FÁBIO ALEX CUSTÓDIO

A NEW GENUS OF MAGNAPORTHACEAE (ASCOMYCOTA) CAUSING TAKE-ALL DISEASE ON *Paspalum guenoarum* IN BRAZIL

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae.*

Orientador: Olinto Liparini Pereira

Coorientador: André Wilson Campos Rosado

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Assentimento:

Fábio Alex Custódio
Autor

Olinto Liparini Pereira
Orientador

"Tudo o que a mente do homem é capaz de conceber e acreditar pode ser alcançado." (Napoleon Hill)

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BIOGRAFIA

Fábio Alex Custódio, filho de Aluízio Custódio e Maria da Conceição Inocêncio Custódio, nasceu em Viçosa, Minas Gerais, no dia 11 de agosto de 1995.

Em 2013 ingressou no curso de Agronomia na Universidade Federal de Viçosa (UFV), graduando-se em janeiro de 2018.

Durante o período de setembro de 2014 até o término da graduação foi estagiário e bolsista de iniciação científica no Laboratório de Micologia e etiologia de Doenças Fúngicas de Plantas do Departamento de Fitopatologia, sob a orientação do Prof. Olinto Liparini Pereira. Neste mesmo laboratório acompanhou e desenvolveu projetos em linhas de pesquisa nas áreas de etiologia de doenças fúngicas de plantas e micologia.

Em agosto de 2018, ingressou no Programa de Pós-graduação, em nível de Mestrado em Fitopatologia na UFV, sob a orientação do Prof. Olinto Liparini Pereira.

RESUMO

CUSTÓDIO, Fábio Alex, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Um novo gênero de Magnaporthaceae (Ascomycota) causando mal-do-pé em *Paspalum guenoarum* no Brasil.** Orientador: Olinto Liparini Pereira. Coorientador: André Wilson Campos Rosado.

No Brasil, espécies de *Paspalum* são comumente utilizadas em gramados esportivos, projetos paisagísticos e principalmente como plantas forrageiras na pecuária. Plantas de *Paspalum guenoarum* com sintomas de mal-do-pé foram observadas no estado do Rio Grande do Sul, Brasil. O fungo *Gaeumannomyces graminis* é a única espécie relatada associada a esta doença em *Paspalum*. Nos últimos anos, novas espécies de *Gaeumannomyces* têm sido propostas após estudos moleculares, demonstrando a existência de um complexo de espécies. No Brasil, o mal-do-pé é relatado em plantas de arroz e trigo, no entanto, a etiologia dessa doença em *P. guenoarum* ainda é desconhecida. Devido à falta de estudos etiológicos adequados até o momento, este trabalho teve como objetivo elucidar a etiologia do mal-do-pé em *P. guenoarum* no Brasil, e buscar hospedeiros alternativos do agente etiológico dessa doença. Com base em análises filogenéticas combinadas das regiões gênicas ITS, LSU, TEF-1α e RPB1, um novo gênero de fungo fitopatogênico pertencente à família Magnaporthaceae foi identificado e será proposto de acordo com o Código Internacional de Nomenclatura para Algas, Fungos e Plantas. Um isolado representativo foi inoculado em plantas sadias de *P. guenoarum* e reproduziu os sintomas do mal-do-pé observados em campo. Além disso, esse fungo também é capaz de causar o mal-do-pé em plantas de trigo, sendo que a temperatura afeta diretamente o desenvolvimento da doença nessa cultura. O mal-do-pé em *Paspalum guenoarum* no Brasil é causado por um novo gênero pertencente à família Magnaporthaceae.

Palavras-chave: Doença radicular. Fungo de solo. *Gaeumannomyces*. Podridão de raíz.

ABSTRACT

CUSTÓDIO, Fábio Alex, M.Sc., Universidade Federal de Viçosa, February, 2020. **A new genus of Magnaporthaceae (Ascomycota) causing take-all disease on Paspalum guenoarum in Brazil.** Adviser: Olinto Liparini Pereira.
Co-adviser: André Wilson Campos Rosado

In Brazil, *Paspalum* species are commonly utilized in sports lawns, landscape projects and specially as forage for livestock. *Paspalum guenoarum* plants showing symptoms of take-all disease were observed in the state of Rio Grande do Sul, Brazil. The fungus *Gaeumanomyces graminis* is the only species reported associated with this disease on *Paspalum*. In recent years, new species of *Gaeumannomyces* have been proposed based on molecular studies, demonstrating the existence of a species complex. In Brazil, take-all is reported on rice and wheat plants, however, the etiology of this disease on *P. guenoarum* plants is still unknown. Due to the lack of suitable diagnostic studies up to now, this work aimed to elucidate the etiology of the take-all on *P. guenoarum* in Brazil, and evaluate possible alternative hosts of agricultural importance. Based on combined phylogenetic analyses of ITS, LSU, TEF-1α, and RPB1 a new genus belonging to the family Magnaporthaceae was identified and it will be proposed in accordance with the International Code of Nomenclature for algae, fungi and plantae. A representative isolate was inoculated on healthy *P. guenoarum* plants and reproduced the same symptoms of take-all observed in the field. Furthermore, this fungus also is able to cause take-all on wheat plants and temperature directly affects the development of the disease in this crop. Take-all on *Paspalum guenoarum* in Brazil is caused by a new genus belonging the family Magnaporthaceae.

Keywords: *Gaeumannomyces*. Root disease. Root rot. Soil fungi.

SUMÁRIO

Introdução Geral

Paspalum L. é um dos maiores gêneros da família Poaceae, e inclui aproximadamente 350 espécies de plantas, distribuídas principalmente nas regiões de clima tropical e subtropical do continente Americano (ZULOAGA; MORRONE, 2005). No Brasil, há a ocorrência de mais de 200 espécies (OLIVEIRA; VALLS, 2002), muitas são nativas e ocorrem naturalmente em diferentes composições vegetais dos biomas Brasileiros (ZULOAGA; MORRONE, 2005).

Espécies de *Paspalum* possuem um grande potencial forrageiro (BATISTA; NETO, 1999), além disso, aquelas que possuem características agronômicas desejáveis como tolerância ao frio e déficit hídrico, baixa exigência nutricional, resistência ao pisoteio, rápida cobertura de solo e baixa necessidade de manutenção são utilizadas em diversas atividades como no paisagismo, gramados esportivos, e na recuperação de áreas degradadas. Entre as espécies de maior interesse, destacam-se: *P. notatum* conhecida popularmente como grama-batatais, *P. guenoarum*, *P. vaginatum* e *P. distichum*.

As áreas de cultivo de *Paspalum* no Brasil têm sido acompanhadas pela ocorrência de diversas doenças que provocam danos à cultura. Dentre as doenças citadas na literatura, destacam-se as ferrugens causadas por *Phakopsora compressa* (Mains) Buriticá & J.F. Hennen e *Puccinia zoysia* Dietel; manchas foliares causadas por espécies de *Curvularia* Boedijn*, Drechslera* S. Ito*, Phyllachora* Nitschke ex Fuckel*, Pyricularia* Saccardo e podridão radicular causada por *Rhizoctonia solani* J.G. Kühn (LENNE, 1990; MENDES *et al*., 2020; HENNEN *et al*., 2005; HERNÁNDEZ-RESTREPO *et al*., 2018).

