Two new *Magnaporthe* species pathogenic to warm-season turfgrassses in Australia

P. T. W. Wong · C. Dong · A. M. Stirling · M. L. Dickinson

Abstract Two new species of ectotrophic root-infecting fungi pathogenic to warm-season turfgrasses are described. *Magnaporthe garrettii* P. T. W. Wong & M. L. Dickinson sp. nov. causes a serious patch disease on couch (*Cynodon dactylon*) bowling greens in South Australia, and *Magnaporthe griffinii* P. T. W. Wong & A.M. Stirling sp. nov. is associated with a disease complex ("summer decline") of hybrid couch (*C. dactylon × C. transvaalensis*) golf greens in New South Wales and Queensland. Both are homothallic, producing perithecia readily on potato dextrose agar. They differ from other *Magnaporthe* spp. in having uniseriate rather than biseriate or multiseriate ascospores, and the absence of a conidial anamorph. Analysis of nuclear rRNA ITS sequences has shown that *M. griffinii* is a new taxon with low homology to *M. grisea, M. poae, M. rhizophila, M. salvinii* and *Gaeumannomyces graminis*. This could not be carried out with *M. garrettii* because there were no living cultures available and the genomic DNA extracted from dead mycelia and perithecia was totally degraded. However, the two new species can be readily distinguished by morphological differences in their perithecia and ascospores. Examination of earlier herbarium specimens has shown that *M. garrettii* was associated with a patch disease of buffalo grass (*Stenotaphrum secundatum*) in New South Wales and *M. griffinii* was associated with diseases of South African couch grass (*C. tranvaalensis*) in South Australia and of kikuyu grass (*Pennisetum clandestinum*) in New South Wales.

Keywords *Magnaporthe* · Patch disease · Summer decline · *Cynodon* · *Pennisetum* · *Stenotaphrum*

P. T. W. Wong (✉)
Amenity Horticulture Research Unit, Plant Breeding Institute,
University of Sydney,
Private Bag 11,
Camden, NSW 2570, Australia
e-mail: percy.wong@sydney.edu.au

C. Dong
Plant Breeding Institute, University of Sydney,
Private Bag 11,
Camden, NSW 2570, Australia

A. M. Stirling
Biological Crop Protection Pty Ltd,
3601 Moggill Road,
Moggill, QLD 4070, Australia

M. L. Dickinson
Turf-Test,
52 Whitmuir Road,
Bentleigh, Victoria 3204, Australia

Introduction

In recent years, patch diseases of couch grass or bermuda-grass [*Cynodon dactylon* (L.) Pers.] and couch hybrids [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] have become more common on golf and bowling greens in Australia. In 2002, a serious patch disease was observed on several couch (cv. Greenlees Park) bowling greens in Adelaide, South Australia (Fig. 1). It appeared in spring (September and October) and increased in size and severity through the warmer months. Field symptoms included patches of unthrifty grass, stunting of stolons, leaf chlorosis and eventual necrosis of stolons. In subsequent years, the numerous small patches (<5 cm) enlarged irregularly and coalesced into large patches that exceeded 0.5 m in diameter.

In Queensland and New South Wales, a disease called "summer decline" by golf superintendents has become increasingly common and severe on hybrid couch, especially cv. Tifgreen, and is becoming more

Fig. 1 Disease patches caused by *M. garrettii* on a couch (cv. Greenlees Park) green at Colonel Light Gardens Bowling Club, Adelaide, South Australia

difficult to control (Stirling 2001). The symptoms are irregular patches of unthrifty turf which are most severe in the summer months, especially when mowing heights are low and/or there is a period of heat and moisture stress (Fig. 2). The roots and rhizomes of plants in affected areas become dark brown and rotten so that eventually very few roots are left. So far, commonly used fungicides have not been effective against either of these diseases. This paper reports on the aetiology of these two new diseases and describes the pathogens.

Methods

Isolation of fungi

Root pieces (0.5–1.0 cm) with disease symptoms were surface-sterilised in equal parts of 10% aqueous sodium

Fig. 2 Summer decline patches caused by *M. griffinii* on a hybrid couch (cv. Tifgreen) green at Carbrook Golf Club, Loganholme, Queensland

hypochlorite (3.5% available chlorine) and 95% ethanol for 2 min, rinsed twice in sterile water and plated onto ¼ strength potato dextrose agar + 100 mg/L novobiocin (¼ PDA + N) in 90 mm diam. Petri plates. The agar plates were incubated in the dark at 25°C for 5–10 d before the most commonly occurring fungi were subcultured onto ¼ PDA and PDA. Pure cultures of the fungi were then tested for pathogenicity to couch grass cv. Greenlees Park or the couch hybrid cv. Tifgreen. Specimens and dried agar cultures of the pathogens were lodged in the Plant Pathology Herbarium, Department of Industry and Investment, Orange, NSW (Herb. DAR).

