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Sporothrix eucalypti (sp. nov.), a shoot and leaf pathogen of *Eucalyptus* in South Africa

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Abstract. A species of *Sporothrix* was consistently isolated from leaf spots and serious shoot infections on a clone of *Eucalyptus grandis* in Northern Natal, South Africa. The fungus was morphologically distinct from other species in the genus and is consequently described as a new taxon, *S. eucalypti*. *Sporothrix eucalypti* was shown to be highly virulent in pathogenicity tests on a number of *E. grandis* clones. Significant differences amongst susceptibility of clones were also detected in these tests. *Sporothrix eucalypti* represents a new pathogen of *Eucalyptus* that has the potential to cause substantial damage to this host in South Africa and probably elsewhere in the world.

Key words: *Eucalyptus* shoot and leaf pathogen, *Sporothrix eucalypti*

Introduction

The South African forestry industry depends on plantations of exotic species of *Pinus*, *Eucalyptus* and *Acacia* [1]. Trends in this industry are to produce plants vegetatively from cuttings and this approach has been particularly successful with *Eucalyptus*. Clonal propagation of *Eucalyptus* in South Africa has resulted in tremendous gains in productivity but has also increased risks due to disease [2]. As might be expected, certain clones have been particularly susceptible to disease whereas others have exhibited considerable tolerance [3, 4]. As part of a strategy to reduce the risks of disease, regular surveys are conducted in clonal nurseries and plantations.

During routine examinations of *Eucalyptus* clonal hedges in the Northern Natal Province of South Africa in 1987, a serious disease was noticed on a single clone (TAG 12) of *E. grandis* Hill: Maid. Diseased plants were characterized by round, light brown leaf spots, and die-back of actively growing shoots. A whitish mycelial growth including masses of spores was associated with the necrotic areas. Isolations from the edges of the diseased tissue consistently yielded a species of *Sporothrix* Hektoen & Perkins: Nicot & Marait in culture. The aim of the present study was, therefore, to compare the *Sporothrix* sp. from *Eucalyptus* with other species in the genus, to test pathogenicity on *Eucalyptus*, and to determine its relative importance as a pathogen of this host in South Africa.

Materials and methods

Single-conidial isolates from diseased plant tissue were obtained on 2% malt-extract agar (Biolab) (MEA). Isolates were studied morphologically by

incubating plates for 10 days at 25 °C under near-ultraviolet light before examination. Optimum growth rate, as well as the temperature requirements for growth were determined by culturing isolates on MEA for 10 days in the dark at 5–40 °C in 5° intervals. An agar disc (3 mm diam.) obtained from the periphery of an actively growing colony was placed in the centre of each plate. Three single-conidial isolates (CMW 917, 918, 1101) were used in the growth studies, with three replicate plates per isolate at each temperature, and the experiment was repeated once. Colony diameters were obtained after 10 days by taking two perpendicular readings of each colony, and determining the averages (mm).

To confirm pathogenicity of the *Sporothrix* sp., a single-conidial isolate (CMW 1101) was cultured on MEA at 25 °C under near-ultraviolet light for 10 days. Conidia were removed from the culture by gently scraping the colony surface with a sterile scalpel blade. A conidial suspension was prepared in 500 ml of sterile water and adjusted to 2.2×10^6 spores/ml. One drop of Tween 80 was added to the suspension to ensure the effective application of spores to *Eucalyptus* leaf-surfaces. Twenty one-year-old potted plants of the *E. grandis* clone on which *Sporothrix* sp. was first recorded (TAG 12) were sprayed with the spore suspension till run-off using an atomizer. Twenty control plants were sprayed with sterile water amended with Tween 80 only. All plants were covered with plastic bags and maintained in a glasshouse at 25 °C to ensure infection. Bags were removed after three days and plants were visually monitored for three weeks.

The number of leaves having at least one necrotic spot 2 mm and larger in size were counted on each plant three weeks after inoculation. A total of 96 symptomatic leaves were counted on all 20 inoculated plants. Control plants displayed no symptoms. Diseased leaves were surface disinfested with 1% NaOCl and *S. eucalypti* was reisolated from the margins of necrotic tissue.

In order to ascertain whether *Eucalyptus* clones varied in susceptibility to *S. eucalypti*, a second inoculation experiment was performed with TAG 12 and two additional *E. grandis* clones, TAG 27 and TAG 70. Ten, one-year-old potted plants of each clone were inoculated with *S. eucalypti* isolate CMW 1101 as previously described. Ten control plants were included and a randomized complete block design was used. Plants were maintained in a glasshouse at 25 °C for three weeks whereupon the number of leaves having at least one necrotic spot 2 mm and larger in size were counted on each plant. A two-way analysis of variance (ANOVA) was conducted and Tukey's HSD procedure for comparison of means was applied.

