First report of *Cylindrocladium* root and petiole rot of *Spathiphyllum* in South Africa

Conrad L. Schoch and Pedro W. Crous*

Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 Republic of South Africa

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This is the first report of the occurrence of *Cylindrocladium spathiphylli* Schoult., El-Gholl & Alfieri on the mainland of Africa. This organism was isolated from commercially cultivated *Spathiphyllum* Schott plants and is responsible for Cylindrocladium root and petiole rot. Sequencing data and morphological characters are utilised to compare the isolate to known cultures of *Cylindrocladium spathiphylli*. Koch's postulates are proven for this fungus and possible disease control measures are discussed.

Keywords: *Calonectria, Cylindrocladium spathiphylli, Spathiphyllum*, fungal systematics, sequencing.

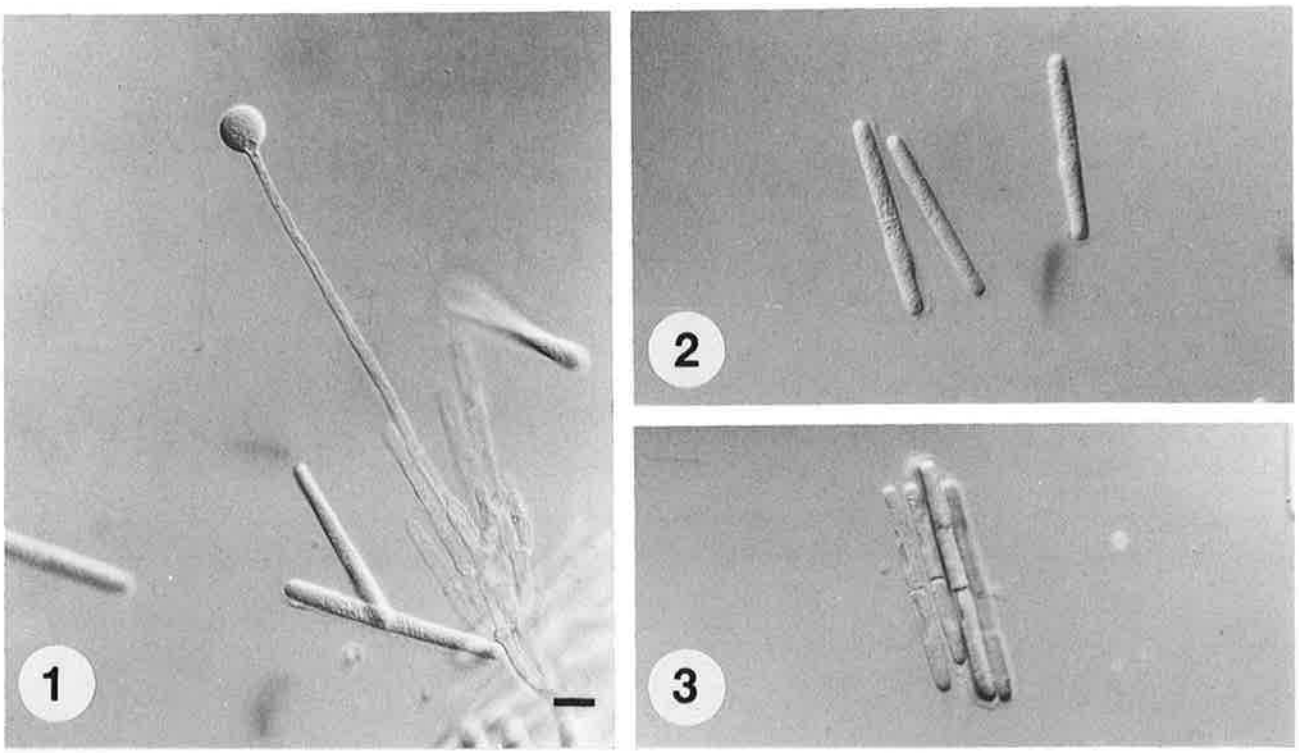
*To whom correspondence should be addressed, (e-mail: pwc@maties.sun.ac.za).

Introduction

A devastating disease of commercially cultivated cultivars of *Spathiphyllum* Schott, a popular indoor ornamental plant, was reported in 1998 from a nursery in the Hazyview area, Mpumalanga, South Africa. Symptoms appeared similar to that of *Cylindrocladium* root and petiole rot. This fungus was first found to cause a disease of *Spathiphyllum* in Florida, U.S.A. in 1978 (Schoulties & El-Gholl 1980), and in 1985 also caused a major disease outbreak in Hawaii (Uchida 1989). Although reported from Mauritius (Crous & Peerally 1996), no records of this disease are known from the mainland of Africa.

