UNIVERSIDADE FEDERAL DE VIÇOSA

ATHUS DIEGO AZEVEDO SILVA

TAXONOMY AND PATHOGENICITY OF FUNGI ASSOCIATED WITH STEM, POD AND SEED DISEASES OF SOYBEAN

VIÇOSA - MINAS GERAIS

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

Adviser: Olinto Liparini Pereira

Co-adviser: Sérgio H. Brommonschenkel

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Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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S586t
2021

Silva, Athus Diego Azevedo, 1991-
Taxonomy and pathogenicity of fungi associated with stem, pod and seed diseases of soybean / Athus Diego Azevedo Silva. - Viçosa, MG, 2021.
59 f. : il. (algumas color.) ; 29 cm.

Inclui anexo.
Orientador: Olinto Liparini Pereira.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Soja - Doenças e pragas. 2. Etiologia. 3. *Colletotrichum*. 4. *Diaporthe*. 5. *Leptosphaerulina*. I. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. II. Título.

CDD 22. ed. 633.3494

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APROVADA: 12 de janeiro de 2021.

Assentimento:

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Autor

Olinto Liparini Pereira
Orientador

Dedico esse trabalho a minha família, em especial minha mãe Evanilde, que nunca deixou de acreditar, apoiar e incentivar, mesmo nos momentos de maiores incertezas e desânimo.

Não posso deixar de agradecer aos incontáveis amigos que formei em toda a jornada na UFV, desde 2010, e que direta ou indiretamente ajudaram muito para que eu chegasse até esse ponto, em especial os amigos da pós graduação, que mesmo com pouco contato se fazem presentes, assim como nunca esquecer os que se fazem presente desde sempre, pois posso me gabar de ter amizades que em breve farão duas décadas de vinculo e muitas histórias.

Aos companheiros do MICOLAB que se tornaram uma enorme família, tanto os ex-membros quanto os atuais.

Não poderia ter chegado aqui sem o apoio e paciência da minha companheira de jornada Thaisa, que se fez presente e me mostrou que posso ir muito além do que acredito ser meu limite.

Obrigado a todos, vocês foram incríveis e muito importantes para mim.

ACKNOWLEDGMENTS

À Universidade Federal de Viçosa e ao Departamento de Fitopatologia pelas oportunidades e estruturas oferecidas para condução deste trabalho.

Ao programa de Pós-graduação em Fitopatologia e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de doutorado e auxílios.

À CAPES, O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Aos professores pelos ensinamentos, conselhos e aprendizado que foram essenciais para o desenvolvimento do projeto assim como crescimento pessoal e acadêmico.

Aos funcionários do Departamento de Fitopatologia que participaram direta ou indiretamente dessa jornada.

Ao Laboratório de Genética e Genômica da Interações Planta-Patógeno pelo apoio técnico e disponibilidade nas análises moleculares, assim como coleta e envio de amostras junto ao FITOLAB.

Ao professor Olinto Liparini Pereira pela Orientação, disponibilidade, apoio e motivação.

À equipe do Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas (MICOLAB), em especial aos estagiários e bolsistas de iniciação científica Thiago, Lucas, Gustavo e Danilo, pois o apoio e dedicação foram essenciais.

“Quando tiver que escolher entre estar certo e ser gentil, escolha ser gentil”.

(R. J. Palacio)

“Pois a vida já é tão complicada por si só, já são tantos sustos, perdas, falhas e desafios que enfrentamos dia a dia, que não deveria sobrar energia para embates desnecessários, com o único objetivo de provar o quanto estamos certos e cheios de verdade em nossos posicionamentos.”

(Fabíola Simões)

ABSTRACT

SILVA, Athus Diego Azevedo, D.Sc., Universidade Federal de Viçosa, January 2020. **Taxonomy and pathogenicity of fungi associated with stem, pod and seed diseases of soybean**. Adviser: Olinto Liparini Pereira. Co-adviser: Sérgio Herminio Brommonschenkel.

Soybean (*Glycine max*) was introduced in Brazil in the late 19th century, in the state of São Paulo, and from 1950 its cultivated area expanded rapidly. It is currently grown in almost all national territory, with Brazil being the largest producer in the world. Diseases play a crucial factor in the management of this culture, being limited to production. In 2017, and in subsequent years, soybean plants with early death, stem and pod drying and showing necrotic lesions along the stem, were detected in production fields in the state of Mato Grosso. The plants had sudden death, however with a root system without visible symptoms of necrosis, suggesting a possible resurgence of stem canker or another disease not yet known. This work aimed to survey fungi species associated with soybean plants with symptoms described previously and pathogenicity tests in different soybean cultivars. Analyzes of reproductive structures under microscope and culture morphology indicated the presence of 6 associated fungal genera, namely *Fusarium*, *Macrophomina*, *Diaporthe*, *Colletotrichum*, *Leptosphaerulina* and *Moniliophthora*. Through phylogenetic analyzes it was possible to identify the species *Colletotrichum truncatum*, *Colletotrichum plurivorum*, *Diaporthe ueckerae*, *Diaporthe longicolla* and a probable new species of *Moniliophthora*. In pathogenicity tests, the incidence (% of dead plants) varied from 0% to 63%, where the pathogenic isolates caused internal necrosis in the stems, which could even lead to plant drought and death. The results indicate the association of different fungal species with the symptoms described on soybean in Mato Grosso. The new pathogenic species of *Monilophthora* will be described and published according to the International Nomenclature Code for fungi, algae and plants.

Keywords: *Colletotrichum*. *Diaporthe*. Etiology. *Leptosphaerulin*. *Moniliophthora*.

RESUMO

SILVA, Athus Diego Azevedo, D.Sc., Universidade Federal de Viçosa, janeiro de 2020. **Taxonomia e patogenicidade de fungos associados a doenças do caule, vagem e sementes de soja**. Orientador: Olinto Liparini Pereira. Coorientador: Sérgio Herminio Brommonschenkel.

A soja (*Glycine max*) foi introduzida no Brasil no final do século XIX, no estado de São Paulo, e a partir de 1950 sua área cultivada expandiu de forma rápida. Atualmente é cultivada em quase todo território nacional, sendo o Brasil o maior produtor do mundo. As doenças desempenham um fator crucial no manejo dessa cultura, sendo limitante à produção. Em 2017, e nos anos posteriores, plantas de soja com morte precoce, secamento de haste e da vagem e apresentando lesões necróticas ao longo da haste, foram detectadas em campos de produção no estado do Mato Grosso. As plantas apresentavam morte súbita, entretanto com sistema radicular sem sintomas visíveis de necrose, sugerindo uma possível ressurgência do cancro da haste ou outra doença ainda não conhecida. Esse trabalho teve como objetivo o levantamento de espécies de fungos associados a plantas de soja com sintomas descritos anteriormente e testes de patogenicidade em diferentes cultivares de soja. Análises de estruturas reprodutivas em microscópio e morfologia de culturas indicaram a presença de 6 gêneros fúngicos associados, sendo eles *Fusarium*, *Macrophomina*, *Diaporthe*, *Colletotrichum*, *Leptosphaerulina* e *Moniliophthora*. Através de análises filogenéticas foi possível identificar as espécies *Colletotrichum truncatum*, *Colletotrichum plurivorum*, *Diaporthe ueckerae*, *Diaporthe longicolla* e uma provável nova espécie de *Moniliophthora*. Nos testes de patogenicidade a incidência (% de plantas mortas) variou de 0% até 63%, onde os isolados patogênicos causaram necrose interna nas hastes, podendo levar até a seca e morte de plantas. Os resultados indicam a associação de diferentes espécies fúngicas aos sintomas descritos em soja no Mato Grosso. A nova espécie patogênica de *Monilophthora* será descrita e publicada segundo o Código Internacional de Nomenclatura para fungos, algas e plantas.

Palavras-chave: *Colletotrichum*. *Diaporthe*. Etiologia. *Leptosphaerulina*. *Moniliophthora*.

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1. INTRODUCTION

The soybean (*Glycine max*) was introduced in Brazil at the end of the 19th century, in the state of São Paulo, and from 1950 the cultivated area expanded rapidly. It is currently cultivated in almost all national territory and Brazil is the largest producer in the world (EMBRAPA, 2014). Diseases play a crucial factor in the soybean growth and development, being one of the main limitations in obtaining higher yields. In Brazil there are around 40 registered diseases, caused by fungi, bacteria, nematodes and viruses. Each disease has a different importance, varying from region to region and from crop to crop, being highly influenced by climatic conditions. Diseases are responsible for losses of 15 – 20%, but under some conditions they can reach 100% (ALMEIDA et al., 2005).

Two diseases whose pathogens are fungi and of great prominence are soybean stem canker, caused by *Diaporthe aspalathi* and *D. caulivora*, and anthracnose mainly associated with *Colletotrichum truncatum*. Soybean stem canker is a disease that has already had a great prominence in the culture of soybean for having been devastating, first detected in 1989 in Paraná state, being quickly spread by all producing regions. Several properties had harvests made unviable by the disease, reaching losses of 80 to 100%. Today it is considered a secondary disease due to the adoption of efficient control measures, mainly due to the use of resistant varieties indicated for producing regions. (YORINORI, 1990; EMBRAPA, 2014). Anthracnose causes a reduction in plant population, seed quality and grain yield. Symptoms occur in petioles, stems and pods. These acquire a blackish color, starting in anasarca streaks and evolving to black spots, being covered by black punctuations that are the structures of the pathogen. It can also infect seeds and may result in seedlings damping-off. Recommendations for controlling this disease do not indicate the use of resistant cultivars, and cultural methods are indicated, such as crop rotation, use of healthy and pathogen-free seeds, treatment with systemic and contact seed fungicides, soil management and potassium fertilization. balanced, larger spacing and lower plant density, in order to reduce the inoculum pressure (DRESSLER et al., 2006; GODOY et al., 2016).

The genus *Diaporthe* and *Colletotrichum* contains species reported as saprophytic, endophytic and phytopathogenic, commonly isolated from many hosts (GOMES et al., 2013; VIEIRA et al., 2014; MA et al., 2018; DA SILVA et al., 2020). In addition to the species already mentioned, we still have reported on soybean in Brazil the species *D. citri*, *D. endophytica*, *D. infertilis*, *D. longicolla*, *D. phaseolorum*, *D. sojae*, *D. ueckerae*, *C. cliviicola*, *C. dematium*, *C. gloeosporioides*, *C. lindemuthianum*, *C. plurivorum* and *C. sojae* (GOMES et al., 2013;

UDAYANGA et al., 2015; GUARNACCIA & CROUS, 2017; BRUMER et al., 2018; DOUANLA-MELI et al., 2018; DAMM et al., 2019; BOUFLEUR et al., 2020; MENDES et al., 2020).