Results and discussion

As far as we have been able to determine, no *Sporothrix* sp. has previously been described as a pathogen on *Eucalyptus*. The fungus is also morphologically different to other species known in the genus [5–7]. The species of *Sporothrix* associated with a leaf and shoot disease of *E. grandis* in South Africa is consequently described as new.

Sporothrix eucalypti Wingfield, Crous & Swart sp. nov. (Figs 1–3)

Coloniae in vitro post 10 dies 28 mm diam., floccosae, vel vevetae, superne, niveae, inferne pallide luteolae. Hyphae hyalinae, glabrae, tenuiter tunicatae. Cellulae conidiogenae dispersae, 5–55 μm longa, 0.5–2.5 μm latus; denticulae truncatae, quam 2 μm longa. Conidia hyalina, nonseptata, glabra; conidia primaria fusiformia, 6–12 × 3–4 μm; conidia secundaria angusta obovoidea ad ellipsoidea, 3.5–5 × 1.5–2.5 μm. Teleomorph ignotus.

Holotype: RSA, Northern Natal, Kwambonambi, *Eucalyptus grandis* clone TAG 12, M.J. Wingfield, 19 May 1987, PREM 51089 (culture ex type, CMW 1101, maintained at the Department of Microbiology and Biochemistry, Univ. OFS).

Colonies in vivo attaining a diameter of 28 mm

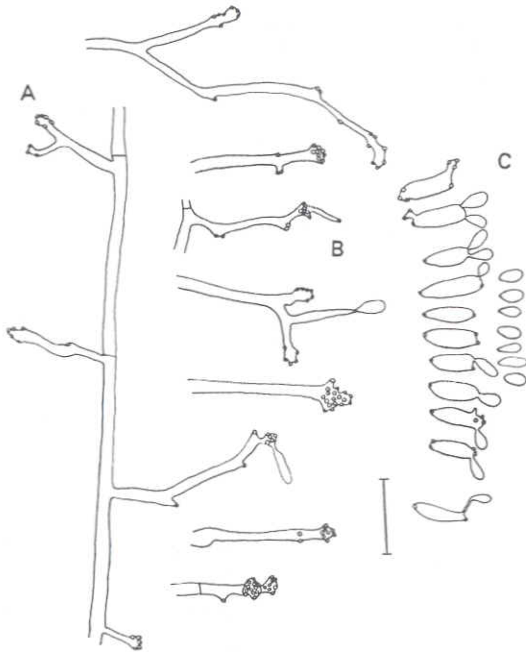


Fig. 1. Morphological structures of *Sporothrix eucalypti* after 10 days on MEA (bar = 10 μm). A, Conidiophores; B, denticulate conidiogenous cells; C, primary and secondary conidia.

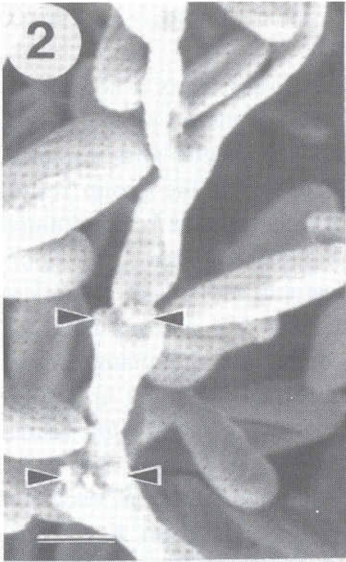
at 25 °C on 2% malt-extract agar (MEA) in the dark after 10 days, appearing finely floccose or velvety, up to 1 mm high; white (viewed from above) and pale yellow (viewed from bottom of plate). Exudate absent, odour present. Aerial hyphae hyaline, smooth, loosely aggregated, irregular, 1–2 μm wide. Conidiogenous cells scattered, terminal or integrated in short side branches, variable in shape and size, usually uniform in width, or widest at swollen apex, seldom tapering to narrower apex, 5–55 μm long, 0.5–2.5 μm wide; apical part forming conidia by sympodial growth, consisting of a not or slightly swollen cluster of conidium-bearing denticles; denticles inconspicuous to protruding, cylindrical, up to 2 μm long. Conidia hyaline, non-septate, smooth and thin-walled, continuous, in dry masses; primary conidia fusiform, with pointed base, 6–12 × 3–4 μm, usually giving rise to one or several narrowly obovoid to ellipsoidal secondary conidia, 3.5–5 × 1.5–2.5 μm. Temperature requirements for growth after 10 days on 2% MEA (Biolab) in the dark: minimum >5 < 10 °C; optimum between 25–30 °C, and maximum >35 < 40 °C. Teleomorph unknown.