Despite the fact that *Spathiphyllum* was cultivated by many growers in Florida, prior to 1980, this disease was not reported. It is, therefore, believed that *Cylindrocladium* root and petiole rot was introduced by importation of diseased plants from tropical Central or South America, as diseased plants with similar symptoms were previously observed in these areas (Chase & Poole 1988). A similar mode of introduction of this disease into South Africa probably occurred.

The causal organism of *Cylindrocladium* root and petiole rot of *Spathiphyllum* was initially identified as *Cylindrocladium floridanum* Sobers and C.P. Seym. (Schoulties & El-Gholl 1980). However, a new species, *Cylindrocladium spathiphylli* Schoult., El-Gholl and Alfieri, was proposed based on its globose vesicles and larger conidia (Schoulties *et al.* 1982). Furthermore, Uchida and Aragaki (1992) also reported that some isolates of this pathogen could be more variable, and have 1-3-septate conidia, with sphaeropedunculate, ellipsoid or spathulate vesicles. El-Gholl *et al.* (1992) subsequently described the teleomorph of this fungus as *Calonectria spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T. S. Schub. and Chase, and further defined it as having a biallelic, heterothallic



Figures 1–3 Conidiophores and conidia of *C. spathiphylli*. **1**. Penicillate conidiophore with sphaeropendunculate vesicle. **2, 3**. One to three septate conidia. Bar = 10 µm.

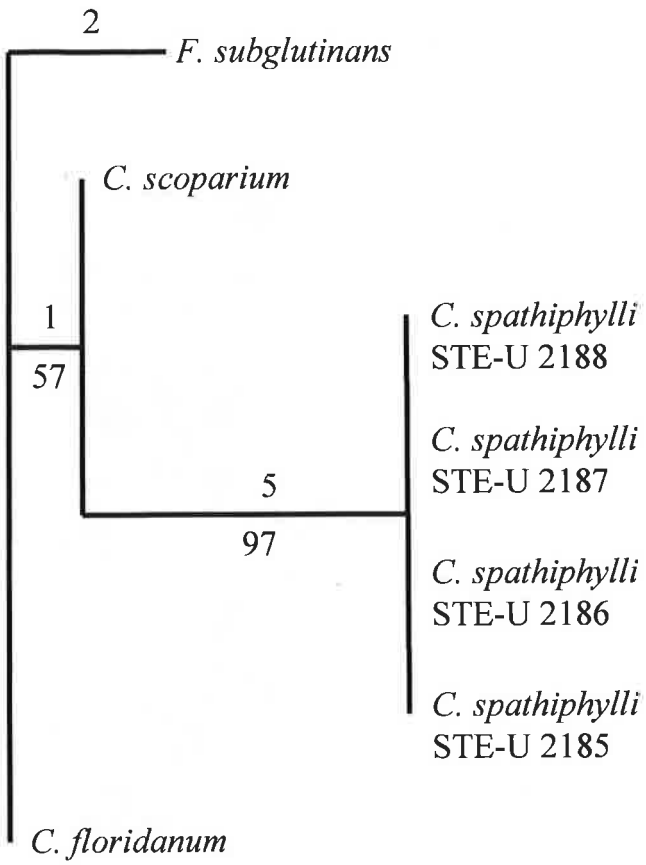


Figure 4 The most parsimonious tree generated with a branch and bound algorithm in PAUP 3.1.1 from aligned sequences of the 5.8S gene and flanking ITS1, ITS2 regions (7 steps, CI =1, RI = 1). Bootstrap values above 50% are shown. A *Fusarium subglutinans* sequence (Genbank no. X94167) was used as outgroup.

mating system.

The aim of the present study is to report *Cylindrocladium* root and petiole rot of *Spathiphyllum* from South Africa, identify and characterise the *Cylindrocladium* species involved, prove Koch's postulates, and also give a brief overview of possible control strategies.