Pathogenicity tests

Pathogenicity tests were carried out in a temperature-controlled glasshouse (12 h at 25°C, 12 h at 30°C). Ten cm diameter plastic pots were filled to 2 cm from the top with steam-sterilised washed river sand and inoculated with the fungus by chopping up a fully-colonised ¼ PDA plate into 1 cm squares and placing them on the soil surface. Three healthy stolons (each 3 cm long and bearing 2 nodes) of couch grass cv. Greenlees Park or the couch hybrid cv. Tifgreen were placed in contact with the agar pieces and covered with a 1 cm layer of sand. Control pots had chopped-up agar colonised by an isolate of non-pathogenic *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen instead of the test fungi. There were 4 replicates per treatment and treatments were randomised in blocks on a glasshouse bench. Pots were watered daily and fertilised fortnightly with 50 ml per pot of a soluble complete fertiliser, Thrive® (Yates Australia, Padstow, NSW, Australia). Above-ground disease symptoms were recorded monthly for 3 months. At the end of that period, the root systems of all the plants were washed free of sand and observed under a stereo microscope (x 40) for disease symptoms. If diseased roots were present, a few roots were plated out as above to re-isolate the causal fungus.

Cultural characteristics of the pathogens

The pathogens, designated *Magnaporthe* sp. 1 and *Magnaporthe* sp. 2, were grown on PDA and ¼ PDA. The agar plates were incubated for up to 3 months at 25°C in the dark and the cultures were observed periodically under a stereo microscope and a compound microscope for colony and mycelial characteristics as well as sporulation. Fungal mycelia and perithecia were mounted in a drop of water or lactophenol acid fuchsin on slides and measurements of hyphal diameter, the dimensions of perithecial characters, asci and 25 random ascospores of each fungus were made using a standard eyepiece micrometer. Photomicrographs were captured with

a digital camera attachment (Tucsen Imaging Technology Co. Ltd., Fuzhou, China). After 3 months, when mature perithecia had formed but there were still no conidia, the plates were exposed to alternating dark and diffuse natural light on a laboratory bench (20–25°C) for another month in an attempt to induce conidial production.

To measure the growth rates of the fungi, 5 mm fungal plugs from the advancing margins of the fungal colonies, grown on PDA, were placed at the centres of 90 mm diam. PDA plates (4 replicate plates per fungus) and incubated in the dark at 28°C. The diameters of the fungal colonies were measured every 5–7 days because of the slow growth rate of the fungi and the radial growth rates (mm/day) of the fungi were calculated.

DNA extraction, amplification and sequence analysis

As there were no living cultures of *Magnaporthe* sp. 1 available, fungal mycelia and perithecia from a dried agar culture of the holotype (DAR 76937) were collected for DNA extraction. For *Magnaporthe* sp. 2, two isolates (TS99 and TY2) were grown for 4 weeks at 25°C on autoclaved, moist cellophane sheets (50 mm × 50 mm) that had been placed on freshly poured PDA plates. Approximately 20 mg of mycelia were scraped off the cellophane, placed in 2.0 ml Eppendorf tubes and left to dry in silica gel for 2 days. Two 4 mm ball bearings and 50 mg of glass beads were added to the dried samples and ground into a fine powder in a Mixer Mill (Retsch MM300, MEP Instruments Pty Ltd, North Ryde, NSW, Australia) at 25 oscillations/second for 4 min. CTAB buffer (2% CTAB, 1.4 M NaCl, 0.2 M EDTA, 0.1 M Tris–HCl pH 8.0, 0.2% β-mercaptoethanol) was pre-warmed at 60°C and 500 μl aliquots were added to the sample, mixed thoroughly and incubated at 60°C for 30 min. The DNA was then extracted using equal volumes of chloroform, mixed well and then centrifuged at full speed in a microcentrifuge (Eppendorf 5415D, Eppendorf, North Ryde, NSW, Australia) for 5 min. The aqueous portion was decanted into clean tubes and an equal amount of isopropanol was added to precipitate the DNA. The DNA pellets were washed with 70% ethanol and resuspended in 50 μl TE (pH8.0). RNase A was added to give a 50 μg/ml concentration. The DNA concentration was determined by electrophoresis and compared with a known concentration of λ DNA. PCR primers used in this study were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990) which amplifies partial 18S rRNA gene, complete internal transcribed spacer 1 (ITS1), complete 5.8S rRNA gene, complete ITS2, and partial 28S rRNA gene. PCR amplification was carried out in a 20 μl reaction containing 5 ng DNA, 1x GoTaq buffer (Promega, Madison, WI, USA), 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.25 μM primers and 0.5