The genus *Sporothrix* represents a heterogenous assemblage of anamorphs of hyphal yeasts (Endomycetes), true Ascomycetes and Phragmobasidiomycetes. In this sense, the group represents an artificial form-genus that is maintained because of morphological similarity [6]. *Sporothrix* was initially established to accommodate the human pathogen *Sporothrix schenkii* Hektoen & Perkins [8]. It also includes the anamorphs of many Ophiostomatoid fungi including important tree pathogens such as *Ophiostoma ulmi* (Buisman) Nannf. [6, 9].

The most notable characteristic of *S. eucalypti* is its high level of pathogenicity on *Eucalyptus* leaves and young shoots. To our knowledge, no other *Sporothrix* species is known as a leaf or shoot pathogen. Morphologically, *S. eucalypti* is most similar to *S. fungorum* De Hoog & De Vries. However, *S. fungorum* has long, cylindrical conidiogenous cells, and well differentiated primary and secondary conidia, the latter being globose, and 2–2.8 μm wide [10]. The secondary conidia of *S. flocculosa* Traquair, Shaw & Jarvis are rough-walled [7], while *S. catenata* De Hoog & Constantinescu has short chains of obovoid to elliptical secondary conidia, 3.5–5.5 × 1.5–3 μm [11]. *S. rugulosa* Traquair, Shaw & Jarvis is similar to *S. eucalypti* in producing narrowly obovoid to ellipsoid secondary conidia, but distinct in having conidiogenous cells that are borne on conical hyphal pedicel-like projections [7], which are not found in other *Sporothrix* spp. [5]. Another species in this group is *S. cyanescens* De Hoog & De Vries, which is distinguished by producing a purple pigment in culture [12].

Two other species similar to *S. eucalypti* are *S. guttiliformis* De Hoog and *S. foliorum* Taylor. *S. guttiliformis* has an optimum growth rate at 35 °C [10], while *S. foliorum* grows at 37 °C, and has smooth secondary conidia, 2.8–4.3 × 2–2.6 μm [5]. *S. eucalypti* can thus be distinguished from these species by its lower optimum growth rate and different conidial dimensions.

Disease symptoms (Fig. 4) in the form of light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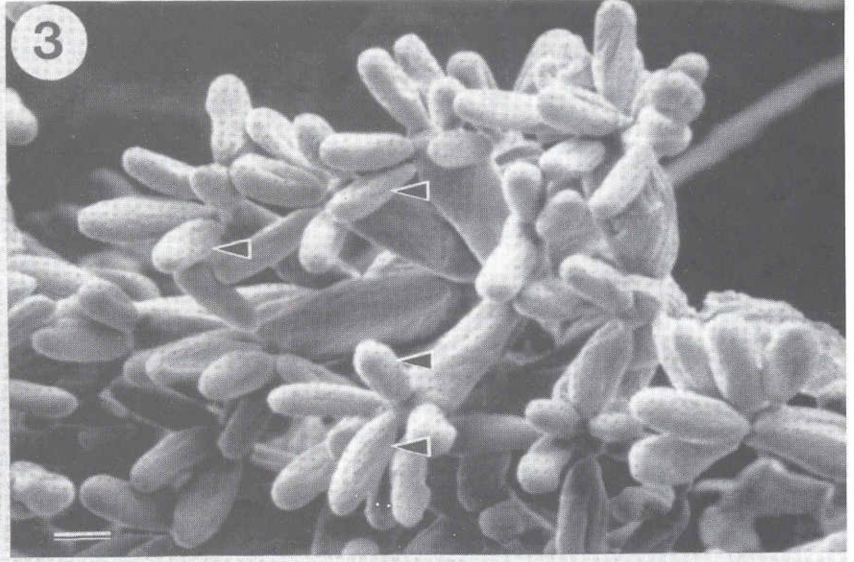
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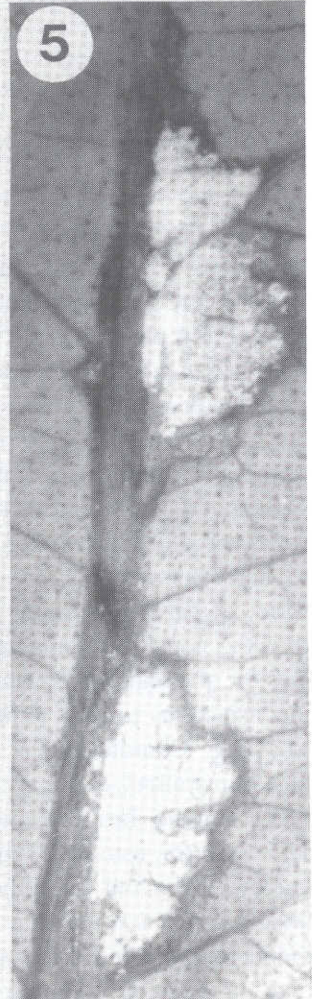
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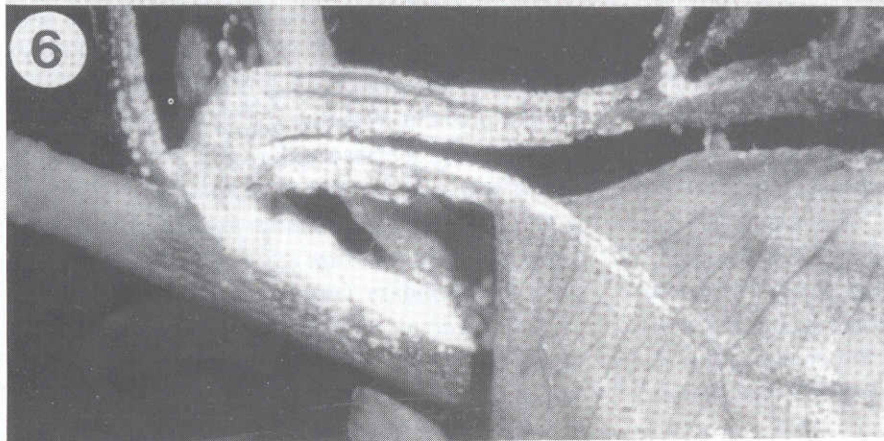
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Table 1. Number of leaves displaying necrotic lesions following glasshouse inoculations with *Sporothrix eucalypi* on three *Eucalyptus grandis* clones