Materials and Methods

Morphology

Diseased leaf and stem pieces of *Spathiphyllum* were incubated in Petri dish moist chambers at approximately 25°C on the laboratory bench to induce sporulation. Single conidium colonies were first established on 2% malt extract agar (MEA) (Oxoid), transferred to plates containing fresh MEA and carnation leaf agar (CLA) (Fisher *et al.* 1982; Crous *et al.* 1992), and incubated at 25°C under continuous near-ultraviolet light. For microscopic examination fungal structures were mounted in lactophenol and measurements made at 1000 × magnification.

The following cultures were used in this study - STE-U 2185, collected by C.L. Schoulties from *Spathiphyllum* sp. in Florida, U.S.A.; STE-U 2186, collected by K.I. Kavouras from *Heliconia psittacorum* in Florida, U.S.A.; STE-U 2187, collected by C.L. Lippincott from *Heliconia* sp. in Florida, U.S.A. and STE-U 2185, collected by A. Thompson from *Spathiphyllum* sp in Mpumalanga, South Africa. All reference cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U).

Sequencing

Single conidial isolates were grown on MEA plates and plugs were transferred to Erlenmeyer flasks containing 100 ml liquid MEA broth. Flasks were shaken on a rotary shaker at 25°C and 125 r.p.m. for approximately 7 days. Mycelium was collected by filtration using Whatman no. 1 filter paper and DNA was extracted as described by Crous *et al.* (1993). Both strands of the 5.8S ribosomal RNA gene, as well as the flanking internally transcribed spacers (ITS1 and ITS2) were sequenced and analysed. DNA was amplified using the primers ITS1 (5'-dTCCGTAGGTGAACCTGCGG) and ITS4 (5'-dTCCTCCGCTTATTGATATGC) (White *et al.* 1990). Polymerase chain reaction (PCR) amplifications were performed on a Hybaid Omnigene Temperature Cycler (Hybaid, Middlesex, UK). Reactions (total volume 100 µl) comprised of 5 units of Expand High Fidelity DNA polymerase (Boehringer Mannheim, Mannheim, Germany) with the buffer as recommended by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM $MgCl_2$, 0.5 µM primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA as target. The reaction mixture was overlaid with liquid paraffm to prevent evaporation. PCR conditions were a denaturing step at 94°C for 1 min followed by 10 cycles of 56°C for 30 s, 72°C for 2 minutes and 94°C for 15 s. This was followed by a further 20 cycles at the same settings except for a 20 s time increase at 72°C. PCR products were purified using Magic PCR Preps (Promega Corporation, Madison, U.S.A.). Both strands of the PCR product were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, New Jersey, U.S.A.). A Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase (Perkin-Elmer) was used for the sequencing reactions. The reactions were carried out with a concentration of 20 to 40 ng of DNA template and 3.2 pmol primer in a total volume of 10 µl. The cycle sequencing reaction was done by PCR under conditions of 96°C for 30 s, 50°C for 15 s and 60°C for 4 min. This was repeated for 25 cycles. DNA was finally purified using Centri-Sep Spin columns (Princeton Separations, New Jersey, U:'S.A..) and loaded onto the sequencing gel. Phylogenetic analysis of the ITS1 and ITS2 DNA sequences was performed by using the PAUP (Phylogenetic Analysis Using Parsimony) 3.1.1 program (Swofford 1993). The branch and bound algorithm, with gaps treated as a fifth character was used. Only informative characters were used. Confidence intervals were determined using 100 bootstrap replications. The consistency (CI) and retention indices (RI) were also determined. All uninformative characters were ignored.

Sequences of *C. scoparium* (Genbank no. U43204) and *C. floridanum* (Genbank no. U36443), previously published by Jeng *et al.* (1997), were used for comparison. All additional sequences were deposited in Genbank. These sequences were obtained from the type culture of *C. spathiphylli* (STE-U 2185, Genbank no. AF124344) two strains of opposite mating type (STE-U 2186, Genbank no. AF12436 and STE-U 2187, Genbank no. AF124345) (Crous & Wingfield 1994) and the strain isolated in this study (STE-U. 2188, Genbank no. AF124347). In addition to this, a sequence of *Fusarium subglutinans* (Wollenw. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas, deposited by Waalwijk *et al.* (1996), was obtained (Genbank no. X94167) and used as outgroup.