U GoTaq Hot Start Polymerase (Promega, Madison, WI, USA). PCR was performed at 94°C for 3 min and then 35 cycles of 94°C 20 s, 55°C 30 s and 72°C 45 s; final extension at 72°C for 2 min. A 2 μl sample of PCR product was checked on an agarose gel and the remaining product was purified by Wizard SV Gel and PCR Clean-Up system (Promega, Madison, WI, USA). Purified PCR product was sequenced in both directions using the PCR primers at the Australian Genome Research Facility (http://www.agrf.org.au/) according to AGRF's instructions. The sequence was assembled in BioEdit v 7.0.9 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and searched for sequence similarity in the GenBank of National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) using BLAST (Altschul et al. 1990).

The new ITS sequence was compared with known ITS sequences retrieved from NCBI (http://www.ncbi.nlm.nih.gov), including sequences of *M. rhizophila* strain ATCC 96043 (Accession No. DQ528790), *M. poae* isolate SCR10 (Accession No. DQ528782), *M. grisea* isolate MG15 Vijayanagaram (Accession No. HQ020359), *M. salvinii* strain ATCC 32149 (Accession No. FJ746639), and *Gaeumannomyces graminis* var. *tritici* strain 9735 (Accession No. AF508789). A multiple sequence alignment was constructed using CLUSTAL W (Thompson et al. 1994) in BioEdit v7.0.9 and a Maximum-Likelihood Tree was constructed using the MEGA5 phylogenetics package (Tamura and Nei 1993) with bootstrap values of 1,000 replicates to evaluate the strength of the internal branches.

Results and discussion

Disease symptoms and isolation of fungi

Washed root systems of the diseased grass from both types of disease patches showed numerous blackened roots, some of which had stelar lesions, similar to those caused by *Gaeumannomyces graminis* (Sacc.) Arx & D. L. Olivier var. *avenae* (E.M. Turner) Dennis on bentgrass (*Agrostis* spp.) and hybrid couch [*C. dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] roots (Smiley et al. 2005; Wong et al. 2000) and by *G. wongoonoo* Wong on buffalo grass (*Stenotaphrum secundatum* Kunze) roots (Wong 2002). There were also dark runner hyphae on the surface of the roots, similar to those of ectotrophic root-infecting fungi (Clarke and Gould 1993).

Several fungi were commonly isolated from the diseased turf from Adelaide: *G. graminis* var. *graminis,* a *Phoma* sp., a *Drechslera* sp. and a slow-growing *Magnaporthe* sp. (designated *Magnaporthe* sp. 1). From the "summer decline" disease samples from Queensland, *Gaeumannomyces incrustans* Landschoot & Jackson, *Drechslera* spp., a *Phoma* sp. and another slow-growing *Magnaporthe* sp. (designated

Magnaporthe sp. 2) were frequently isolated. Pure cultures of the above fungi were maintained on ¼ PDA slopes at 4°C.

Pathogenicity tests

Two months after inoculating Greenlees Park couch with the putative pathogens isolated from the Adelaide turf, only the grass in the pots inoculated with *Magnaporthe* sp. 1 showed symptoms of moderate chlorosis and stunting of stolons. The grass in the control pots as well as in those inoculated with *G. graminis* var. *graminis,* the *Phoma* sp., and the *Drechslera* sp. was healthy and green. After 3 months, the experiment was terminated and the root systems were washed free of sand. When observed under a stereo microscope (x 40), only the root systems of plants inoculated with *Magnaporthe* sp. 1 showed dark-coloured roots with dark brown stelar lesions, similar to those of diseased field specimens. The surfaces and cortices of the roots were extensively colonised by black ectotrophic mycelium. Occasionally, unlobed appressoria were observed on infected stolons. The root systems of the plants infected with *Magnaporthe* sp. 1 were reduced in size compared to the healthy controls (Fig. 3) and there was stunting and chlorosis of the shoots. Re-isolation of fungi from the diseased roots on ¼ PDA + N yielded the original fungus, thereby completing Koch's postulates.