Plantas de *Paspalum guenoarum* com sintomas de mal-do-pé foram observadas em áreas de pastagem no município de Eldorado do Sul, no estado do Rio Grande do Sul, o que pode representar um entrave para o cultivo comercial de espécies de *Paspalum* no Brasil, uma vez que a doença nunca foi relatada em plantas desse gênero no país. No campo, os sintomas foram observados em reboleiras com as plantas apresentando folhas cloróticas a necróticas, podridão de raízes e estolões, em casos de maior severidade da doença, o patógeno pode a matar a planta. Em outros países, a ocorrência da doença em *P. dilatatum* e *P. vaginatum,* é frequentemente associada ao fungo *Gaeumannomyces graminis* (Saccardo) von Arx e Olivier (LENNE**,** 1990;

ELMORE *et al*., 2002; WANG, 2015). Entretanto, o gênero *Gaeumannomyces* compreende um complexo de espécies crípticas (HERNÁNDEZ-RESTREPO *et al.,* 2016).

O gênero *Gaeumannomyces* (Magnaporthaceae*,* Magnaporthales, Sordariomycetes) foi descrito por von Arx e Olivier (1952) para acomodar *Ophiobolus graminis,* pois com base nas características morfológicas da fase sexuada, o mesmo se diferenciava do holótipo de *Ophiobolus*. A fase assexuada deste fungo, foi por muito tempo classificada como *Phialophora*, por possuir células conidiogênicas semelhantes a este gênero. O gênero *Harpophora,* foi proposto para acomodar os fungos com características de *Phialophora* que possuem conídios falcados, rápido crescimento das colônias e que apresentam diferença de pigmentação das estruturas vegetativas de acordo com a idade, dentro da família Magnaphortaceae (GAMS, 2000), entretanto, o mesmo foi considerado sinônimo de *Gaeumannomyces*, a partir de análises filogenéticas (LUO *et al*., 2015).

A família Magnaporthaceae foi descrita por Cannon (1994), para classificar o gênero *Magnaporthe* R. A. Krause & R.K. Webster e os gêneros relacionados, *Buergenerula* Sydow, *Clasterosphaeria* Sivan., *Gaeumannomyces* Arx & D.L. Olivier, *Herbampulla* Scheuer & Nograsek e *Omnidemptus* P.F. Cannon & Alcorn. Os fungos pertencentes a essa família são cosmopolitas e incluem espécies saprófitas, parasitas e endofíticas (HUHNDORF *et al*., 2008). Magnaporthaceae possui fungos que são importantes patógenos de plantas da família Poaceae e Cyperaceae, onde se destaca o gênero *Gaeumannomyces* que é o agente causal do mal-do-pé a doença radicular mais importante da cultura do trigo no mundo e também por atacar o arroz e outras gramíneas de importância econômica e social (COOK, 2003).

Os fungos pertencentes à família Magnaporthaceae, são morfologicamente caracterizados por possuírem peritécios escuros, globosos a subglobosos, com rostro longo, que ficam separados e imersos no substrato. Asca com 8 ascósporos, subcilíndrica, unitunicada, apresentando coloração do anel apical em reação ao iodo (corante de Melzer). Ascósporos curvados a sigmoides, septados, filiformes a fusiformes, hialinos a oliváceos, com as extremidades arredondadas e sem bainha. As formas assexuadas produzem conidióforos simples, não ramificados ou ramificados. Células conidiogênicas integradas, pigmentadas, fialídicas com colaretes ou

dentículos. Conídios hialinos a castanho-claro, septados ou não, de forma variável sendo retos ou curvos. Em alguns gêneros são observados apressórios lobados e escleródios (CANNON, 1994; KLAUBAUF *et al*., 2014).

As características morfológicas, juntamente com dados ecológicos, foram por muitos anos a base para a taxonomia desse grupo de fungos, entretanto, nos últimos anos, análises moleculares têm sido utilizadas para um melhor entendimento das relações filogenéticas nesse grupo e auxíliado na delimitação de gêneros e espécies (HERNÁNDEZ-RESTREPO *et al*., 2016).

Recentemente, vários trabalhos têm combinado às análises morfológicas, análises filogenéticas multilocus que possibilitaram a distinção de espécies pertencentes a complexos, como no caso de *Gaeumannomyces*, e também na descrição de novos gêneros com formas assexuadas semelhantes a *Phialophora* e *Harpophora* (LUO; ZHANG, 2013; KLAUBAUF *et al*., 2014; LUO *et al*., 2014; LIU *et al*., 2015; HERNÁNDEZ-RESTREPO *et al*., 2016; SILVA *et al*., 2019).

Além disso, após análises filogenéticas da subunidade maior do rDNA nuclear (LSU), os gêneros que antes eram incluídos em apenas uma família dentro da ordem Magnaporthales, passaram a ser divididos em quatro famílias, Magnaporthaceae, Pyriculariaceae, Ophioceraceae e Pseudohalonectriaceae, compreendendo um total de 36 gêneros e mais de 100 espécies conhecidas (KLAUBAUF *et al*., 2014, WIJAYAWARDENE *et al*., 2017; SILVA *et al*., 2019).

No Brasil, o mal-do-pé é relatado em áreas de produção de trigo e arroz, incluído o Rio Grande do Sul, principal estado produtor dessas culturas no país (AMORIM *et al*., 2016; CONAB, 2019). Entretanto, não há relatados dessa doença em plantas de *Paspalum* no país. Assim, baseado na importância das espécies de *Paspalum*, os danos causados pela doença nessa cultura, ausência de estudos etiológicos e da grande importância das culturas do trigo e arroz, o presente estudo teve como objetivo elucidar a etiologia do mal-do-pé em *Paspalum guenoarum* no Brasil e avaliar possíveis hospedeiros do agente etiológico dessa doença.

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Artigo

According to the guidelines of Plant Pathology

A new genus of Magnaporthaceae (Ascomycota) causing take-all disease on *Paspalum guenoarum* in Brazil

A new genus of Magnaporthaceae (Ascomycota) causing take-all disease on *Paspalum guenoarum* in Brazil

Abstract

In Brazil, *Paspalum* species are commonly utilized in sports lawns, landscape projects and specially as forage for livestock. *Paspalum guenoarum* plants showing symptoms of take-all disease were observed in the state of Rio Grande do Sul, Brazil. The fungus *Gaeumanomyces graminis* is the only species reported associated with this disease on *Paspalum*. In recent years, new species of *Gaeumannomyces* have been proposed based on molecular studies, demonstrating the existence of a species complex. In Brazil, take-all is reported on rice and wheat plants, however, the etiology of this disease on *P. guenoarum* plants is still unknown. Due to the lack of suitable diagnostic studies up to now, this work aimed to elucidate the etiology of the take-all on *P. guenoarum* in Brazil, and evaluate possible alternative hosts of agricultural importance. Based on combined phylogenetic analyses of ITS, LSU, TEF-1α, and RPB1 a new genus belonging to the family Magnaporthaceae was identified and it will be proposed in accordance with the International Code of Nomenclature for algae, fungi and plantae. A representative isolate was inoculated on healthy *P. guenoarum* plants and reproduced the same symptoms of take-all observed in the field. Furthermore, this fungus also is able to cause take-all on wheat plants and temperature directly affects the incidence and development of the disease in this crop. Take-all on *Paspalum guenoarum* in Brazil is caused by a new genus belonging the family Magnaporthaceae.

Running head: Take-all on *Paspalum*

Keywords: *Gaeumannomyces*, Root disease, Root rot, Soil fungi, *Triticum*

Introduction

Paspalum is one of the largest genera in the family Poaceae and includes approximately 350 species of plants, distributed mainly in the tropical and subtropical regions of the American continent (Zuloaga & Morrone, 2005). More than 200 species occurs in Brazil (Oliveira & Valls, 2002), most of them native and naturally found in different vegetable compositions of the Brazilian biomes (Zuloaga & Morrone, 2005).