Initially, the taxonomy of these groups was based on host specificity, morphology and geographic distribution, but some studies have show that a species could colonize different hosts, just as different species could occur in the same host, and different species were considered as cryptic species, with a very close and little differential morphology, so species are currently identified through phylogenetic analyzes using sequences from different gene loci, using as phylogenetic markers internal transcript spacer (ITS) of DNA and genomic regions 1-alpha elongation factor (TEF1), β-tubulin 2 (TUB2), among others (CANNON et al., 2012; GOMES et al., 2013; GAO et al., 2016; DAMM et al., 2019). The taxonomy of fungi of the genus *Colletotrichum* is somewhat confusing and problematic, mainly due to the designation of species based only on morphological characters and host specificity, but a summary of names (HYDE et al., 2009a) and a review of the confusion with names in the genus (HYDE et al., 2009b) enable the development of studies for the correct identification of species of the genus and the determination of the correct etiological agent of a disease is of great importance for breeding programs, since each species, and even isolated, can have an epidemiological behavior and interaction with a host different from the others. Accurate identification is still important in the management and adequate control of the pathogen, as each species may have specific characteristics of aggressiveness, geographic distribution and resistance to fungicides (LIMA et al., 2015; VIEIRA et al., 2017).

Although many pathogens are already reported on soybean, the emergence of new diseases or the introduction of pathogens in regions previously unreported is a constant concern, as an example we have the introduction of *Phakopsora pachyrhizi* in 2001 in Brazil and in 2005 in the USA and that today the soybean rust, caused by this pathogen, is the main disease and requires greater care during the crop (ROSSI, 2003; YORINORI et al., 2005; GOELLNER et al., 2010; GODOY et al., 2016). Other examples are the introductions of *Fusarium proliferatum* in Canada and the USA, causing root rot on soybean, and *Meloidogyne graminicola* in China, as well as the recent identifications of *C. cliviicola* and *C. musicola* causing anthracnose on soybean in Brazil (DÍAZ ARIAS et al., 2011; CHANG et al., 2015; BARBIERI et al., 2017; LONG et al., 2017; BOUFLEUR et al., 2020).

In 2017, and in subsequent years, soybean plants with early death, stem and pod drying, pod rot and showing necrotic lesions along the stem, were detected in production fields in Mato

Grosso state (FARMING BRASIL, 2017; PACHECO, 2020; AGROLINK, 2021). The plants had sudden death, however with a root system without visible symptoms of necrosis, suggesting a possible resurgence of stem canker, an anthracnose epidemic or another disease not yet known. In view of the need for the correct identification of the disease and the etiology of the causal agents, this work aimed to carry out a survey of species of fungi associated with stem, pod and seed of soybean in Mato Grosso production fields with plants that exhibit that symptoms. The specific objectives were describe the species found and build a collection of fungi cultures associated (deposited in the COAD collection), identify species found through phylogenetic analysis, make DNA sequences, alignments and phylogenetic trees available in public databases, determine pathogenicity of isolates with inoculation in different soybean cultivars and determine the etiological agents of the diseases.

2. MATERIAL AND METHODS

2.1 Obtaining and preserving isolates

Samples of symptomatic soybean plants with early death, stem and pod drying, pod rot and showing necrotic lesions along the stem were obtained from different soybean fields, naturally infected, from the cities of Sorriso and Lucas do Rio Verde at Mato Grosso state in Brazil, in partnership with the company Fitolab, between the years 2018 and 2020. Samples were taken to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas at the Universidade Federal de Viçosa, photographs and description of associated symptoms were taken. Direct isolations in PDA medium (Potato-Dextrose-Agar) were carried out from stems, pods and seeds of plants showing symptoms already described. After 7 days, the isolates were transferred to plates with 2% AA medium (Agar-Water) and after 48 hours, isolates were obtained from the hyphae tip to obtain pure culture. The isolates were incubated at a temperature of 25 °C.

Indirect isolation of samples without sporulation was carried out by cutting the stem in small pieces, placed in 70% alcohol for 1 min, in 0.2% sodium hypochlorite for 1-3 min and then in distilled water to remove the excess of hypochlorite, after this they were placed in 3 points of plates with 3% AA medium and with 48h pure cultures were obtained through the hyphae tipping to PDA medium.

For the preservation of cultures, three storage methods were used. First method in silica gel, where the fungal structures of each isolate, grown in PDA medium for 7 days, were suspended in sterilized skim milk (10%) and impregnated in paper strips that were stored in bottles containing sterilized silica gel. The bottles were kept in a refrigerator at 4 ºC. Second

method in glycerol 10%, where the fungal structures of each isolate, grown in PDA medium for 7 days, were deposited in the form of discs in 2.0mL microtubes, containing 1000µL of 10% glycerol and kept at -80ºC. The third method was Castellani, where the fungal structures of each isolate, grown in PDA medium for 7 days, were deposited in the form of discs in 2.0mL microtubes, containing 1000µL of 0.85% NaCl aqueous solution and kept at room temperature. The storage protocols and isolation methods were adapted according to ALFENAS & MAFIA (2016). Representative cultures were deposited in the culture collection Octávio de Almeida Drumond at the Universidade Federal de Viçosa (COAD) (Table 02).

2.2 Pathogenicity of isolates

The pathogenicity tests were conducted in the greenhouses and growth chambers at the Universidade Federal de Viçosa and the soybean cultivars used were M7739, TMG4182, TMG238, NS7901, DESAFIO and BRAGG. Pots of 4L, containing soil and substrate mixture were used for planting with a total of 8-10 seeds of each cultivar per pot. These were kept in a greenhouse with daily irrigation for 15 days until pathogenicity tests were performed. The fungal isolates were grown in PDA medium in the presence of 15-20 wooden toothpicks pieces, with approximately 1.5 cm long, for 15 days until the mycelium completely covered the toothpicks.

The seedlings with 15 days were inoculated by inserting the toothpicks, colonized by fungi, into the stem, as described by PLOETZ & SHOKES (1989) and CAMPBELL et al. (2017), with non-colonized toothpicks as a negative control. After inoculation, the plants were kept in a fog chamber for 48 hours and then placed in growth chambers at 25ºC until symptoms appear, about 15-21 days.

Symptons were evaluated 2, 3 and 4 weeks after inoculation, determining the incidence of the disease. The severity/incidence was assessed according to the diameter of the lesion developed and the percentage of dead plants (%DP), this were calculated according to the following formula %DP = ((nDP + nPL/2) / nTP) x 100: number of dead plants (nDP), plus half the number of plants with lesions larger than 1cm (nPL) and this sum were divided by the total of plants (nTP) and multiplied by 100 to obtain the values as percentage according to YORINORI (1990). To view the results, bar graphics were generated in the R software using the GGplot2 data packages (WICKHAM, 2016).

Isolates belonging to the genus *Colletotrichum* and obtained in 2019 have been inoculated yet into cultivars TMG4182 and M7739 by sprinkling suspension with 10^4 spores mL^{-1}. Previously to the inoculation, wounds were made with sterile needles in the leaves.

Subsequently, the plants were kept in a fog chamber for 48 hours and then placed in growth chambers at 25ºC. Negative control was done by spraying water.

2.3 Morphological characterization of the isolates

Slides were prepared from colony fragments taken from of plates colonies using a sterile needle. Microscope slides were mounted with lactoglycerol solution and examined under light microscope Olympus BX 53. Fungal structures were measured with a minimum of 30 measurements taken from each structure, the highest and lowest values obtained in the measurements were described. Photographs were obtained with a Q-color 5 camera (Olympicus America INC.). The software Corel Draw graphics suite X8 was used to edit the photos and prepare photo plates.

2.4 Extraction of genomic DNA and amplification

Monosporic isolates were grown in PDA medium for 7 days at 25ºC. Subsequently, the mycelium was removed from the culture medium, using a sterile wooden toothpick, and transferred to a 2 mL microcentrifuge tube. For physical maceration, metallic spheres (beads) were added to the tube and the tube placed in a cell disruptor (L-Beader 6, manufacturer Loccus) for 30 seconds at 4000rpms. The total DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) according to the protocol described by the manufacturer and modifications made by PINHO et al. (2013).

Each PCR reaction was prepared using 18 µL of Platinum® PCR SuperMix, 0.4 µL of each forward and reverse primer at 10 µM (Table 1) synthesized by Invitrogen (Carlsbad, USA), 1.2 µL of genomic DNA (25 ng/µL). The amplifications were performed according to the specifications of each primer and the manufacturer of Platinum® PCR SuperMix and the reactions were incubated in a thermal cycler with Initial denaturation 94ºC/2 minutes and 35 cycles of Denaturing 94ºC/30 seconds, Annealing (Table 1)/30 seconds and Extending 72ºC/90 seconds. The PCR products were purified and sequenced by the Laboratório de Genética e Genômica das Interações Planta-Patógeno at the Universidade Federal de Viçosa. The isolate codes, collection, hosts, location and Genbank numbers can be viewed in Table 2.

Table 1. List of primers

Gene	Primer	Sequence 5'-3'	References	Annealing temperature
Internal transcribed spacer (nrITS)	ITS-1F	CTTGGTCATTTAGAGGAAGTAA	GARDES & BRUNS (1993)	53
	ITS-4	TCCTCCGCTTATTGATATGC	WHITE et al. (1990)	
b-Tubulin 2 (TUB2)	T1	AAC ATG CGT GAG ATT GTA AGT	O'DONNELL & CIGELNIK (1997)	55
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	GLASS & DONALDSON (1995)	
Translation elongation factor 1-α gene (TEF1)	EF1-728F	CAT CGA GAA GTT CGA GAA GG	CARBONE & KOHN (1999)	55
	EF1-986R	TAC TTG AAG GAA CCC TTA CC	CARBONE & KOHN (1999)	

2.5 Molecular phylogeny

The nucleotide sequences obtained were edited with the DNAbaser software. The sequences were submitted to multilocus phylogenetic analysis, involving all sequenced genes. Additional species sequences of each fungal genus were obtained from GenBank to create databases, according to the megaBlast tool of NCBI portal and specific literature for each fungal group. All sequences were aligned using the MUSCLE software, implemented in the MEGA X program (TAMURA et al., 2013). Bayesian Inference (BI) analysis using the Markov Monte Carlo (MCMC) chain method and Maximum Likelihood (ML) were perfomed. The software Mr MODEL TEST 2.3 (POSADA & BUCKLEY, 2004) was used to select the nucleotide replacement model for BI analysis. The models were estimated separately for each genomic region and selected according to Akaike Information Criterion (AIC). The BI analysis was completed with MrBayes v.3.1.1 (RONQUIST & HUELSENBECK, 2003). The MCMC algorithm was performed using 10,000,000 generations. The trees were sampled every 1,000 generations, resulting in 10,000 trees. The first 2,500 trees were discarded from the analysis. The subsequent probability values (RANNALA AND YANG, 1996) were determined from the

consensus tree through the remaining 7,500 trees. The analyzes were considered finished after 10,000,000 generations if the standard deviation of split frequencies was below 0.01.