For the pathogenicity tests of the fungi isolated from turf affected by "summer decline", only *Magnaporthe* sp. 2 showed characteristic disease lesions with associated dark runner hyphae on roots and unlobed appressoria (Fig. 4) on infected stolons similar to those in field specimens. However, it appeared to be less pathogenic than *Magnaporthe* sp. 1 as there was no visual reduction in the size of the root systems and the shoots appeared healthy. It is possible that more severe symptoms may have developed had the hybrid couch plants in the glasshouse been subjected to the same type of stresses that

Fig. 3 Washed root system of couch inoculated with *M. garrettii* showing blackened roots and reduced size (*right*) compared with the healthy control (*left*) in the pathogenicity test (bar=60 mm)
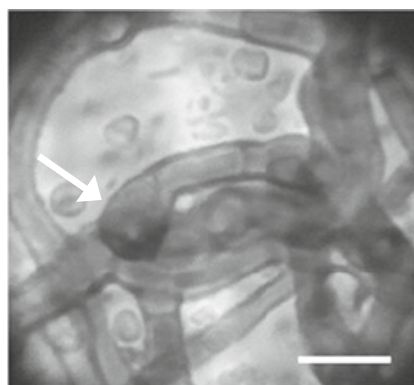

Fig. 4 Appressorium (*arrowed*) and mycelium of *M. griffinii* on a couch stolon (bar=10 μm)

are associated with summer decline in the field, such as moisture and heat stress. However, *Magnaporthe* sp. 2 was consistently re-isolated from the diseased roots on ¼ PDA + N, thereby confirming its pathogenic status.

Cultural characteristics of the pathogens

Both fungi grew very slowly on PDA. *Magnaporthe* sp. 1 (DAR 76937) grew at the rate of 1.2 mm/day at 28°C on PDA while *Magnaporthe* sp. 2 (DAR 80512) grew at the rate of 0.8 mm/day at 28°C on PDA. The relatively thin mycelium (1–3 μm) of both fungi was brownish in colour, with lighter brown aerial growth but giving the mature fungal colony an overall dark brown to black colour. The reverse side of the Petri plate was also black. Hyphae at the margins of the colonies were mostly straight and without the "curling back" characteristic of *Phialophora* spp. or *G. graminis* (Walker 1972; 1980). No conidia were produced in the cultures even after 3 months of incubation at 25°C or 28°C in the dark or when subsequently exposed to alternating dark and diffuse natural light on a laboratory bench (20–25°C) for another month.

Both fungi produced abundant perithecia (Figs. 5 and 6) on PDA after 4–6 weeks' incubation at 25°C and 28°C. In 6–8 week old cultures, numerous ejected ascospores were often found on the lids of Petri plates, especially when the plates were placed upside down. Perithecia also developed on the stolons and leaf sheaths of diseased couch kept in a humid chamber for 4–6 weeks at 20–25°C on a laboratory bench, which was exposed to diffused natural light. When the mature perithecia of the fungi were placed in a drop of water on a microscope slide and lightly squashed with a cover slip, large numbers of asci (Figs. 7 and 8) and ascospores (Figs. 9a, b and 10) were observed. Ascospores of both fungi germinated readily on PDA with germ tubes growing out only from the end cells. Cultures from single ascospores of both fungi produced perithecia readily on PDA, showing that the fungi were homothallic. On both
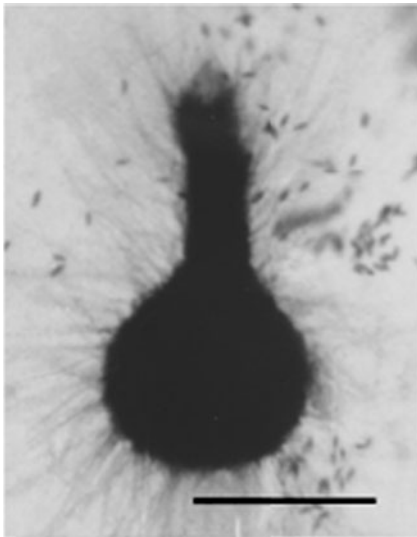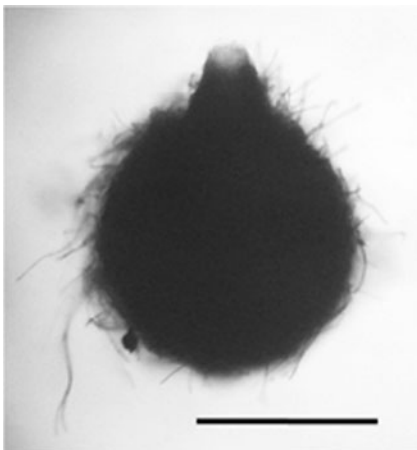
Fig. 5 Perithecium of *M. garrettii* produced on potato dextrose agar showing a characteristic long neck (bar=200 μm)

