Phylogenetic tree of the *Stemphylium* genus showing the relationships between strains isolated in Mauritius (in bold) and species previously described by Woudenberg et al. (2017). The tree was inferred with the maximum likelihood method (TN93+ Γ model) from the concatenated data set including ITS, cmdA and GAPDH sequences. Branches with bootstrap values lower than 80 % are represented by dotted lines. The triangles represent the collapsed clades; the height of the triangle is proportional to the genetic variability within the clade.

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