Paspalum species have a great morphological and physiological diversity (Sartor *et al*., 2009). Those that have desirable agronomic characteristics such as cold tolerance and water deficit, low nutritional requirement, resistance to trampling, good palatability, fast soil cover, and low maintenance need are used in sports lawns, landscape projects, and as forage for livestock. Among the species of greatest economic interest, we highlight *P. notatum* popularly known as Pensacola Bahiagrass, *P. guenoarum*, *P.vaginatum* and *P. distichum*. However, the occurrence of fungal diseases affects the production and quality of these plants, causing economic and ecological losses.

Plants of *P. guenoarum* ecotype "Azulão" with symptoms of take-all disease were observed in Eldorado do Sul, Rio Grande do Sul state, Brazil. The symptoms observed in the field were spots that vary in shape and size and have well-defined borders between diseased and healthy plants. The diseased plants have chlorotic or dark foliage, rot of roots and culms (Fig 1). In others *Paspalum* species, the take-all has commonly been associated with the fungus *Gaeumannomyces graminis* **(**Lenne**,** 1990; Elmore *et al*., 2002; Wang, 2015**).** However, *G. graminis* comprises a complex of species (Hernández-Restrepo *et al*., 2016).

The genus *Gaeumannomyces* (Magnaporthaceae, Magnaporthales, Sordariomycetes) was described by von Arx & Olivier (1952) to accommodate *Ophiobolus graminis*, based on morphological characteristics of the sexual morph, which differed from the *Ophiobolus* holotype. The asexual morph of *Gaeumannomyces* for a long time was classified as *Phialophora,* due to its phialidic conidiogenous cells and lunate conidia similar to this genus. The genus *Harpophora* has been proposed to accommodate *Phialophora*-like fungi that have sickle-shaped conidia, fast growing-colonies and differences in pigmentation of vegetative structures according to age, within the Magnaphortaceae family (Gams, 2000). However, it has

been considered synonymous with *Gaeumannomyces* based on results from phylogenetic analysis (Luo *et al.*, 2015).

Morphological characteristics, together with ecological data, have been the basis for the taxonomy of this group of fungi for many years. However, in recent years, molecular analyzes have been used to better understand the phylogenetic relationships in this group and to aid in the delimitation of genera and species (Luo & Zhang, 2013; Klaubauf *et al*., 2014; Luo *et al*., 2015; Hernández-Restrepo *et al*., 2016; Silva *et al*., 2019).

In Brazil, take-all is reported in the areas of wheat and rice production, including the state of Rio Grande do Sul, the main producing state of these crops in the country (Amorim *et al*., 2016; CONAB, 2019). However, there are no report of this disease on *Paspalum* species in the country. Thus, based on the importance of the *Paspalum* species, the severe damage caused by disease on these crops and the lack of suitable etiologic studies and the great importance of wheat and rice crops, the aims of this present work were to elucidate the etiology of the take-all on *Paspalum guenoarum* in Brazil and evaluate alternative hosts of agricultural importance.

Materials and methods

Fungal isolation

Plants of *Paspalum guenoarum* ecotype "Azulão" showing take-all were collected in Eldorado do Sul, Rio Grande do Sul state, Brazil. The plants were sent to the Laboratório de Fitopatologia (Departamento de Fitossanidade, Universidade Federal do Rio Grande do Sul). Small fragments of areas of transition between asymptomatic and symptomatic tissue were obtained to proceed with indirect isolation of fungal. The fragments were disinfected superficially in 70% ethanol for 1 min, followed by immersion in 1% sodium hypochlorite for 3 min and subsequently washed two times in sterile distilled water. Later, these fragments were placed in Petri dishes containing potato dextrose agar (PDA; Acumedia) added with antibiotic to prevent the bacterial growth and incubated at 25 °C.

The isolates obtained were sent to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas from the Departamento de Fitopatologia of the Universidade Federal de Viçosa. These cultures were purified by the single hyphal-tip method prepared on 2% water agar (WA; type I Himedia) (Dhingra & Sinclair, 1995)

and maintained on 2 mL microtubes containing distilled water. The isolates were stored in sterile distilled water, in anhydrous silica gel at 5 ºC and in 2 mL microtubes containing 10% glycerol solution at -80 ºC (Castellani, 1939; Dhingra & Sinclair, 1995). The isolates were deposited in the culture collection of fungi "Coleção Octávio Almeida Drummond" (COAD), and metabolically inactive cultures were deposited in the herbarium VIC, both belonged at the Universidade Federal de Viçosa. Nomenclatural novelties and descriptions will be deposited in MycoBank (www.mycobank.org).

DNA extraction, sequencing, and phylogenetic analyses

Single hyphal-tip isolates were grown on PDA at 25 °C under a 12-h photoperiod for a week. Approximately 40 mg of mycelia were collected and placed in a 2 mL microcentrifuge tube containing 600 µL of Nuclei Lysis Solution of the Wizard Genomic DNA Purification kit (Promega), 100 mg polyvinylpyrrolidone (PVP; Sigma-Aldrich) and four steel beads. Next, the samples were mixed and crushed in the L-Beader 6 (Loccus Biotecnologia). After the maceration, the extraction was proceeded as described by Pinho *et al.* (2013). The samples were deposited in the DNA collection (CDA), from at the "Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas", Viçosa, Minas Gerais, Brazil.

Target sequences of the internal transcribed spacer region (ITS), including internal transcribed spacer 1, 5.8S rRNA and internal transcribed spacer 2, partial large subunit (LSU) of nuclear rDNA, translation elongation factor 1-a (TEF1- α) and large subunit of RNA polymerase II (RPB1) were amplified using primers ITS1 and ITS4 for ITS (White *et al*., 1990); LR0R and LR5 for LSU (Vilgalys & Hester, 1990) EF1-983F and EF1–2218R for TEF1- α (Rehner & Buckley, 2005); RPB1-Af and RPB1-Cr for RPB1 (Stiller & Hall, 1997; Matheny *et al*., 2002), respectively. PCR was prepared with 18 µL of Platinum® PCR SuperMix (Life Technologies, Brazil); 0,4 µL of 10 pmol/µL of each primer (Invitrogen, Carlsbad, CA, U.S.A.); 1.2 µL of genomic DNA (25ng/µL). Negative controls with nuclease-free water instead of DNA were performed at each PCR.

The PCR conditions consisted of initial denaturation at 94 ºC for 5 min for ITS, 94 ºC for 2 min for LSU and TEF1-α or 94 ºC for 3 min for RPB1; followed by 30, 35, 35 or 40 cycles of denaturation at 94 ºC for 30 s for ITS or 94 ºC for 60 s for

LSU, TEF1-α and RPB1; primer annealing at 52 ºC for 40 s, 53 ºC for 60 s, 57 ºC for 60 s or 54 ºC for 90 s, primer extension at 72 ºC for 40 s, 60 s, 60 s or 70 s and a final extension at 72 ºC for 5 min for ITS, LSU, RPB1 or 10 min for TEF1-α, respectively. PCR products were stained with GelRed (Biotium Inc.) and analyzed by electrophoresis in 2% agarose gels at 80 V for 45 min in 1 × TAE buffer and visualized under UV light to check for amplification size and purity. Amplification products were purified and sequenced by Macrogen Inc., South Korea (http://www.macrogen.com).

The nucleotide sequences were edited with the BIOEDIT software (Hall, 2012). All sequences were checked manually. New sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov; Table 1). Consensus sequences were compared against GenBank's database using their MEGA BLAST program for a first identification. The ITS, LSU, TEF1-α and RPB1 sequences of all genera of Magnaporthaceae were retrieved from GenBank (Table 1). The sequences generated in this study were aligned with sequences retrieved using the MUSCLE® algorithm (MUltiple Sequence Comparison by Log-Expectation; Edgar, 2004), performed in MEGA 7 software (Kumar *et al*., 2016) using the default parameters. Alignments were checked and manual adjustments were made when necessary. The absence of some locus in the concatenated alignment was treated as missing data. The resulting alignment will be deposited into TreeBASE (http://www.treebase.org/).