Trial No.	Clone No.	Treatment	Symptomatic leaves		
			Total number on plants	Mean number per plant[c]	Range per plant[d]
1[a]	TAG 12	Inoculated	96	4.8	0–11
		Control	0	0.0	–
2[b]	TAG 12	Inoculated	64	6.4 a	4–11
		Control	0	0.0 c	–
2[b]	TAG 27	Inoculated	36	3.6 b	0–10
		Control	0	0.0 c	–
2[b]	TAG 70	Inoculated	21	2.1 b	0–4
		Control	0	0.0 c	–

[a] Twenty plants per treatment.
[b] Ten plants per treatment.
[c] Values followed by the same letter are not significantly different ($p < 0.05$) according to Tukey's HSD procedure.
[d] An average of 15 leaves per plant.

brown necrotic lesions appeared on leaves three weeks after inoculation with *S. eucalypti*. Necrotic spots varied in diameter from approximately 2 mm to 12 mm. The number of symptomatic leaves per plant, out of an average of 15 leaves, varied from 0 on three plants to 11 on one plant (Table 1). The mean number of symptomatic leaves per plant was significantly different ($p < 0.05$) between TAG 12 and the other two clones, TAG 27 and TAG 70, which were not significantly different from each other (Table 1). These results suggest, therefore, that although other clones can also be infected by *S. eucalypti*, they are much less susceptible than clone TAG 12.

Sporothrix eucalypti represents a new and unique pathogen of *Eucalyptus*. The fungal genus has not previously been associated with leaf and shoot diseases of plants and this pathogen is also unknown in areas of origin of *Eucalyptus*. It is possible that the pathogen does occur in Australasia where *Eucalyptus* is native but that ecological homeostasis has precluded its proliferation. It might alternatively have originated from native plants in South Africa, having thus adapted to pathogenicity on exotic *Eucalyptus*. If the latter is true, this pathogen could pose a threat to native *Eucalyptus* species in Australasia.

At this stage, *S. eucalypti* is not resulting in serious losses to *Eucalyptus* propagation in South Africa. It is apparently restricted to the hot humid areas of Northern Natal and also exhibits a strong preference to pathogenicity on certain clones. Where a clone such as TAG 12 is susceptible, *S. eucalypti* can cause considerable damage and, in fact, this clone has had to be abandoned due to the occurrence of the pathogen (Figs 5–6). The significant differences observed in susceptibility between clones in this study, might explain why *S. eucalypti* is rarely isolated from other clones of *E. grandis* propagated in Northern Natal. However, given the high degree of pathogenicity of this fungus to clone TAG 12, it must be considered amongst the potentially serious pathogens of *Eucalyptus*.

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Figs 2–6. Disease symptoms, conidiophores, conidiogenous cells and conidia of *Sporothrix eucalypti* (bar = 5 μm). *Fig. 2.* Denticulate conidiogenous cell (arrowed) with conidia. *Fig. 3.* Primary and secondary (arrowed) conidia. *Fig. 4.* Necrotic leaf spots on an inoculated *Eucalyptus grandis* seedling. *Fig. 5.* Profuse sporulation on the hypophyllous leaf surface of an *E. grandis* seedling collected in the field. *Fig. 6.* Leaf, petiole and stem necrosis with fluffy mycelial growth and profuse sporulation.

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