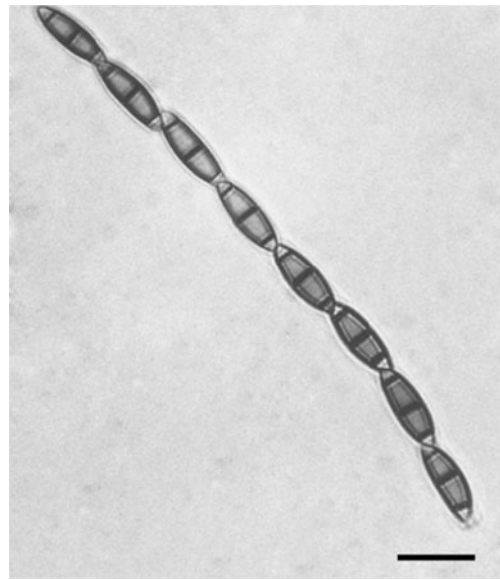**Fig. 7** Ascus of *M. garrettii* showing eight uniseriate ascospores (bar=20 μm)

field material and on PDA, the perithecia of *Magnaporthe* sp. 1 (Fig. 5) had characteristically longer necks than those of *Magnaporthe* sp. 2 (Fig. 6 & Table 2).

ITS sequence and phylogenetic analysis

A 550 bp DNA fragment was obtained from ITS PCR of *Magnaporthe* sp. 2 (isolates TS99 and TY2); however, no product was obtained from *Magnaporthe* sp. 1 (isolate DAR 76937) because the genomic DNA of the dried culture had been totally degraded. The sequences of TS99 (GenBank Accession No. JQ390311) and TY2 (GenBank Accession No. JQ390312) were identical (GenBank accession no. xxxx); therefore, only the TS99 sequence was used in sequence comparison and tree construction. The percentages

of sequence identity among *M. rhizophila, M. poae, M. grisea, M. salvinii, G.graminis* and TS99 are shown in Table 1. It was found that TS99 has low homology compared with the other sequences. BLAST search (using BLASTN) in GenBank (http://www.ncbi.nlm.nih.gov/) against nucleotide collections showed no identical sequence to TS99 indicating that it is a new species. The closest matches (>90%) are: an uncultured Sordariales clone (Accession No. GQ924037.1), 96%; a fungal endophyte isolate (Accession No. FN392299.1), 96% and an uncultured fungus clone (Accession No. GU053815.1), 94%.

Fig. 6 Perithecium of *M. griffinii* produced on potato dextrose agar showing a relatively short neck compared with that of *M. garrettii* (bar=150 μm)

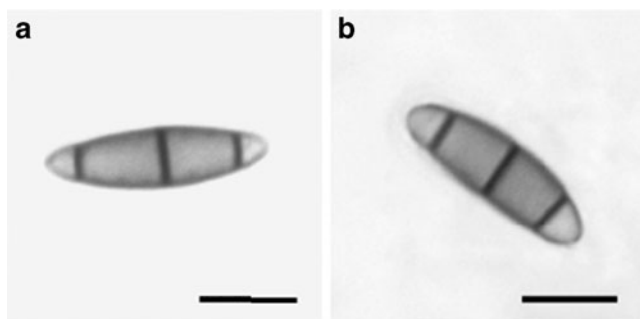**Fig. 8** Ascus of *M. griffinii* showing eight slightly overlapping uniseriate ascospores (bar=20 μm)

Fig. 9 **a** & **b**. Ascospores of *M. garrettii* showing hyaline end cells that are shorter than the brown median cells (bar=10 μm)

A molecular phylogenetic tree was constructed using the Maximum-Likelihood method in MEGA5 with bootstrap values of 1,000 (Fig. 11). It showed that the *Magnaporthe* genus is quite divergent. Only *M. rhizophila* and *M. poae* are grouped with 100% bootstrap support. *M. salvinii* is separated from this group with only 77% bootstrap support. *M. grisea* and *M. griffinii* (TS99) are outgrouped. As the genus *Magnaporthe* is somewhat heterogeneous with species having *Phialophora, Nakataea* and *Pyricularia* anamorphs, the relationship of *M. griffinii* and *M. garrettii* to the other species cannot be resolved at this stage as the latter species have no known anamorphs. However, the fact that *M. grisea* with a *Pyricularia* anamorph is also outgrouped and is less closely related to the other *Magnaporthe* species than *G. graminis* (Fig. 11) suggests that further work is required to clarify the genus concepts of *Magnaporthe* and *Gaeumannomyces*. Morphologically, the ascospores of the two new species are characteristic of *Magnaporthe* (elongated ascospores with median cells pigmented) rather than *Gaeumannomyces* (hyaline, filiform ascospores) [Cannon 1994].