Bayesian inference (BI) concatenated analyses employing a Markov Chain Monte Carlo (MCMC) method were performed with all sequences, first with each gene/locus separately, and then with the concatenated sequences (ITS, LSU, RPB1 and TEF1-α). Before launching the BI, the best nucleotide substitution model was determined for each gene by MRMODELTEST v. 2.3 (Posada & Buckley, 2004) according to the Akaike Information Criterion (AIC). Phylogenetic analyses were performed with the CIPRES Science Gateway V. 3.3 (Miller *et al*., 2015) using MRBAYES v. 3.2.6 (Ronquist & Huelsenbeck, 2003). In MRBAYES, data were partitioned by locus, and the parameters of the nucleotide substitution models for each partition were set as described above. Were realized two runs simultaneously, in each run were four MCMC chains were run simultaneously, starting from random trees for 10 000 000 generations. Trees were sampled every 1 000th generation for a total of 20 002 trees. The first 2 500 trees were discarded as the burn-in phase of each analysis.

Posterior probabilities (Rannala & Yang, 1996) were determined from a majority-rule consensus tree generated with the remaining trees.

Maximum likelihood (ML) analyses were conducted in RAxML-HPC v.8 on XSEDE in the CIPRES Science Gateway V. 3.3 (Miller *et al*., 2015). The evolution model selected was GTRGAMMA and 1 000 replications of bootstrap were performed. Trees were visualized in FIGTREE v. 1.4.2 (Rambaut, 2009), and exported to graphics programs. The trees were rooted with *Pyricularia grisea* BR0029.

Morphological study

Microscopic preparations were made by slide culture, as described by Riddell (1950) and cited by Rosado *et al*. (2019). For sporulation, the fungi were grown on 2 % PDA plugs, placed directly onto a sterile microscope slide. Petri dishes with slides were incubated at 25 °C under a photoperiod of 12 h for 1-3 wk. Microscopy slides were mounted in lactoglicerol. Photographs were taken with an Olympus BX53 microscope equipped with a digital camera, Olympus Q-Color5™. Measurements (n=30) of the relevant morphological characteristics (conidia, conidiophores and conidiogenous cells) were made using the Olympus cellSens Dimension 1.9 software. For cultural characteristics, colonies were cultivated on PDA, MEA and OA for 7 days at 25 ºC a photoperiod of 12 h.

Pathogenicity test

Paspalum guenoarum

To confirm fungal pathogenicity, healthy 1-month-old plants of *P. guenoarum* were inoculated with a representative isolate. For inoculation, the isolate were grown in Petri dishes containing PDA for 7 days at 25 °C with a photoperiod of 12 h. Two methods of inoculation were used, a with injury and a without injury. In the firstone, the culm of plants was perforated with a sterile toothpick immersed in a conidial suspension adjusted to 10^6 conidia mL^{-1}. In the second method, 50 mL of the same conidial suspension adjusted to 10^6 conidia mL^{-1} were mixed with soil. In the control, the culm of plants was wounded with sterile toothpicks immersed in sterile distilled water or 50 mL of sterile distilled water were spread in the soil, respectively. After inoculation, the plants were maintained in moist chambers for 48h . Posteriorly, the plants were maintained in a greenhouse at 28 ºC ± 5 °C for 30 days and were then evaluated for the presence or absence of symptoms (incidence).

Pensacola Bahiagrass, rice and wheat

Plants of Pensacola Bahiagrass, rice (cultivar BRSMG SELETA) and wheat (cultivar BRS 394) were inoculated to verify if these species are possible hosts of the fungal. In the plants of Pensacola Bahiagrass, were realized the same the methods of inoculation utilized on *P. guenoarum*. For rice and wheat plants, in the firstone method, 10µL of the conidial suspension were injected in the culm of each plant, on the control plants 10µL of sterile distilled water were injected. The second method of inoculation, were same utilized on *P. guenoarum*. After inoculation, the plants were maintained in moist chambers for 48h. Posteriorly, five repetitions of each treatment were maintained in chambers with temperature controlled of 19 ºC, 25 ºC and 28 ºC for 30 days and were then evaluated for the presence or absence of symptoms.

The disease incidence data under different conditions of temperature and inoculation, were analyzed by Kruskal-Wallis test to check for if there was variance between the treatments, what was observed (p-value = 0.0001408). Posteriorly, the treatments were compared by Wilcox test at 5 % significance level, the data were processing and analyses with software R version 3.6.3.

Results

Fungal isolation

Thirty-six homogenous dark pigmented colonies, similar to *Gaeumannomyces* colonies were obtained from plant tissues with symptoms of take-all. From these colonies, four representative isolates were randomly selected for molecular and morphological characterization. The isolates were deposited in the COAD culture collection of fungi, with accession numbers COAD 2959, COAD 2960, COAD 2961 and COAD 2962.

PCR amplification, sequencing, and phylogenetic analyses

PCRs were conducted successfully for all gene regions used. Sequencing was successfully performed for most samples. However, the sequence from ITS of COAD 2961 showed low quality. Thus, this sequence was excluded from further analyses. The high-quality sequences were deposited in GenBank (Table 1). The concatenated alignment from BI and ML analyses consisted of 32 strains (including the outgroup sequence) and 2515 characters (570 for ITS, 754 for LSU, 700 for RPB1 and 491 for TEF1-α) including gaps. From the phylogenetic analyses (Fig 2), the isolates COAD

2959, COAD 2960, COAD 2961 and COAD 2962 were clustered and formed a single well-supported clade distinct from the known genera belonging to Magnaporthaceae, representing a possible new genus. The topology of tree generated from Bayesian inference analysis was essentially similar to that from Maximum likelihood analysis.

Taxonomy

Sordariomycetes, Magnaporthales, Magnaporthaceae (To be proposed as new genus)

Mycelium septate, branched, smooth, and hyaline to pale brown. *Conidiophores* are commonly reduced to conidiogenous cells, sometimes macronematous. *Conidiogenous cells* phialidic, hyaline to pale brown, solitary or grouped, terminal or intercalary, lageniform, straight or curved with a cylindrical to funnel-shaped collarette. *Conidia* falcate, hyaline, smooth, and non-septate. *Hyphopodium* not observed.

(To be proposed as a new species) (Fig 3)

Description on PDA: *Mycelium* is septate, branched, smooth, and hyaline to pale brown, 2-6 µm diam hyphae. *Conidiophores* are commonly reduced to conidiogenous cells, sometimes macronematous, 5–12.5 × 2.5–3.5 µm. *Conidiogenous cells* are phialidic, hyaline to pale brown, solitary or grouped, terminal or intercalary, lageniform, straight or curved, 2.5–18 × 1.5–4 µm, cylindrical to funnel-shaped collarette up to 1.5 µm, 1–2 µm diam. *Conidia* are falcate, hyaline, smooth, non-septate, 5–18.2 × 1.5–3.5 µm. *Hyphopodium* not observed.

Culture characteristics: After 7 days at 25 °C: On PDA reaching 55 mm diam, aerial mycelium sparse, dense in the center cottony, with olivaceous-grey surface and dark-grey on the reverse. On MEA reaching 40 mm diam, aerial mycelium abundant, cottony, with a white surface and dark-grey on the reverse, colorless to the periphery. On OA reaching 44 mm diam, aerial mycelium scarce to moderate, dense in the center cottony, with dark-grey surface and pale brown on reverse.