ML and bootstrapping analyses were conducted in RAxML-HPC v.8 algorithm on XSEDE software, using default parameters established in the CIPRES web portal (http://www.phylo.org/portal2/; MILLER et al., 2010) and calculating bootstrap statistics from 1,000 replicates and separation of partitions for each gene loci (STAMATAKIS, 2014). The tree was visualized in the FigTree software (RAMBAUT, 2009) and exported to graphics programs.

Five databases were built, one with sequences belonging to isolates of the genus *Diaporthe*, another with sequences of isolates belonging to species of the *Colletotrichum orquidearum* complex, the third with sequences of isolates belonging to species to the "*C. truncatum* complex", the fourth with sequences of isolates belonging to species of the genus *Leptosphaerulina* and the last with sequences of isolates belonging to species of the genus *Moniliophthora*. The isolate codes, collection, hosts, location and Genbank numbers can be viewed in Table 2 and Supplementary table 1.

The *Diaporthe* database was formed by sequences of ITS, TUB2 and TEF1. The data set of the ITS region has a total of 552 sites, of which 331 are conserved, 210 variables and 109 informative for parsimony. The alignment has 98 singletons, but none of them in the sequences obtained in this work. The Btub2 data set has a total of 490 sites, of which 260 are conserved, 219 variables and 191 informative for parsimony. The alignment has 25 singletons, but none of them in the sequences obtained in this work. The TEF1 data set has a total of 408 sites, of which 93 are conserved, 262 variables and 214 informative for parsimony. The alignment has 40 singletons, with only one of them belonging to a sequence of this work, in this case at position 76 of the alignment, where only the sequence of the isolated COAD3255 has a thymine instead of a guanine.

A database using the sequences together from the ITS, Btub2 and TEF1 region was set up, totaling a matrix with 53 taxa and 1450 characters. The nucleotide replacement model selected for the ITS and TEF1 database was the GTR (General Time Reversible), assuming that part of the region is evolutionarily invariable (+ I) and that non-uniform evolution rates can be modeled using a discrete gamma distribution (+ G). The nucleotide replacement model selected for the Btub2 database was HKY (Hasegawa-Kishino-Yano), assuming that part of this region is evolutionarily invariable (+ I) and that non-uniform evolution rates can be modeled using a discrete gamma distribution (+ G).

The *Colletotrichum orquidearum* species complex database was formed by sequences of ITS and TUB2. The data set of the ITS region has a total of 541 sites, of which 518 are conserved, 21 variables and 11 informative for parsimony. The alignment has 10 singletons, but none of them in the sequences obtained in this work. The TUB2 dataset has a total of 540 sites, of which 440 are conserved, 99 variables and 44 informative for parsimony. The alignment has 55 singletons, but none of them in the sequences obtained in this work.

A database using the sequences together from the ITS and TUB2 region was set up, totaling a matrix with 42 taxa and 1081 characters. The nucleotide replacement model selected for the ITS and TUB2 was the Kimura 2-parameter.

The "*Colletotrichum truncatum* species complex" database was formed by sequences of ITS and TUB2. The data set of the ITS region has a total of 541 sites, of which 415 are conserved, 125 variables and 96 informative for parsimony. The alignment has 29 singletons, but none of them in the sequences obtained in this work. The TUB2 dataset has a total of 569 sites, of which 306 are conserved, 261 variables and 225 informative for parsimony. The alignment has 36 singletons, but none of them in the sequences obtained in this work.

A database using the sequences together from the ITS and TUB2 region was set up, totaling a matrix with 56 taxa and 1110 characters. The nucleotide replacement model selected for the ITS and TUB2 was the Kimura 2-parameter and that non-uniform evolution rates can be modeled using a discrete gamma distribution (+ G).

The *Leptosphaerulina* database was formed by sequences of ITS and has a total of 31 taxons and 492 characters, of which 430 are conserved, 61 variables and 33 parsimony information. The alignment has 28 singletons, but none of them in the sequence obtained in this work. The nucleotide replacement model selected for the database was GTR (General Time Reversible) and assuming that part of this region is evolutionarily invariable (+ I).

The *Moniliophthora* database was formed by sequences of ITS and has a total of 25 taxa with 575 characters, of which 287 are conserved, 270 variables and 201 informative for parsimony. The alignment has 63 singletons, but none of them in the sequences obtained in this work. The nucleotide replacement model selected for the analyses was the GTR (General Time Reversible), assuming that part of the region is evolutionarily invariable (+ I) and that non-uniform evolution rates can be modeled using a discrete gamma distribution (+ G).

Table 2. Host / substrate, locality, collector and GenBank accession numbers of strains obtained in the study.

Specie	Collection number	Host	Country	GenBank accession number		
				ITS	TEF1	TUB2
C. plurivorum	COAD 3227	*Glycine max*	Brazil	MW264947	-	MW273490
C. plurivorum	COAD 3228	*Glycine max*	Brazil	MW264948	-	MW273491
C. plurivorum	COAD 3229	*Glycine max*	Brazil	MW264949	-	MW273492
C. plurivorum	COAD 3230	*Glycine max*	Brazil	MW264950	-	-
C. plurivorum	COAD 3231	*Glycine max*	Brazil	MW264951	-	MW273493
C. plurivorum	COAD 3232	*Glycine max*	Brazil	MW264952	-	MW273494
C. truncatum	COAD 3207	*Glycine max*	Brazil	MW264926	-	MW295986
C. truncatum	COAD 3208	*Glycine max*	Brazil	MW264927	-	MW295987
C. truncatum	COAD 3209	*Glycine max*	Brazil	MW264928	-	MW295988
C. truncatum	COAD 3210	*Glycine max*	Brazil	MW264929	-	-
C. truncatum	COAD 3211	*Glycine max*	Brazil	MW264930	-	MW295989
C. truncatum	COAD 3212	*Glycine max*	Brazil	MW264931	-	MW295990
C. truncatum	COAD 3213	*Glycine max*	Brazil	MW264932	-	MW295991
C. truncatum	COAD 3214	*Glycine max*	Brazil	MW264933	-	MW295992
C. truncatum	COAD 3215	*Glycine max*	Brazil	MW264934	-	-
C. truncatum	COAD 3216	*Glycine max*	Brazil	MW264935	-	MW295993
C. truncatum	COAD 3217	*Glycine max*	Brazil	MW264936	-	MW295994
C. truncatum	COAD 3218	*Glycine max*	Brazil	MW264937	-	MW295995
C. truncatum	COAD 3219	*Glycine max*	Brazil	MW264938	-	MW295996
C. truncatum	COAD 3213b	*Glycine max*	Brazil	MW264939	-	MW295997
C. truncatum	COAD 3220	*Glycine max*	Brazil	MW264940	-	-
C. truncatum	COAD 3221	*Glycine max*	Brazil	MW264941	-	MW295998
C. truncatum	COAD 3222	*Glycine max*	Brazil	MW264942	-	MW295999
C. truncatum	COAD 3223	*Glycine max*	Brazil	MW264943	-	MW296000
C. truncatum	COAD 3224	*Glycine max*	Brazil	MW264944	-	MW296001
C. truncatum	COAD 3225	*Glycine max*	Brazil	MW264945	-	MW296002
C. truncatum	COAD 3226	*Glycine max*	Brazil	MW264946	-	MW296003
D. longicolla	COAD 3243	*Glycine max*	Brazil	MW264962	MW296017	MW273693
D. longicolla	COAD 3244	*Glycine max*	Brazil	MW264963	MW296018	MW273694
D. longicolla	COAD 3245	*Glycine max*	Brazil	MW264964	MW296019	MW273695
D. longicolla	COAD 3246	*Glycine max*	Brazil	MW264965	MW296020	MW273696
D. longicolla	COAD 3247	*Glycine max*	Brazil	MW264966	-	MW273697
D. longicolla	COAD 3248	*Glycine max*	Brazil	MW264967	-	MW273698
D. longicolla	COAD 3249	*Glycine max*	Brazil	MW264968	MW296021	MW273699
D. longicolla	COAD 3250	*Glycine max*	Brazil	MW264969	MW296022	MW273700
D. longicolla	COAD 3251	*Glycine max*	Brazil	MW264970	MW296023	MW273701
D. longicolla	COAD 3252	*Glycine max*	Brazil	MW264971	MW296024	MW273702
D. longicolla	COAD 3253	*Glycine max*	Brazil	MW264972	MW296025	MW273703
D. longicolla	COAD 3254	*Glycine max*	Brazil	MW264973	MW296026	MW273704
D. ueckerae	COAD 3233	*Glycine max*	Brazil	MW264953	-	-
D. ueckerae	COAD 3234	*Glycine max*	Brazil	MW264954	-	-
D. ueckerae	COAD 3235	*Glycine max*	Brazil	MW264955	-	MW296004
D. ueckerae	COAD 3236	*Glycine max*	Brazil	-	-	MW296005
D. ueckerae	COAD 3237	*Glycine max*	Brazil	MW264956	MW296012	-
D. ueckerae	COAD 3238	*Glycine max*	Brazil	MW264957	MW296013	MW296006
D. ueckerae	COAD 3239	*Glycine max*	Brazil	MW264958	MW296014	MW296007
D. ueckerae	COAD 3240	*Glycine max*	Brazil	MW264959	MW296015	MW296008
D. ueckerae	COAD 3241	*Glycine max*	Brazil	MW264960	MW296016	MW296009
D. ueckerae	COAD 3242	*Glycine max*	Brazil	MW264961	-	MW296010
Leptosphaerulina sp.	COAD 3256	*Glycine max*	Brazil	MW264975		
Moniliophthora sp.	COAD 3204	*Glycine max*	Brazil	MW264924		
Moniliophthora sp.	COAD 3205	*Glycine max*	Brazil	MW264923		
Moniliophthora sp.	COAD 3206	*Glycine max*	Brazil	MW264925		

3. RESULTS

3.1 Symptoms and isolates

In 2019 the samples collected belonged to the cultivars CORUMBÁ, NS7505, TMG4182 and TEC7548 and the associated symptoms were stem blight (Fig 1 a-d), with the presence of reddish to dark lesions rounded to elliptical (Fig 1 b-d), pods blight with coloration whitish (Fig 1 e,f), wrinkling of the seeds (Fig 1 g), grayish-white mycelium in the pods and seeds (Fig 1 e-i) and the presence of pycnidia linearly along the stem. This symptomatology is typical of the disease known as stem and pod blight, whose etiologic agent is fungi of the genus *Diaporthe*, with greater relevance *D. sojae* and *D. longicolla*. In addition, acervuli with setae were also found on the stems and pods of some materials, these structures being typical of fungi belonging to the genus *Colletotrichum* that cause anthracnose on soybean (Fig 1 j-l).