Although DNA data were not obtained for *M. garrettii* to show its relationship in the phylogenetic tree (Fig. 11), its morphological characteristics such as uniseriate ascospores, twice as long asci, much slower growth rate (1.2 mm/day vs
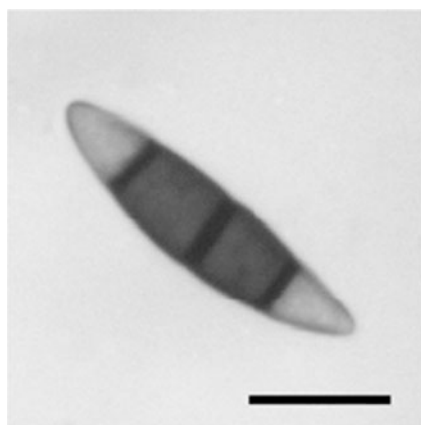
Fig. 10 Ascospore of *M. griffinii* showing hyaline end cells that are similar in length to the brown median cells (bar=10 μm)

~4 mm/day at 28°C) and lack of a *Phialophora* anamorph (Table 2) easily separate it from *M. rhizophila,* the only other homothallic *Magnaporthe* species. However, it is interesting that the examination of all turfgrass specimens bearing perithecia accessioned as *M. rhizophila* in Herb. DAR, Orange, NSW, has shown that DAR 51653 on *Stenotaphrum secundatum* is, in fact, *M. garrettii* and was the cause of a patch disease of buffalo grass in Bourke, New South Wales. Two other specimens of *M. rhizophila* were identical to *M. griffinii* and were the cause of a disease of kikuyu grass in Kanwal, New South Wales (DAR 58461) and a disease of South African couch in Renmark, South Australia (DAR 50508). In all these specimens, when intact asci were present, the ascospores were uniseriate whereas those of *M. rhizophila* are biseriate. It is, therefore, likely that *M. rhizophila* and *M. poae*, have not yet been found in Australia.

Taxonomy

Comparison with other *Magnaporthe* species found on *Poaceae*

Although the anamorphs of the two new taxa are unknown, the teleomorphs belong to the genus *Magnaporthe* according to the concept of Cannon (1994). The four *Magnaporthe* spp. previously recorded on Poaceae are compared with the two recent isolates in Table 2. Of the described species (Krause and Webster 1972; Yaegashi and Udagawa 1978; Scott and Deacon 1983; Landschoot and Jackson 1989), the pathogens most resemble *M. rhizophila* Scott & Deacon in ascospore size and in being homothallic (Table 2). However, they grow more slowly (~1 mm/day vs ~4 mm/day at 28°C) and do not have the *Phialophora* anamorph (Scott and Deacon 1983; Gams 2000) or the biseriate ascospores of *M. rhizophila* (Scott and Deacon 1983). The asci of the pathogens, bearing uniseriate ascospores (Figs. 7 and 8), are also longer and narrower than the asci in the other *Magnaporthe* spp. Evidence from earlier herbarium specimens indicates that these two *Magnaporthe* species have been confused with *M. rhizophila* in Australia, probably because of their similarity in ascospore size (Table 2). The two pathogens are considered to be distinct from the known species of *Magnaporthe* (Cannon 1994) and are described below as new.

Magnaporthe garrettii P. T. W. Wong & M.L. Dickinson sp. nov.

MycoBank: (MB 564204)

Etymology: to honour the late Professor S.D. Garrett, Cambridge University, for his significant work on the ecology of root-infecting fungi of Poaceae.

Table 1 Comparison of the ITS sequence of *M. griffinii* isolate TS99 (Accession No. JQ390311) with known sequences of *M. rhizophila* strain ATCC 96043 (Accession No. DQ528790), *M. poae* isolate SCR10 (Accession No. DQ528782), *M. grisea* isolate MG15 Vijayanagaram (Accession No. HQ020359), *M. salvinii* strain ATCC 32149 (Accession No. FJ746639), and *Gaeumannomyces graminis* var. *tritici* strain 9735 (Accession No. AF508789). Percentages of sequence identity are shown

Sequence	*M. rhizophila* (DQ528790)	*M. poae* (DQ528782)	*M. grisea* (HQ020359)	*M. salvinii* (FJ746639)	*G. graminis* (AF508789)	*M. griffinii* (JQ390311)
M. rhizophila (DQ528790)	100.0%					
M. poae (DQ528782)	97.5%	100.0%				
M. grisea (HQ020359)	76.8%	77.3%	100.0%			
M. salvinii (FJ746639)	81.6%	82.0%	82.9%	100.0%		
G. graminis (AF508789)	80.2%	80.8%	82.3%	89.3%	100.0%	
M. griffinii (TS99)	63.3%	63.5%	66.9%	68.9%	67.2%	100.0%