Material examined: Brazil, Rio Grande do Sul state, municipality of Eldorado do Sul, isolated from *Paspalum guenoarum*, January. 2016, R. Ramos Lopes (to be proposed as holotype VIC 47360, to be proposed as ex-type culture COAD 2960). Other material examined: Brazil, Rio Grande do Sul state, municipality of Eldorado do Sul, isolated

from *Paspalum guenoarum*, January. 2016, R. Ramos Lopes.: COAD 2959, COAD 2961 and COAD 2962.

Pathogenicity tests

Paspalum guenoarum

Plants of *P. guenoarum* showed the same symptoms observed in the field in both inoculation methods, namely roots rot, internal darkening of the culm and severe wilt, resulted in the death of the plants (Fig 4). No symptom was observed on control plants. The fungus was re-isolated from all inoculated plants and its identity was confirmed as previously described, thereby satisfying Koch's Postulates. No fungus was re-isolated from control plants.

Pensacola Bahiagrass, rice and wheat

The wheat plants showed symptoms of take-all in all tested temperature conditions, but with different levels of disease incidence (Fig 5). The plants inoculated without injury kept at 19 ºC no showed symptom of take-all. Although there is no statistical difference in the incidence of the disease, between the plants inoculated by with injury kept at 19 ºC and those in the chamber at 25 ºC (both methods of inoculation), were observed differences in symptoms. The plants at 19 ºC developed only small necrotic lesions on culm base (Fig 6), while those maintained at 25 ºC symptoms of roots rot, internal darkening of the culm and wilt (Fig 7). The major incidence of the disease occurred at 28 ºC, were observed symptoms of roots rot, internal darkening of the culm, severe wilt and also an elevated number of dead plants. No symptom was observed on control plants. The fungus was re-isolated from plants with showed symptoms, and no fungus was re-isolated from control plants.

The plants of Pensacola Bahiagrass and rice showed no symptom of take-all in conditions tested.

Discussion

Some works repost *Gaeumannomyces graminis* as the causal agent of take-all in *Paspalum* in other countries (Lenne**,** 1990; Elmore *et al*., 2002; Wang, 2015). However, in this study from combined phylogenetic analyses of ITS, LSU, TEF-1α

and RPB1, using sequences of know genera belonging to Magnaporthaceae, a probable new genus in this family causing this disease in Brazil was identified.

The possible new genus is morphologically similar to the genera *Falciphora*, *Falciphoriella*, *Gaeumannomycella* and *Gaeumannomyces,* producing a *Harpophora*-like asexual state, but is phylogenetically different. The possible new genus is phylogenetic close to *Buergenerula spartinae,* but this last genus is associated to aerial part of *Spartina* in marine habitat, moreover, the genera are phylogenetically different (Kohlmeyer & Gessner, 1976; Luo & Zhang, 2013). Corroborating previous studies, phylogenetic analyses are necessary for correct identification of fungi with asexual morphs of *Phialophora*-like and *Harpophora*-like on Magnaporthaceae (Luo & Zhang, 2013; Klaubauf *et al*., 2014; Luo *et al*., 2015; Hernández-Restrepo *et al*., 2016; Silva *et al*., 2019).

According to the results of this study, take-all on *P. guenoarum* is caused by a probable new genus of Magnaporthaceae. This fungus can also cause take-all in wheat plants and temperature directly affects the development of the disease in this crop. In Brazil, the take-all of wheat caused by *Gaeumannomyces graminis* is favored in temperature conditions around of 12–20 ºC (Amorim *et al*., 2016). However the disease caused by new genus is favored in conditions of more elevated temperatures, in milder temperature conditions, the fungal causes only small necrotic lesions on culm base of plants, while at higher temperatures are observed symptoms of roots rot, culm rot, severe wilt and dead plants.

In Brazilian state of Rio Grande do Sul, many farmers utilize the same areas of cultivation of *P. guenoarum* for plantation of wheat. This practice should be avoid in areas of occurrence of take-all on *P. guenoarum*, since the possible new genus is able to cause disease in both crops.

Take-all is the most important root disease of the wheat crop in the world and was traditionally been associated with the fungus *Gaeumannomyces graminis* (Cook, 2003). Recetly, Hernández-Restrepo *et al*. (2016) realized a taxonomic revision of *Gaeumannomyces* and *Gaeumannomyces*-like and found different species of *Gaeumannomyces* associated with the wheat crop. The authors used isolates collected in different countries and hosts. However, in this revision, a small number of isolates from South America was used. In the present study, we showed that the possible new

genus causes take-all on wheat. Therefore, additional studies are necessary to verify the diversity of other Magnaporthaceae associated with take-all on wheat and other cultures in Brazil.

To our knowledge, this is the first study employing multilocus molecular phylogenies on etiology of take-all on *Paspalum*, which may be considered an important disease to this crop, since it can result in the death of the plants. The information shown herein will certainly be useful for future studies involving the etiology of take-all on wheat using a polyphasic approach in Brazil, control measures on *P. guenoarum* and wheat. Furthermore, it will provide data for quarantine programs and possibly for the development of *Paspalum* and wheat varieties resistant to take-all.

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Figure legends

Figure 1. Symptoms of take-all on *Paspalum notatum*. (a - d) Symptoms in the field; (e) Symptoms of culm rot.

Figure 2. Phylogenetic tree inferred from the Bayesian analysis using a combined ITS, LSU, TEF-1a, and RPB1sequences of 31 members of the Magnaporthaceae family. Bootstrap values above 70% and Bayesian posterior probabilities are indicated above the nodes. The tree was rooted to *Pyricularia grisea* Br0029. The bar represents the number of changes in the nucleotide sequence of each 100 bp. The isolates obtained in this study are highlighted in bold.

Figure 3. Possible new genus of Magnaporthaceae (COAD 2960). (a – d) Conidiophores (black arrows) and conidiogenous cells (white arrows); (e) Conidia. Scale bars: (a–e) = 10 µm.

Figure 4. Pathogenicity tests with injury on *Paspalum guenoarum***.** (a) Inoculated plants. (b). Control plants. (c) Roots of inoculated plants. (d) Roots of control plants.

Figure 5. Mean incidence of take-all on wheat plants inoculated with the new genus, under different conditions of inoculation and temperature. Columns with the same letter are not significantly different according to Wilcox test ($P \geq 0.05$).

Figure 6. Pathogenicity tests with injury on wheat plants maintained at 19ºC. (a) Inoculated plants. (b). Control plants. (c) Roots of inoculated plants. (d) Roots of control plants.

Figure 7. Pathogenicity tests with injury on wheat plants maintained at 25ºC. (a) Inoculated plants. (b). Control plants. (c) Roots of inoculated plants. (d) Roots of control plants.

Figure 8. Pathogenicity tests with injury on wheat plants maintained at 28ºC. (a) Inoculated plants. (b). Control plants. (c) Roots of inoculated plants. (d) Roots of control plants.

Table 1. GenBank accession numbers of DNA sequences of Magnaporthaceae used in phylogenetic analyses.