In 2020 the samples collected belonged to the cultivars TMG1180 and CORUMBÁ and the associated symptoms were stem blight (Fig 2 a-c), with the presence of reddish to dark lesions rounded to elliptical (Fig 2 b, d, e), grayish-white mycelium in the pods and seeds (Fig 2 f-h) and the presence of pycnidia linearly along the stem (Fig 2 i). This symptomatology is typical of the disease known as stem and pod blight, whose etiologic agent is fungi of the genus *Diaporthe*, with greater relevance *D. sojae* and *D. longicolla*. In addition, acervuli with setae were also found on the stems and pods of some materials and depressed lesions on pods (Fig 2 j), these structures being typical of fungi belonging to the genus *Colletotrichum* that cause anthracnose on soybean. In addition samples showing whitish mycelium on stems and pods rot were collected and isolation performed (Fig 2 k, l).

In 2019, 40 isolates were obtained, 26 from seeds, 9 from the stem and 5 from pods. These 17 belonged to the genus *Colletotrichum*, 19 to the genus *Diaporthe*, 3 to the genus *Fusarium* and one isolate with the presence of micro-sclerotia belonging to the genus *Macrophomina*. In 2020 the number of isolates were 83, 22 from seeds, 43 from the stems and 18 from the pods. These 43 belong to the genus Colletotrichum, 12 to the genus Fusarium, 18 to the genus *Diaporthe* with 3 isolates obtained by indirect isolation from stem (COAD3251, COAD3252 and COAD3254), 6 to the genus *Macrophomina*, 3 to *Moniliophthora* and one to the *Leptosphaerulina*. The morphological variation of some cultures can be seen in Fig 3.

The isolates of the genus *Diaporthe* did not sporulate on PDA, AA or MEA medium (Malt extract and agar). The isolates frequently showed clear and cottony mycelium (Fig 3 a, b) with darkening in areas of the colony (Fig 3c). The identify of the isolates was also possible due the presence of pycnidia and alpha conidia in slides made during isolations (Fig 4 a, b).

Figure 1: Symptoms observed on soybean plants and infected tissues in the year 2019. (a) Plants with stem blight on the field. (b) Stem with reddish lesions rounded to elliptical. (c,d) Stem with dark lesions rounded to elliptical and pycnidia linearly along the stem. (e, f) Pods blight with coloration whitish. (g) Wrinkling of the seeds. (h, i) Grayish-white mycelium in the pods and seeds. (k-l) Stem and pods with presence of acervuli and setae.

Figure 2: Symptoms observed on soybean plants and infected tissues in the year 2020. (a) Plants with stem blight. (b-e) Stem with reddish to dark lesions rounded to elliptical. (f-h) Pods blight with coloration whitish. (i-j) Stem and pods with presence of acervuli, setae and depressed lesions. (k,l) Pods and stem with whitish mycelium and pod rot.

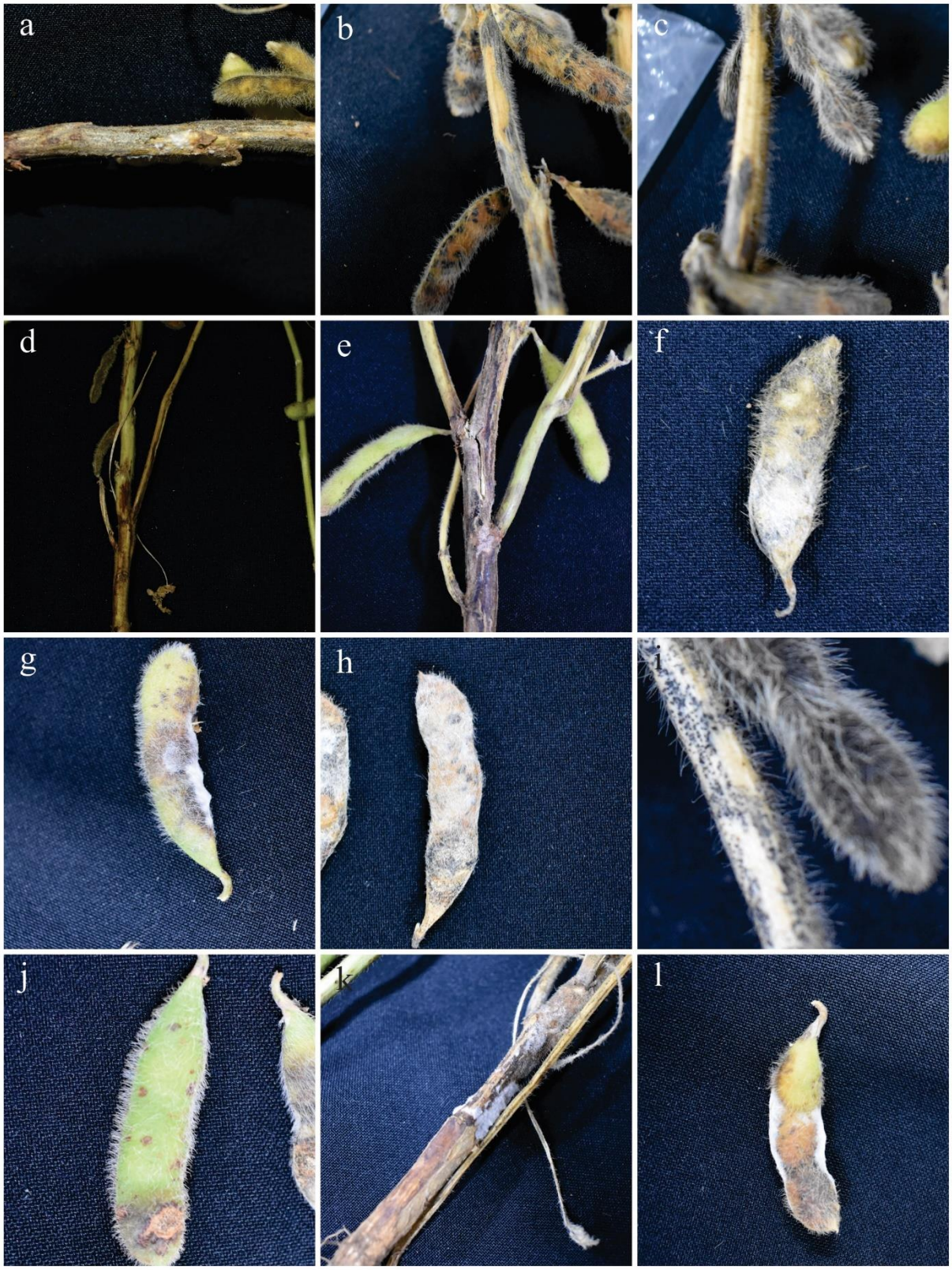


Figure 3: Cultures on MEA and PDA medium. (a-b) *Diaporthe* cultures with 15 days on MEA. (c) Reverse view of *Diaporthe* culture on MEA. (d-f) *Colletotrichum* isolates on MEA. (g-i) *Fusarium* isolates on MEA. (j-l) *Moniliophthora* isolates on BDA with 15 days.