Perithecia in agaro superficiaria et immersa, nigra, praecipue singularia vel 1–5 aggregata, corpore globoso 180–450 μm diam. et colo decrescenti usque ad 260 μm longo, 90–160 μm lato ad basim. Paries perithecii pseudoparenchymatus, cellulis externis fuscatis. *Asci* unitunicati, numerosi, cylindrici, praecipue recti, octospori, 165–205 × 7–8 μm, stipite breve, apice rotundato, cum annulo refractivo non amyloideo. *Ascosporae* uniseriatae, cylindricae vel fusiformes, rectae vel leniter curvatae cum extremis rotundatis et septis tribus transversis, 19–25 × 5–7 μm, cellulis duobus centralibus atrobrunneis et cellulis duobus extremis hyalinis. *Paraphyses* delicatae, hyalinae, filamentosae, septatae, et in peritheciis maturis languescentes. *Hyphae* in agaro brunneae, rectae vel flexuosae, 1–3 μm latae, in hospite 3–5 μm latae et cum appressoriis lateralibus non-lobatis circa 7 × 10 μm.

Holotypus hic designatus: in cultura agaro sicca ex radicibus *Cynodontis dactylonis* cv. Greenlees Park, Colonel Light Gardens Bowling Club, Adelaide, South Australia, Australia, 30 October 2004, M. L. Dickinson, DAR 76937.

Perithecia superficial and immersed, globose, black, mostly single, sometimes aggregated in small groups, 180–450 μm diameter with a tapering neck up to 260 μm long, 90–160 μm wide at the base. Perithecial wall pseudoparenchymatous, external cells much darker. *Asci* unitunicate, numerous, cylindrical, mostly straight, 8-spored, short-stalked, 165–205 × 7–8 μm, with a tapering but rounded apex and a light refractive, non-amyloid apical ring. *Ascospores* uniseriate, cylindrical to fusiform, straight or slightly curved with rounded ends, 3-septate, with dark brown middle cells and hyaline end cells shorter than the coloured cells, 19–25 × 5–7 μm. *Paraphyses* delicate, hyaline, filamentous, septate and shrivelling in mature perithecia. *Hyphae* brown, straight or flexuous, 1–3 μm wide on potato dextrose agar and 3–5 μm on host. *Appressoria* simple, unlobed, about 7 × 10 μm.

Other specimens examined: On *Stenotaphrum secundatum* (Walter) Kuntze, Bourke, New South Wales, Australia, 14 May, 1985, R. Stone, DAR 51653 (as *M. rhizophila* D.B. Scott & Deacon).

The holotype (DAR 76937) has been deposited in the Plant Pathology Herbarium of the Department of Industry and Investment (Herb. DAR), Orange, New South Wales, Australia. It consists of dried PDA cultures with numerous perithecia of the fungus. The fungus was isolated from diseased roots of *C. dactylon* cv. Greenlees Park from Colonel Light Gardens Bowling Club in Adelaide, South Australia.

Magnaporthe griffinii P.T.W. Wong & A.M. Stirling sp. nov. MycoBank: (MB 564205)

Etymology: to honour Emeritus Professor D.M. Griffin, Australia National University, for his major contribution to the understanding of the role of soil physical factors in the ecology of soil fungi.
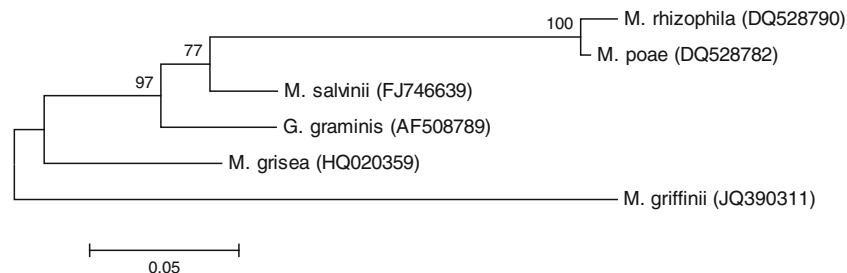
Fig. 11 Molecular phylogenetic analysis by Maximum-Likelihood method using MEGA5 with bootstrap of 1,000 replicates. Bootstrap values are shown for the internal branches (only values >50% are shown). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 456 positions in the final dataset

Table 2 Comparison of the characteristics of six *Magnaporthe* species on Poaceae