Taxa	Strain numbers[a]	Country: locality	Host/substrate	GenBank accession numbers			
				ITS	LSU	RPB1	TEF1
New genus	**COAD 2959**	**Brazil: Rio Grande do Sul**	***Paspalum guenoarum***	**MN747361**	**MN749075**	**MN755608**	**MN698637**
New genus	**COAD 2960**	**Brazil: Rio Grande do Sul**	***Paspalum guenoarum***	**MN747362**	**MN749076**	**MN755609**	**MN698638**
New genus	**COAD 2961**	**Brazil: Rio Grande do Sul**	***Paspalum guenoarum***	**-**	**MN749077**	**MN755610**	**MN98639**
New genus	**COAD 2962**	**Brazil: Rio Grande do Sul**	***Paspalum guenoarum***	**MN747363**	**MN749078**	**MN755611**	**MN698640**
Bifusisporella sorghi	URM 7442	Brazil: Pernambuco	*Sorghum bicolor*, endophyte from leaves	MK060155	MK060153	MK060159	MK060157
Bifusisporella sorghi	URM 7864	Brazil: Pernambuco	*Sorghum bicolor*, endophyte from leaves	MK060156	MK060154	MK060160	MK060158
Budhanggurabania cynodonticola	BRIP 59305	Australia	*Cynodon dactylon,*	KP162134	KP162140	KP162143	KP162138

			rotted roots and stolons				
Bussabanomyces longisporus	CBS 125232	Thailand	*Amomum siamense*, leaves	KM484832	KM484951	KM485046	KM009202
Buergenerula spartinae	ATCC 22848	USA	*Spartina alterniflora*, leaves	JX134666	DQ341492	JX134720	JX134692
Falciphora oryzae	CBS 125863 = R5-6-1	China	*Oryza sativa*, root, endophyte	EU636699	KJ026705	KJ026706	JN857963
Falciphoriella solaniterrestris	CBS 117.83	Netherlands	Soil in potato field	KM484842	KM484959	KM485058	-
Gaeumannomyces arxii	CBS 903.73 = DAR 23471	Australia	*Pennisetum clandestinum*, (kikuyu grass), stolon	KM484837	KM484854	KM485053	KX306681
G. graminis	CPC 26020 = CBS 141384	USA	*Cynodon dactylon* × *C. transvaalensis*	KX306498	KX306568	KX306633	KX306701
G. graminicola	CPC 26025 = CBS 141381	USA	*Stenotaphrum secundatum*	KX306495	KX306565	KX306630	KX306698
Gaeumannomycella caricis	CPC 26262 = CBS 141374	UK	*Carex rostrata*	KX306478	KX306548	KX306671	KX306675
Gaeumannomycella caricis	CBS 388.81	UK	*Carex rostrata*	KM484843	KM484960	-	KX306674

Kohlmeyeriopsis medullaris	CBS 117849 = JK5528S	USA	*Juncus roemerianus*	KM484852	KM484968	KM485068	-
Magnaporthaceae, *incertae sedis*	CPC 26284 = GP57	UK	*Triticum aestivum*	KX306546	KX306616	-	KX306677
Magnaporthiopsis sp.	CPC 26038	USA	*Cynodon dactylon* × *C. transvaalensis*	KX306545	KX306615	KX306672	KX306676
M. incrustans	M35			JF414843	JF414892	JF710437	-
M. maydis	CBS 133165 = ATCC MYA-3356	Israel	*Zea mays*	KX306544	KX306614	-	-
M. maydis	CBS 662.82A	Egypt	*Zea mays*	KM484856	KM484971	KM485072	-
M. poae	M23		*Poa pratensis*	JF414834	JF414846	JF710432	-
	M48	USA	*Poa pratensis*	JF414837	-	JF710434	-
Nakataea sp.	CBS 332.53	USA: Arkansas	*Oryza sativa*	KM484867	KM484981	KM485083	-
N. oryzae	CBS 252.34	Burma	*Oryza sativa*	KM484862	KM484976	KM485078	-
	CBS 288.52	Japan: Takada	*Oryza sativa*, stem	KM484864	KM484978	KM485080	-
Neogaeumannomyces bambusicola	MFLUCC11-0390	Thailand	Dead culm of bamboo	KP744449	KP744492	-	-
Omnidemptus affinis	ATCC 200212	Australia	*Panicum effusum* var. effusum grass leaves	JX134674	KX134686	JX134728	JX134700
Pseudophialophora eragrostis	CM12m9	USA	*Eragrostis* sp.	KF689648	KF689638	KF689618	KF689628

Pyricularia grisea	BR0029	Brazil: Goias	*Digitaria sanguinalis*	KM484880	KM484995	KM485100	-

Sequences obtained in this study are shown in bold

Figures

Figure 1.

Figure 2.