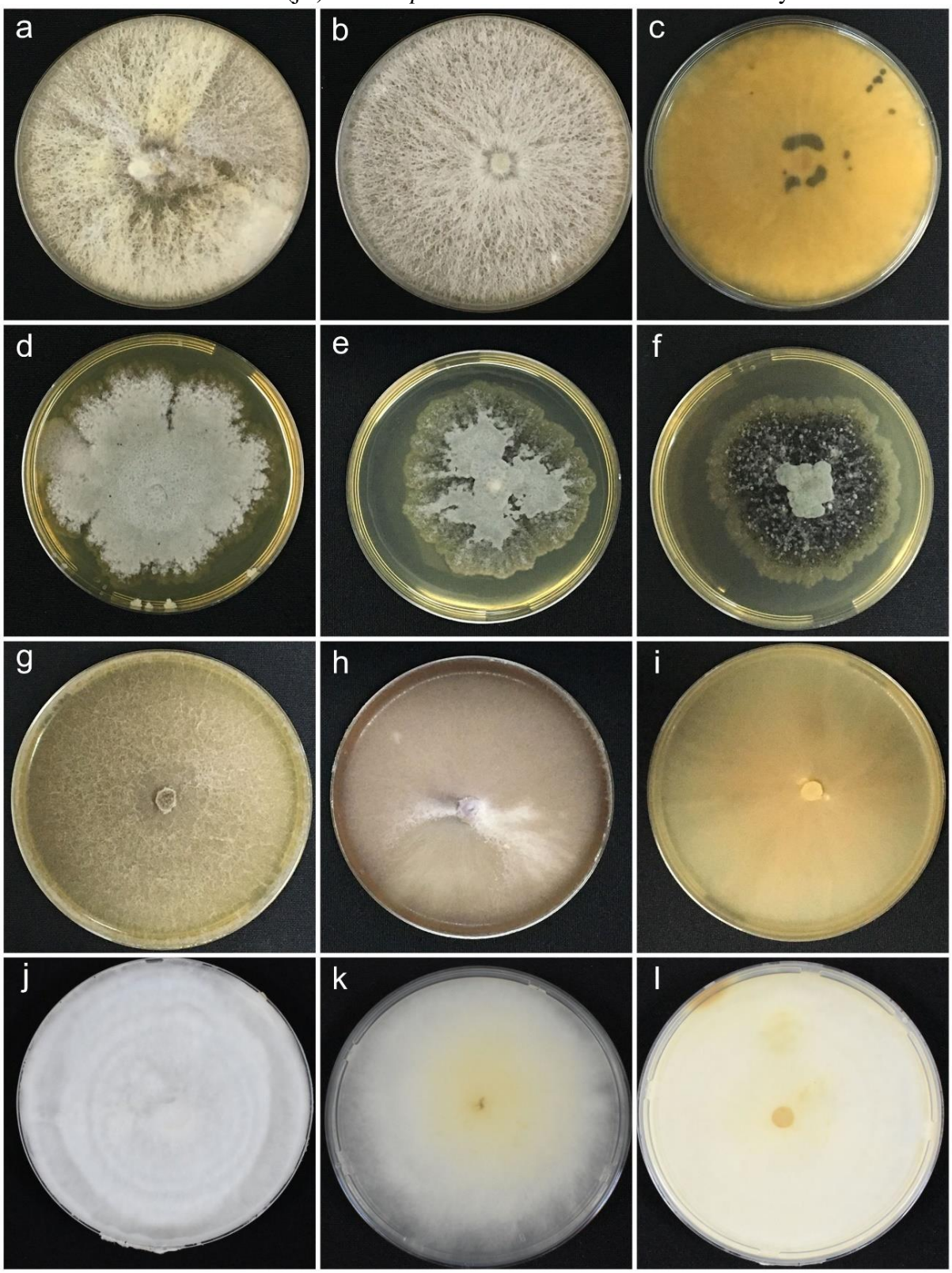
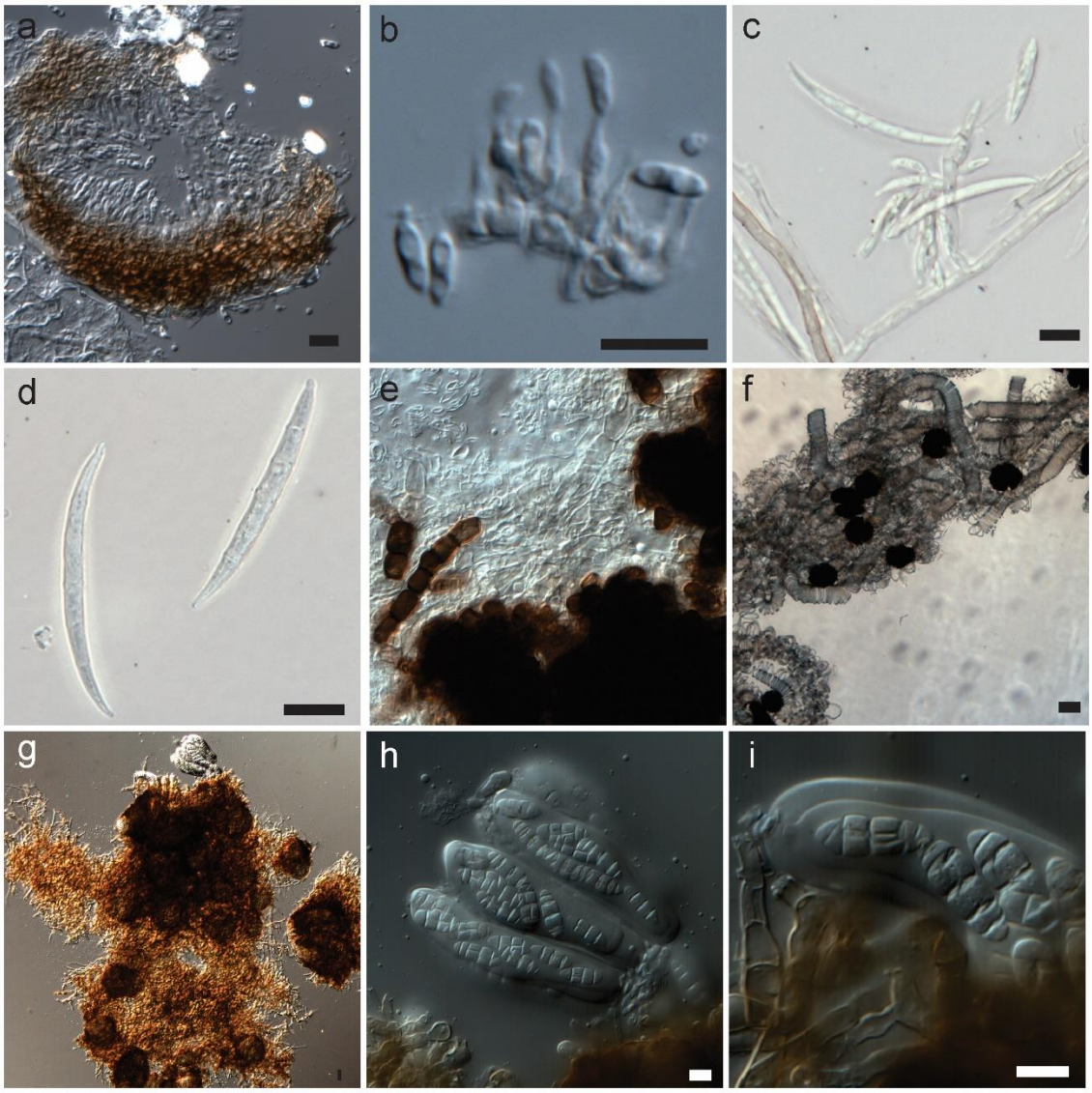


Figure 4: Morphology of *Diaporthe* (a-b); *Fusarium* (c-d), *Macrophomina* (e, f) and *Leptosphaerulina* (g-i) isolates. (a) Picnydia. (b) Conidiogenic cell of *Diaporthe* sp. (c) Conidiophore and conidiogenic cells of *Fusarium* sp. (d) Conidia falcate. (d, e) Micro sclerotia of *Macrophomina* sp. (g) Pseudothecia of *Leptosphaerulina* sp. (h, i) Bitunicate asci with ascospores.



The isolates belonging to the genus *Fusarium* presented similar morphology of the *F. incarnatum-equiseti* species complex, with falcated macroconidia and presence of chlamydospores in chain (Fig 4 c, d). The isolates of *Moniliophthora* sp. showed rapid growth in PDA, with whitish, abundant and cottony mycelium, but there was no formation of conidia or sexual structures (Fig. 2 j-l). The isolates of *Macrophomina* sp. they did not form reproductive structures in PDA or MEA medium, presenting a whitish culture, with dense mycelium and formation of micro-sclerotia (Fig 4 d, e). The isolate of *Leptosphaerulina* sp.

formed the pseudothecium on PDA medium with 15 days growth. It presented bitunicated asci, with 8 hyaline ascospores with 3-4 longitudinal and 0-2 transversal septa (Fig 4 h-j).

The isolates of *Colletotrichum* had grayish color on PDA and MEA (Fig 3 d-f). Two distinct morphologies were found in the *Colletotrichum* materials when examined under the microscope, the first morphology showed setae, with 77-181 x 3.5-6 µm in length, and falcated conidia that were 20-27 x 4-7 µm in length (Fig 5 a-c), this morphology is typical of the species *C. truncatum*. The other isolates formed perithecia after 15 days on PDA, being superficial, dark brown, 100-200 x 95-150 µm, with unitunicated asci, 50-65.5 x 10.5-12.5 µm (Fig 5 d-g). The ascospores could be septate or non-septate, allantoid to fusiform, with both ends rounded, 14-20 x 4-7 µm (Fig 5 h-k). The asexual form was not commom, but some isolates had septate conidia and verruculose setae (Fig 5 l). This morphology is similar to species from *C. orquidearum* complex and only some isolates collected in 2020 had this morphology.

3.2 PCR, sequencing and phylogeny

We obtained sequences of 22 isolates belonging to the genus *Diaporthe*, obtained in the years 2018 and 2020 and from the stems, seeds and pods. Amplification and sequencing of the ITS, TEF1 and TUB2 were successful, with the exception of the COAD3236 isolate which had only one TUB2 sequence, isolates COAD3235, COAD3247, COAD3248 which do not have TEF1 sequences and the COAD3233 and COAD3234 isolates which only had ITS sequences (Table 2). The sequences from *Diaporthe* obtained in this study were grouped into 2 clades, one together with sequences of strains of *D. ueckerae* including type and the second with sequences of *D. longicolla* including type (Fig 6). The clade of *D. longicolla* sequences obtained high support in the BI analyses with 100% of posterior probability and 100% of bootstrap by ML analyses. The clade of *D. ueckerae* sequences obtained high support in the BI analyses with 100% of posterior probability and 84% of bootstrap by ML analyses.

We obtained sequences of 28 isolates belonging to the genus *Colletotrichum*, obtained in the years 2018 and 2020, and from stems, seeds and pods. Amplification and sequencing of the ITS and TUB2 were successful, with exception of the isolates COAD3220, COAD3210, COAD3215 and COAD3230 that do not have TUB2 sequences. The *Colletotrichum* sequences were divided into two databases for analysis, as previously described. Twenty-two sequences were used in the first database and all of them grouped together with sequences of *C. truncatum*, including type strain, and obtained high support in the BI analyses with 100% of posterior probability and 100% of bootstrap by ML analyses (Fig 7).

Figure 5: Morphology of isolates from the genus *Colletotrichum*. *C*. *truncatum* (a-c) and *C*. *orquidearum* species complex (d-i). (a, b) Sporodoquia and setae of isolates with typical morphology of *C*. *truncatum*. (c) Falcate conidia typical of *C*. truncatum. (d, e) Perithecium of *C*. *orquidearum* species complex. (f, g) Asci with ascospores. (h-k) Ascospores. (l) Conidia.