Confirming Koch's Postulates

Agar plugs containing *C. spathiphylli* (STE-U 2188) were plated on 2% MEA agar plates and incubated at 25°C for 7 days. Conidia were washed from the plates using sterile water with the help of a sterile glass rod. The final conidial concentration in a 0.1% Tween 20® solution was emended to 70 000 conidia/ml by means of a haemocytometer. The inoculum was applied with a hand held sprayer to *Spathiphyllum* plants until run-off. Control plants were sprayed with water. Plants were placed in a moisture chamber at 25°C for 3 days and leaf symptoms noted.

Lesions were cut from infected leaves using a sterile scalpel blade. Leaf portions were surface sterilized by immersion in 70% ethanol for

Figures 5 and 6 Symptoms of *Cylindrocladium* leaf rot on *Spathiphyllum*. **5**. Inoculated plants with leaf spots (left) and control (right). **6**. Close-up of leaf to show leaf spots caused by *C. spathiphylli*.

30 s, 1% hypochloride for 1 min and again in 70% ethanol for 30 s. They were placed in Petri dishes containing water agar and incubated for three days at 25°C. Sub-cultures were made from fungal growth and identified as discussed above.

Results

Species identification

Sporulating cultures on CLA had conidiophores with stipe extensions terminating in sphaeropedunculate vesicles typical of *C. spathiphylli* (Figure 1). Conidia were predominantly one septate, occasionally three septate, with dimensions of 60–90 × 5–7 µm (Figures 2 and 3). These characteristics closely correspond to those of *C. spathiphylli* (Crous & Wingfield 1994). The sequence obtained from isolate STE-U 2188 was compared with those from *C. scoparium* (Genbank number U43204), *C. floridanum* (Genbank number U36443), as well as the type culture of *C. spathiphylli* (STE-U 2185) and its two mating types (STE-U 2186 and STE-U 2187). The South African isolate yielded a 100% homology with the latter three cultures (Figure 4). *C. spathiphylli* proved quite distinct from *C. floridanum* in having a 6 base pair deletion, and 12 other single base substitutions. These data provide unambiguous results, confirming the identity of the South African isolate as *C. spathiphylli*.

Koch's postulates

The inoculated plants showed severe disease symptoms three days after inoculation (Figures 5 and 6). Early symptoms of *Cylindrocladium* root and petiole rot were a slight wilting and chlorosis of the lower leaves, which gradually turned necrotic. Elliptical, dark brown spots with a bright yellow halo could also be observed on leaves. The bases of the petioles became rotten and petioles eventually detached from the plants. The causal organism was re-isolated from the margins of lesions, and its identity confirmed in culture. This concluded Koch's postulates.

Discussion

The present study is the first record of *C. spathiphylli* occurring on the mainland of Africa. At present it is known from several countries including the U.S.A. (Florida, Hawaii), Central America (Uchida 1989), Australia (Forsberg 1988), Italy (Carrai & Caribaldi 1990), Mauritius, Switzerland (Crous & Peerally 1996), Taiwan and China (Jiang & Qi 1997). Other than *Spathiphyllum*, it has also been reported from hosts such as *Heliconia* spp., *Strelitzia nicolai* Regel & Körn and *Ludwigia palustris* (L.) Elliott (Schoulties *et al.* 1982; El-Gholl *et al.* 1992).

According to Henny and Chase (1986) several species and cultivars of *Spathiphyllum* have yielded varying degrees of resistance to *C. spathiphylli*. Of all species and cultivars tested, *S. floribundum* yielded the most resistance, of which the commercial cultivars Tasson, Bennett, Mauna Loa and Queen Amazonica showed different degrees of susceptibility (Henny & Chase 1986).

Because *Cylindrocladium* root and petiole rot of *Spathiphyllum* is a new disease to South Africa, no research on its control has yet been done. In Florida U.S.A., a major focus of research on *C. spathiphylli* has been disease control. Soil temperature, potting medium, compaction and soil pH were all found to influence the disease to a varying extent, with the fungus favouring warm, moist conditions with lower pH and higher soil compaction (Chase & Poole 1988). However, higher pH only appeared to delay the onset of disease development. The application of

fungicide drenches were also investigated and triflumizole, prochloraz and benomyl provided the best disease control (Chase & Poole 1988). Only preventative measures were found effective, while no curative effects could be obtained after infection had taken place. In Florida this disease could be controlled by the use of fungicides in association with good nursery practices, including immediate removal of diseased plants, use of new, uninfected potting medium, and minimal watering (Chase & Poole 1988). It is believed that the same approach will be fruitful for controlling the disease under South African conditions.

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