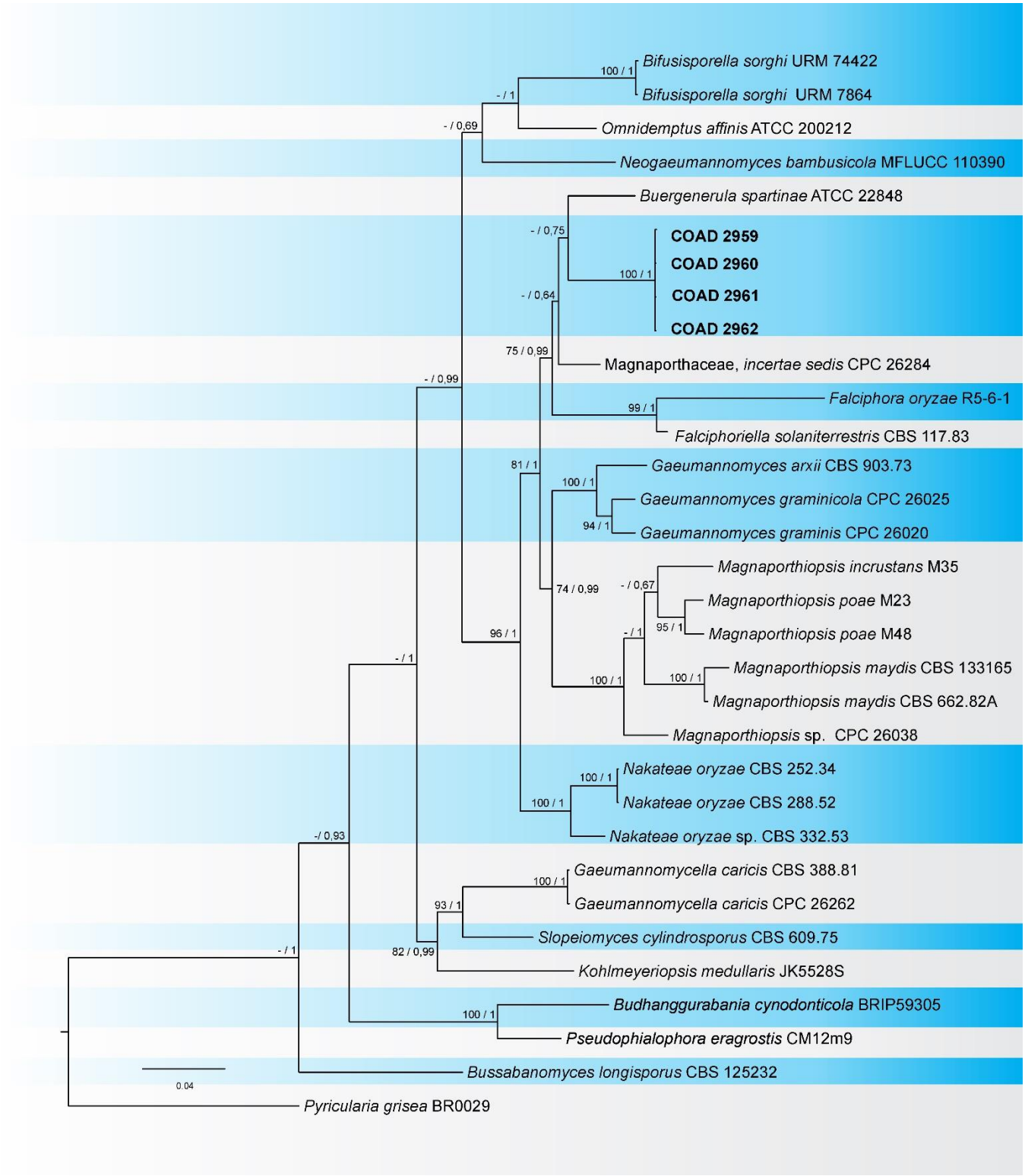

Bifusisporella sorghi URM 74422
Bifusisporella sorghi URM 7864
Omnidemptus affinis ATCC 200212
Neogaeumannomyces bambusicola MFLUCC 110390
Buergenerula spartinae ATCC 22848
COAD 2959
COAD 2960
COAD 2961
COAD 2962
Magnaporthaceae, incertae sedis CPC 26284
Falciphora oryzae R5-6-1
Falciphoriella solaniterrestris CBS 117.83
Gaeumannomyces arxii CBS 903.73
Gaeumannomyces graminicola CPC 26025
Gaeumannomyces graminis CPC 26020
Magnaporthiopsis incrustans M35
Magnaporthiopsis poae M23
Magnaporthiopsis poae M48
Magnaporthiopsis maydis CBS 133165
Magnaporthiopsis maydis CBS 662.82A
Magnaporthiopsis sp. CPC 26038
Nakateae oryzae CBS 252.34
Nakateae oryzae CBS 288.52
Nakateae oryzae sp. CBS 332.53
Gaeumannomycella caricis CBS 388.81
Gaeumannomycella caricis CPC 26262
Slopeiomyces cylindrosporus CBS 609.75
Kohlmeyeriopsis medullaris JK5528S
Budhanggurabania cynodonticola BRIP59305
Pseudophialophora eragrostis CM12m9
Bussabanomyces longisporus CBS 125232
Pyricularia grisea BR0029
0.04

Figure 3.

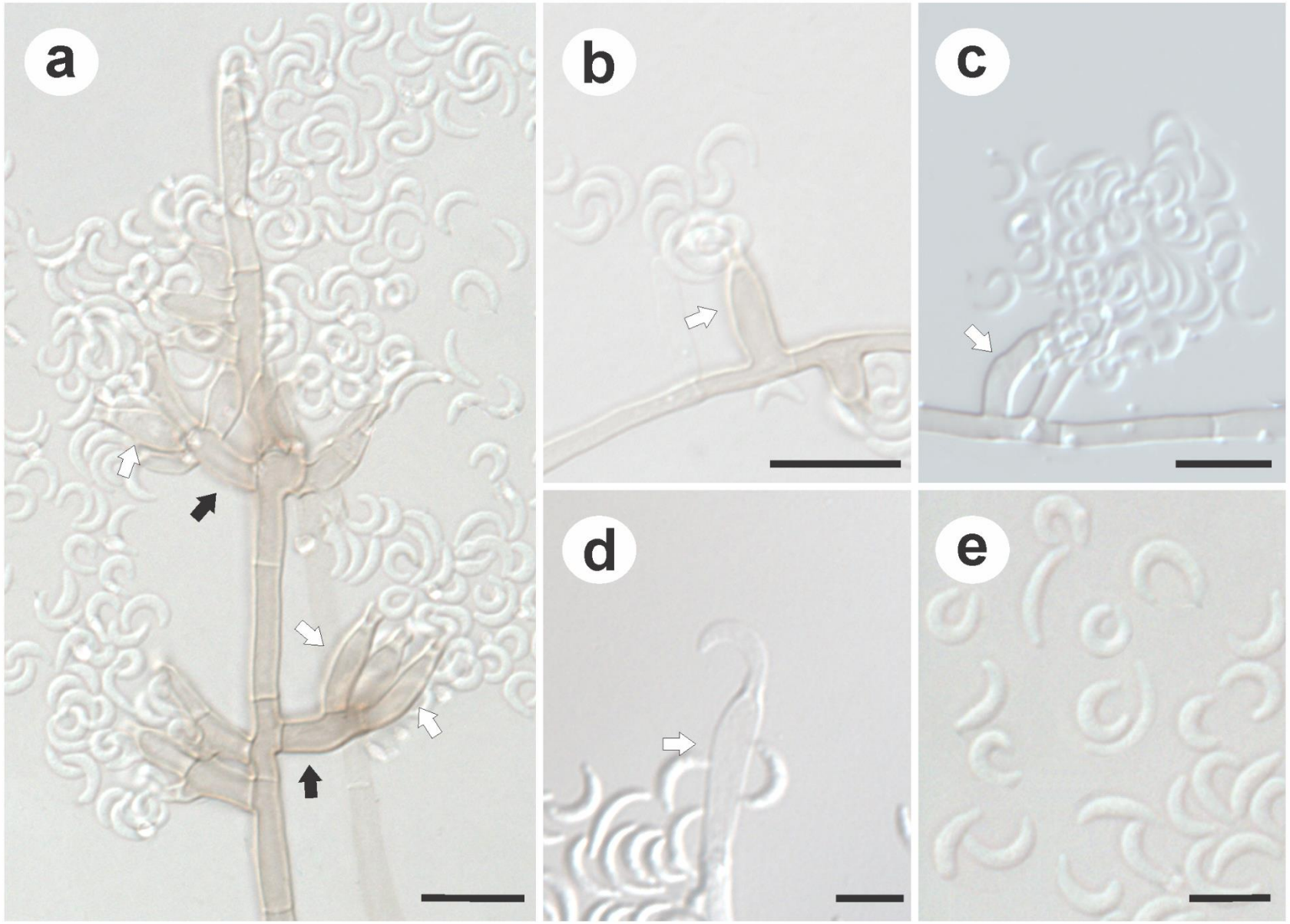
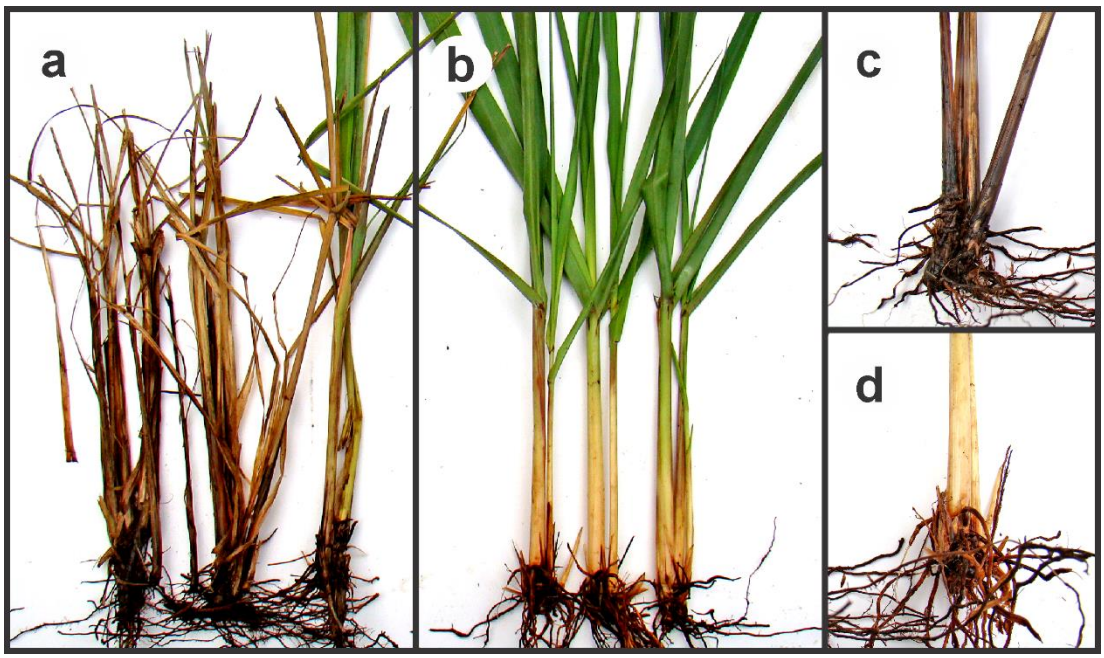


Figure 4.