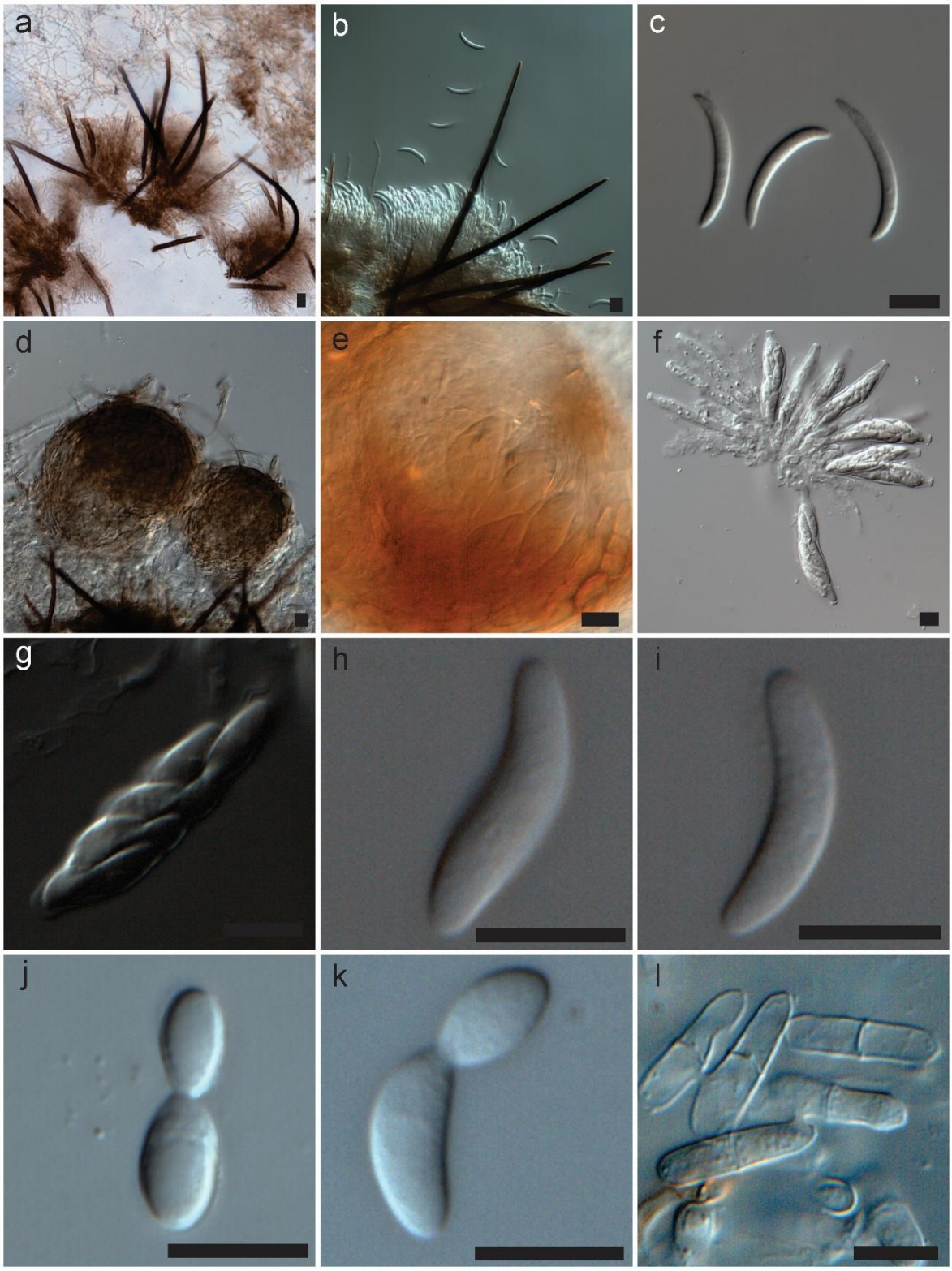
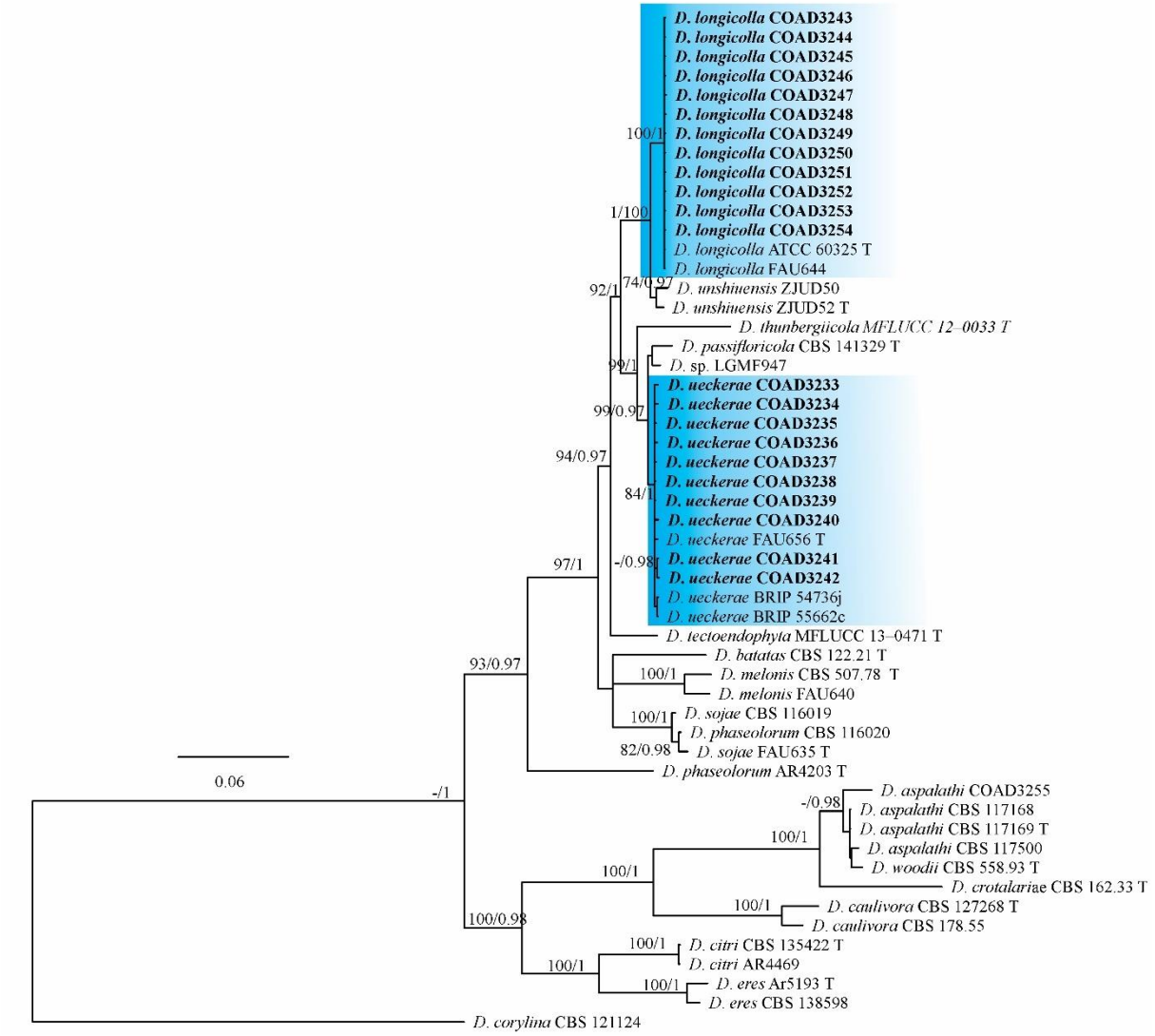


Figure 6: Phylogenetic tree of *Diaporthe* spp. obtained by Maximum-Likehood method using ITS, TUB2 and TEF1 sequences combined. Bootstrap (over 70%) and posterior probability (over 90%) values are indicated on the nodes. The sequences in bold were obtained in this study. The tree was rooted with *Diaporthe corylina* (CBS 121124). T= Type material.



Eight sequences were used in the second database of *Colletotrichum* and all of them grouped together with sequences of *C. plurivorum*, including type strain, and obtained high support in the BI analyses with 94% of posterior probability and 98% of bootstrap by ML analyses (Fig 8). Four clades with high support were formed within the clade of *C. plurivorum*, but none of the sequences from this work were grouped together with sequences in those clades.

The sequence of the COAD3256 isolate grouped together with other sequences of the genus *Leptosphaerulina* with 98% posterior probability by the BI analysis and 94% of bootstrap by ML analyses. Despite this grouping, only the ITS region had no resolution for the separation of species within the genus *Leptosphaerulina* (Fig 9).

Figure 7: Phylogenetic tree of Colletotrichum spp. obtained by Maximum-Likehood method using ITS and TUB2 sequences combined. Bootstrap (over 70%) and posterior probability (over 90%) values are indicated on the nodes. The sequences in bold were obtained in this study. The tree was rooted with *C. lindemuthianum* (CBS 15128). T= Type material.