Characteristic	M. salvinii[a]	M. grisea[b]	M. poae[c]	M. rhizophila[d]	M. garrettii	M. griffinii
Ascocarp body (μm)	200–400×150–300	200–450	280–565	179–420	180–450	160–340
Neck length (μm)	100–400	ND	<242	252–672	<260	<120
Neck width (μm)	70–100	ND	48–116	84–147	90–160	50–70
Ascus length (μm)	104–165	55–90	63–103	80–100	165–205	145–190
Width (μm)	8.7–7.4	7–10	7–15	8–12	7–8	8–10
Ascospore arrangement	Biseriate	Biseriate	Multiseriate	Biseriate	Uniseriate	Uniseriate
Ascospore Length (μm)	35–65	17–24	23–42	24–28	19–25	24–35
Width (μm)	8.7	4–7	4–6	6–8	5–7	6–9
Anamorph	Nakataea sigmoidea	Pyricularia grisea	Phialophora sp.	Phialophora sp.	Unknown	Unknown
Growth rate on PDA at 28°C (mm/day)	Not determined	Not determined	~6 (at 28–30°C)	~4	1.2	0.8
Thallism	Heterothallic	Heterothallic	Heterothallic	Homothallic	Homothallic	Homothallic
Pathogenicity	Pathogenic to Oryza sativa	Pathogenic to many graminaceous hosts, including Oryza sativa, Stenotaphrum secundatum, Digitaria spp.	Pathogenic to Festuca spp., Poa spp., Lolium perenne, Agrostis stolonifera.	Pathogenic to Triticum aestivum	Pathogenic to Cynodon dactylon and Stenotaphrum secundatum	Pathogenic to Cynodon dactylon × C. transvaalensis, C. transvaalensis and Pennisetum clandestinum

[a] Data from Krause and Webster (1972); [b] Data from Yaegashi and Udagawa (1978); [c] Data from Landschoot and Jackson (1989); [d] Data from Scott and Deacon (1983)

Perithecia in agaro superficiaria et immersa, nigra, praecipue singularia vel in gregibus parvis disposita, corpore globose 160–340 µm diam. et colo decrescenti usque ad 120 µm longo, 50–70 µm lato ad basim. Paries perithecii pseudoparenchymatus, cellulis externis fuscatis. *Asci* unitunicati, numerosi, cylindricae, praecupue recti, octospori, stipite breve, 145–190 × 8–10 µm, apice decrescenti et rotundato cum annulo aefrectivo non amyloideo. *Ascosporae* uniseriatae vel leviter superpositae, fusiformes, rectae vel leviter curvatae, cum extremis obtusis-acutis et septis tribus transversis, 24–35 × 6–9 µm, cellulis duobus centralibus atrobrunneis et cellulis duobus extremis hyalinis. *Paraphyses* delicatae, hyalinae, filamentosae, septatae, et in peritheciis maturis languescentes. *Hyphae* in agaro brunneae, rectae vel flexuosae, 1–3 µm latae, in hospite 3–5 µm latae et cum appressoriis lateralibus non-lobatis circa 8 × 15 µm.

Holotypus hic designatus: in cultura agaro sicca ex radicibus *Cynodontis dactylonis × C. transvaalensis* cv. Tifgreen, Hyatt Coolum Golf Club, Coolum, Queensland, Australia, 13 March, 2008, M. Whatman, DAR 80512.

Perithecia superficial and immersed, globose, black, mostly single, sometimes aggregated in small groups, 160–340 µm diameter with a short tapering neck less than 120 µm long, 50–70 µm wide at the base. Perithecial wall pseudoparenchymatous, external cells much darker. *Asci* unitunicate, numerous, cylindrical, mostly straight, 8-spored, short-stalked, 145–190 × 8–10 µm, with a tapering but rounded apex and a light refractive, non-amyloid apical ring. *Ascospores* uniseriate, slightly overlapping, fusiform, straight or slightly curved with bluntly pointed ends, 3-septate, with dark brown middle cells and hyaline end cells of similar length to coloured cells, 24–35 × 6–9 µm. *Paraphyses* delicate, hyaline, filamentous, septate and shrivelling in mature perithecia. *Hyphae* brown, straight or flexuous, 1–3 µm wide on potato dextrose agar and 3–5 µm on the host. *Appressoria* simple, unlobed, about 8 × 15 µm.

Other specimens examined: On *Cynodon transvaalensis* Burtt-Davy, Renmark, South Australia, Australia, November 1984, G. E. Walker, DAR 50508 (as *M. rhizophila*). On *Pennisetum clandestinum* Hochst. ex Chiov., Kanwal, New South Wales, Australia, 21 November 1986, R. Swan, DAR 58461 (as *M. rhizophila*). On *C. dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy, Adelaide, South Australia, Australia, December, 2010, P. Toy, TY2; Brisbane, Queensland, Australia, January, 2000, A.M. Stirling, TS99.

The holotype (DAR 80512) has been deposited in the Plant Pathology Herbarium of the Department of Industry and Investment (Herb. DAR), Orange, New South Wales, Australia. It consists of dried PDA cultures with numerous perithecia of the fungus. The fungus was isolated from diseased roots of hybrid couch cv. Tifgreen (*C. dactylon × C. transvaalensis*) from Hyatt Coolum Golf Club, Coolum, Queensland, Australia.

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