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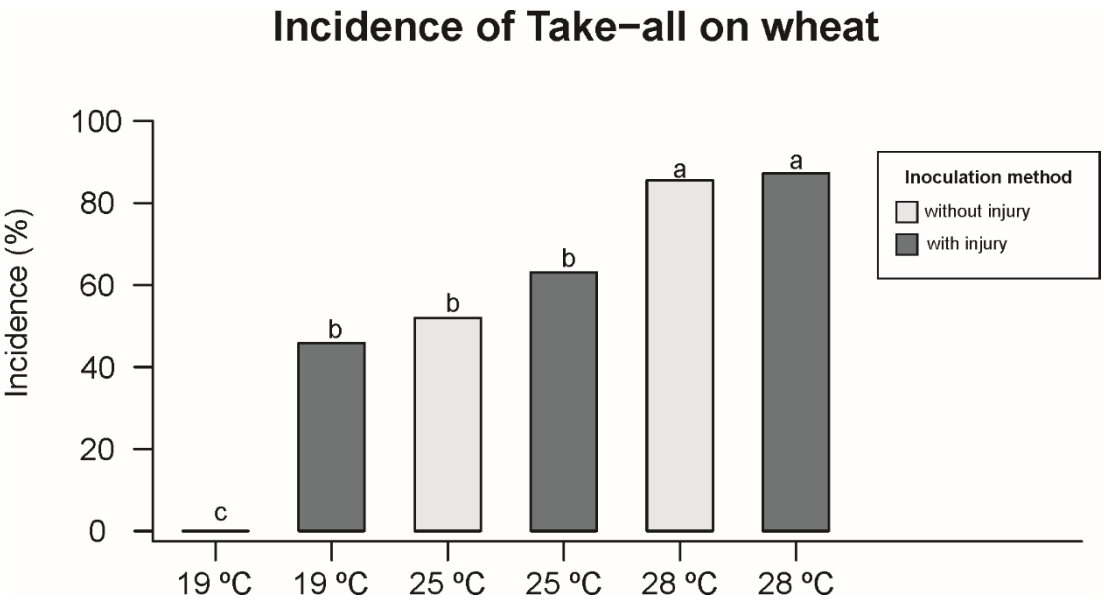


Figure 6.

Figure 7.

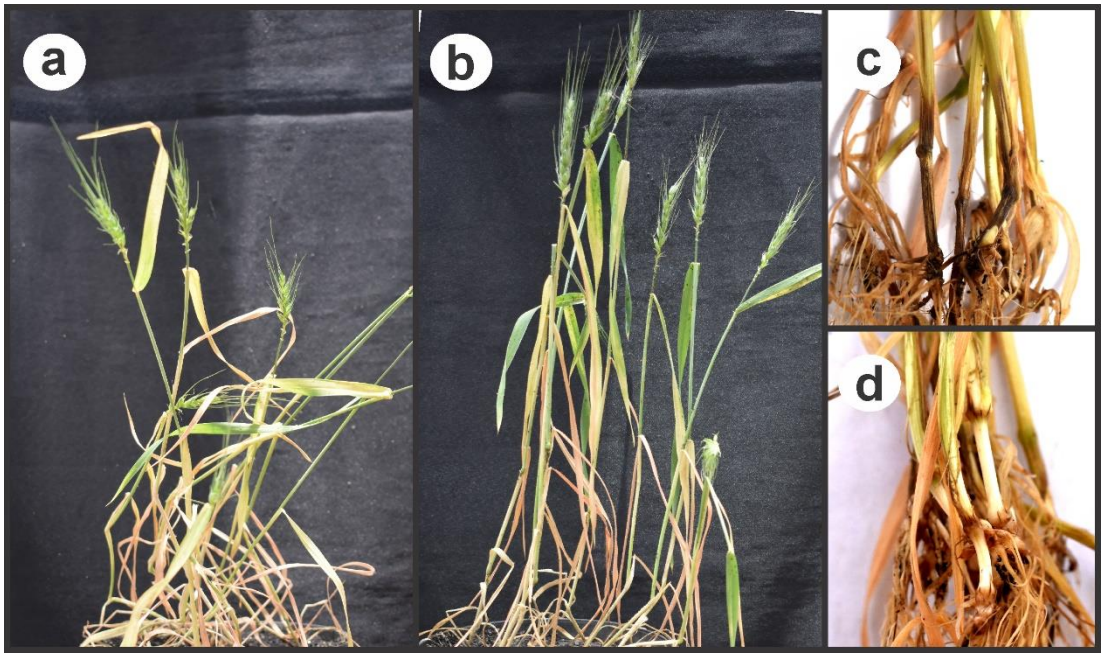
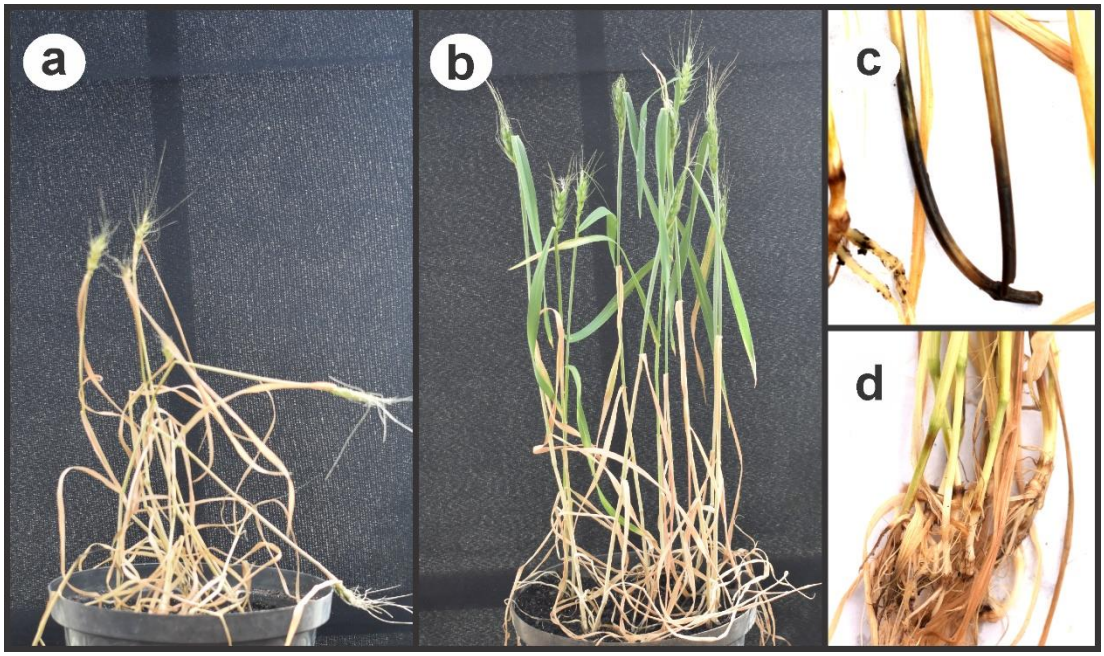


Figure 8.



Conclusões Gerais

Este é o primeiro relato da ocorrência do mal-do-pé em plantas de *Paspalum* no Brasil, sendo a doença causada por um provável novo gênero fúngico pertencente à família Magnaporthaceae.

Além do gênero *Gaeumannomyces*, o agente causal do mal-do-pé do *Paspalum* também é capaz de causar o mal-do-pé do trigo.

A incidência e desenvolvimento do mal-do-pé do trigo causado pelo possível novo gênero é diretamente relacionado as condições de temperatura.