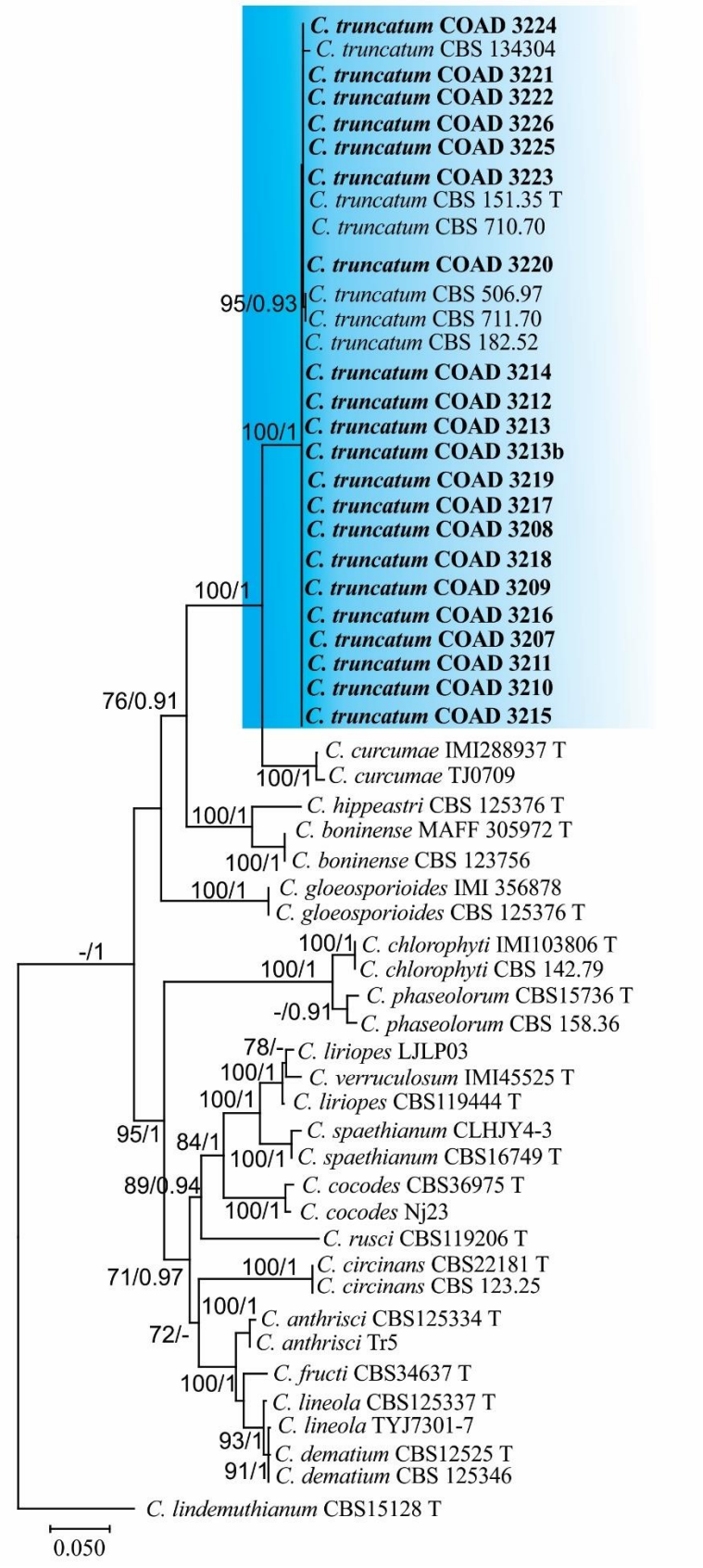


Figure 8: Phylogenetic tree of Colletotrichum spp. obtained by Maximum-Likehood method using ITS and TUB2 sequences combined. Bootstrap (over 70%) and posterior probability (over 90%) values are indicated on the nodes. The sequences in bold were obtained in this study. The tree was rooted with *C. brevisporum* (BCC 38876). T= Type material.

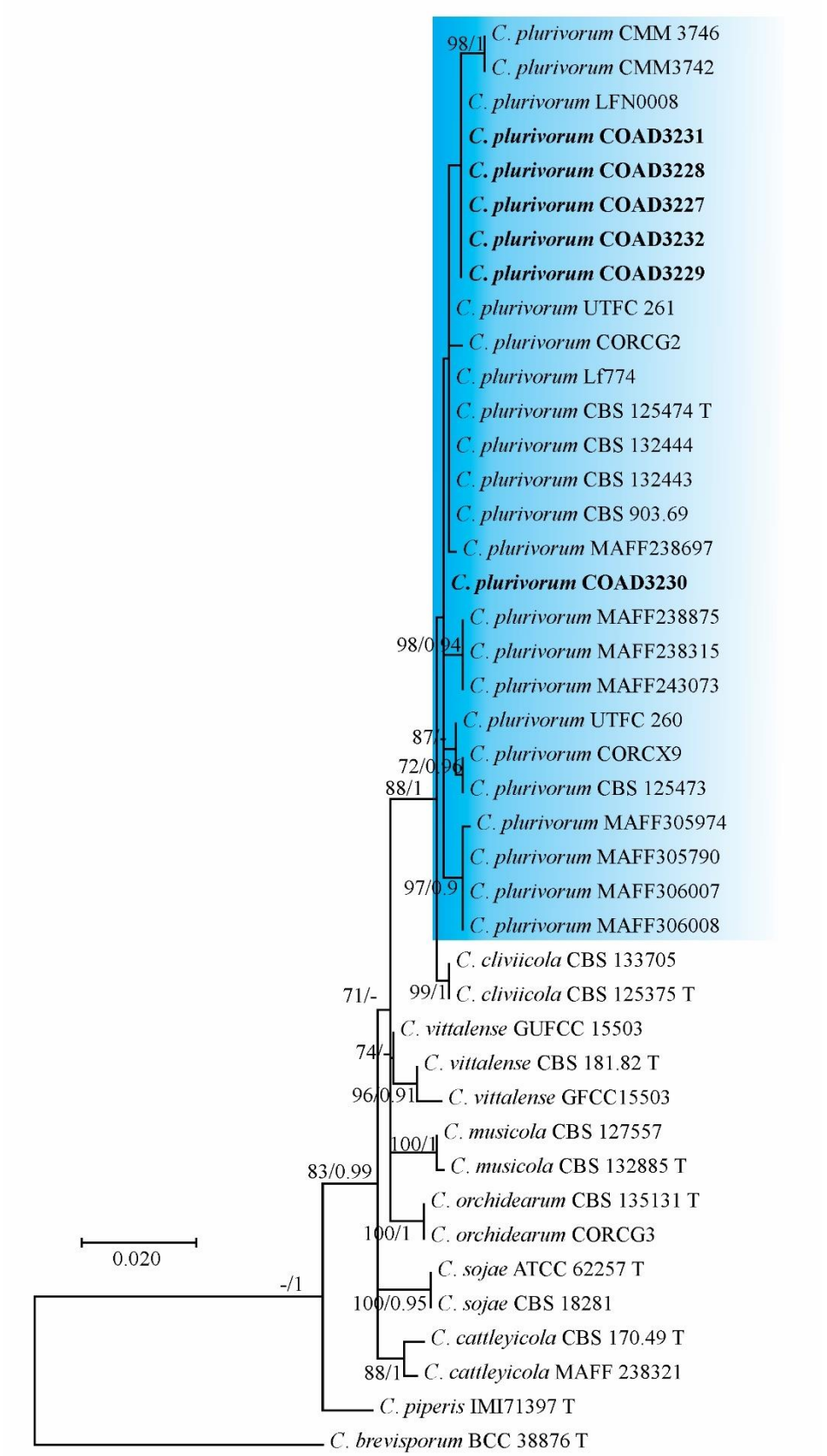
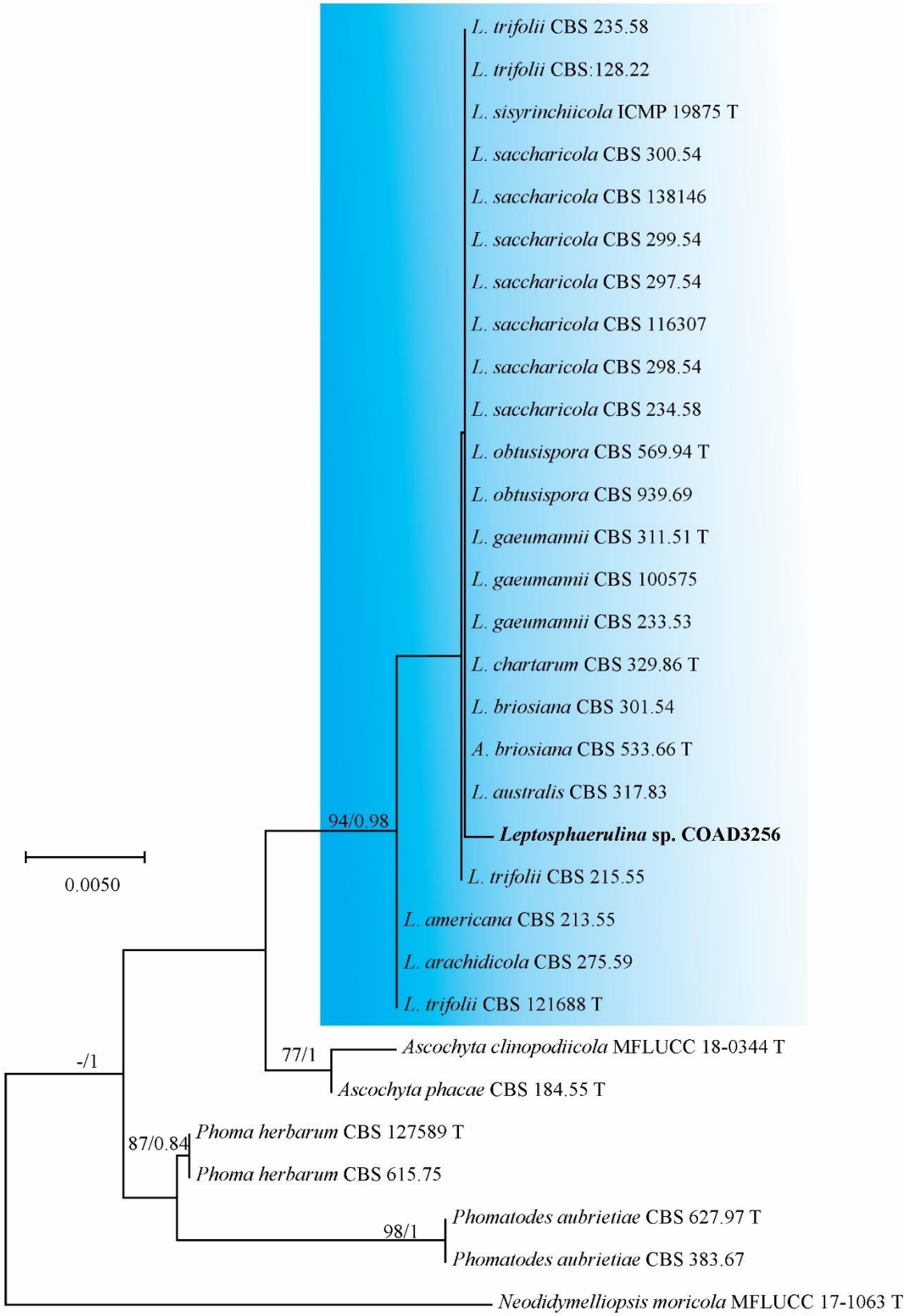


Figure 9: Phylogenetic tree of *Leptosphaerulina* spp. obtained by Maximum-Likehood method using ITS sequences. Bootstrap (over 70%) and posterior probability (over 90%) values are indicated on the nodes. The sequence in bold were obtained in this study. The tree was rooted with *Neodidymelliopsis moricola* (MFLUCC 17-1063). T = Type material.



The three sequences of *Moniliophthora* obtained in this study were grouped together within the monophyletic clade of the genus *Moniliophthora* (Fig 10). The isolates COAD3206, COAD3205 and COAD3204 grouped into a single clade highly supported with 99% posterior probability by the BI analysis and 96% of bootstrap by ML analyses and sister of *M. conchata*. This distinct lineage will be proposed as a new species. Two strains (MCA 2500 and MCA 2501) obtained by AIME & PHILLIPS-MORA (2005) as endophytic on *Bouteloua eriopoda*, in New Mexico USA, formed a sister clade of the new lineage obtained here. That clade well supported clearly represents a distinct uncharacterized taxon.

Taxonomy

Moniliophthora sp. VIC47431 (to be proposed as a new species)

Type: Brazil: Mato Grosso: Sorriso, on Glycines max seeds, cultivar TMG1180, Jan 2019, T. C. Brommonschenkel (VIC 47431, preserved in a metabolically inactive state – holotype; living culture ex-type COAD 3205). Additional cultures examined: Brazil: Mato Grosso: Sorriso, on *Glycine max* stem, cultivar Corumba, Jan 2019, T. C. Brommonschenkel (VIC 47430; COAD 3204). Brazil: Mato Grosso: Sorriso, on *Glycine max* seed, cultivar TMG1180, Jan 2019, T. C. Brommonschenkel (VIC 47432; COAD 3206). All isolates obtained from direct isolation.

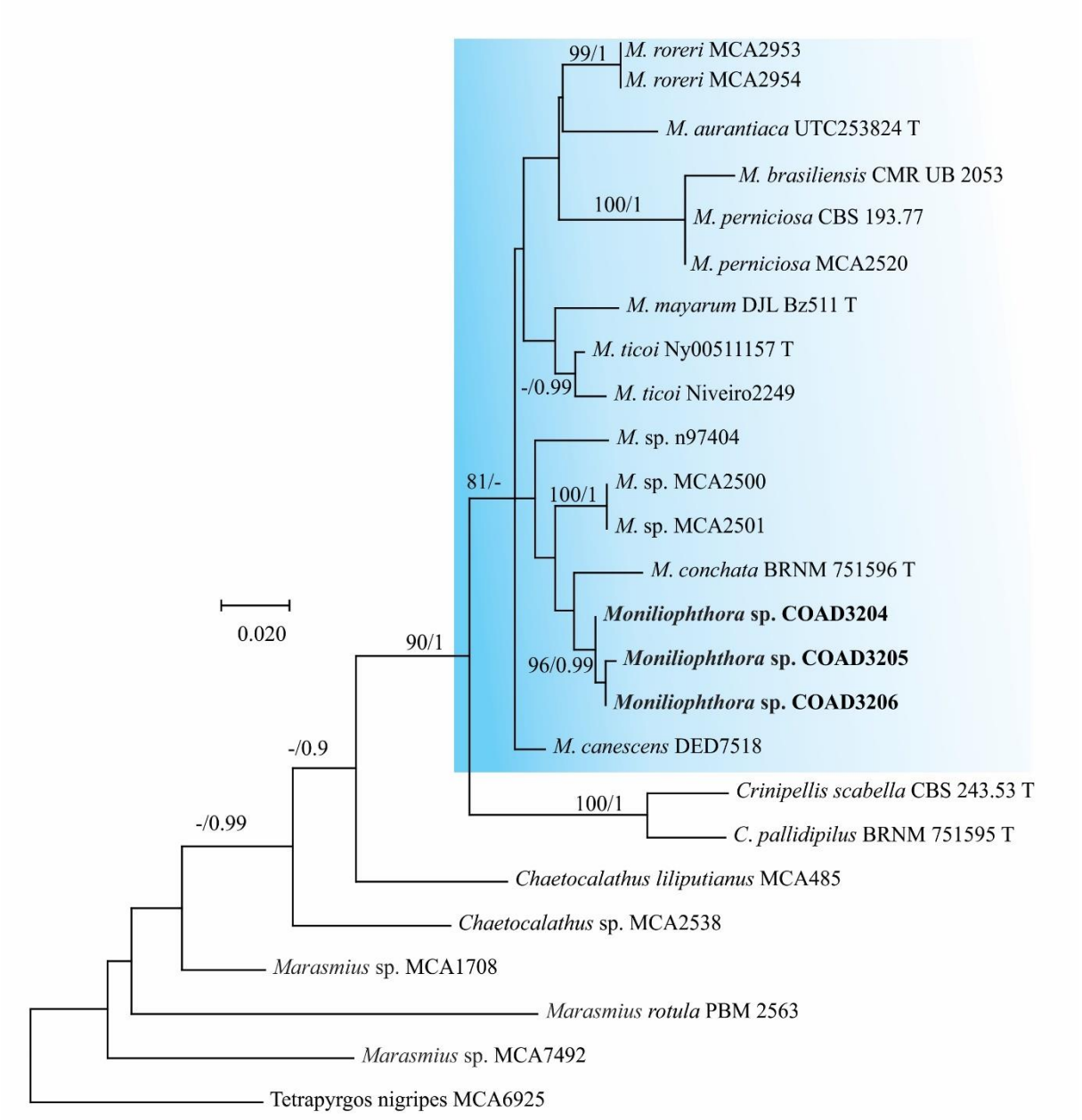
The isolates have only one nucleotide polymorphism in the ITS sequences at position 265, where the COAD3206 and COAD3205 isolate has a Cytosine (C) and the COAD3204 isolate a Thymine (T).

Notes: *Moniliophthora* sp. VIC47431/COAD3205 is phylogenetically distinct from others *Moniliophthora* species, but cultures of the new specie failed to sporule. It was therefore not possible to carry out a morphological analysis. Thus, the new taxon is here introduced based only on molecular data. The differences within the alignment with species / isolates that grouped together in phylogenetic analyzes are shown below.

Differs from *M. conchata* by nucleotide polymorphisms in ITS sequences based on alignment: 51 (C), 237 (C), 255 (C/t/-), 442 (G), 449 (C), 451 (T), 452 (A), 466 (G), 476 (T), 490 (G), 509 (C).

In addition, *Moniliophthora* sp. VIC47431 differs from *Moniliophthora* sp. MCA 2500 and M. sp. MCA 2501 (AIME & PHILLIPS-MORA, 2005) by nucleotide polymorphisms in ITS sequences based on alignment: 2 (T), 3 (C), 4 (T), 5 (A), 8 (G); 9 (C); 11 (A), 24 (C), 67 (C), 77 (A), 121 (T), 123 (G), 124 (A), 131 (T), 132 (C), 141 (A), 219 (T), 235 (A), 237 (C), 245 (T), 255 (C/t/-), 442 (G), 545 (C), 561 (G).

Figure 10: Phylogenetic tree of Marasmiaceae s.s. obtained by Maximum-Likehood method using ITS sequences. Bootstrap (over 70%) and posterior probability (over 90%) values are indicated on the nodes. The sequences in bold were obtained in this study. The tree was rooted with *Tetrapyrgos nigripes* (MCA 6925). T= Type material.



3.3 Pathogenicity

The three isolates of *Moniliophthora* sp., 13 of *C. truncatum*, six of *C. plurivorum*, 10 of *D. ueckerae*, 11 of *D. longicolla* and the only *Leptosphaerulina* sp. were selected to use during pathogenicity tests. The plants of negative control did not show necrotic lesions on the stem within 48 hours, neither signs of necrosis with 15 days after inoculation and no developed necrosis was observed when the stem was sectioned in the toothpick insertion area (Fig 11 a-c).

Figure 11: Negative control, pathogenicity test and visualization of symptoms on soybean cultivar M7739 with 21 days after inoculation. Negative control with 48h after inoculation (a); (b, c) Cross-section of the stem without internally developed of necrosis of negative control with 21dai. (d-g) *Colletotrichum truncatum*: (d) COAD3208, (e) COAD3210, (f) COAD3213, (g) COAD3220. (h-l) *Diaporthe ueckerae*: (h) COAD3233, (i) COAD3234, (j) COAD3235, (k) COAD3239, (l) COAD3240.

The isolates of *C. truncatum* COAD3208, COAD3210 and COAD3213 were all pathogenic to cultivar M7739 (Fig 11 d-f), with the incidence of dead plants (% DP) varied from 7% (COAD3210) to 32% (COAD3208) (Fig 12). Only the COAD3220 isolate was not pathogenic with any dead plant or necrosis with more than 1 cm (Fig 11 g; Fig 12). The isolates of *D. ueckerae* COAD3233, COAD3234, COAD3235, COAD3239 and COAD3240 were all pathogenic to cultivar M7739 (Fig 11 h-l) and the %DP varied from 17% (COAD3239 and COAD3238) to 57% (COAD3234 and COAD3240) (Fig 12). The isolate COAD3240 formed pycnidia linearly on stem of dead plants, but without the production of conidia (Fig 11 l). The isolate COAD3247 of *D. longicolla* were pathogenic to cultivar M7739 with 11% of dead plants (Fig 12).

The isolates of *C. truncatum* COAD3208, COAD3210, COAD3213, COAD3215 and COAD3216 were all pathogenic to cultivar TMG4182 (Fig 14 a-e), with the %DP varying from 6% (COAD3210) to 31% (COAD3213) (Fig 13). Only the COAD3220 isolate was not pathogenic with any dead plant or necrosis with more than 1 cm (Fig 13, Fig 14 f). The isolates of *D. ueckerae* COAD3233, COAD3234, COAD3235, COAD3238, COAD3239 and COAD3240 were all pathogenic to cultivar TMG4182 (Fig 14 g-k) and the %DP varied from 21% (COAD3235) to 40% (COAD3234 and COAD3240) (Fig 13). The isolates COAD3234 and COAD3235 formed pycnidia linearly on stem of dead plants, but without the production of conidia (Fig 11 h, i). The isolate COAD3247 of *D. longicolla* were pathogenic to cultivar TMG4182 with 28% of dead plants (Fig 13, Fig 14 l).

The isolates of *Moniliophthora* sp. COAD3204 and COAD3205 were pathogenic to cultivar TMG238 (Fig 16 a, b), with %DP varying from 25% (COAD3204) to 38% (COAD3205) (Fig 15). The isolates of *C. truncatum* COAD3223 and COAD3226 were pathogenic to cultivar TMG238 (Fig 16 c, d), with the %DP of 25% for both isolates (Fig 15). The isolates of *C. plurivorum* COAD3227, COAD3228, COAD3229, COAD3230, COAD3231 and COAD3232 were all pathogenic to cultivar TMG238 (Fig 16 e-h) and the %DP varied from 25% (COAD3228 and COAD3231) to 50% (COAD3227, COAD3229, COAD3230 and COAD3232) (Fig 15). The isolates of *D. ueckerae* COAD3236, COAD3237, COAD3241 and COAD3242 were pathogenic to cultivar TMG238 (Fig 16 g-k) and the %DP varied from 13% (COAD3236) to 50% (COAD3232, COAD3237 and COAD3241) (Fig 15). The isolates of *D. longicolla* COAD3244 (Fig 16 k), COAD3243, COAD3245, COAD3249, COAD3250, COAD3251 and COAD3253 were pathogenic to cultivar TMG238.

Figure. 12: Pathogenicity test on cultivar M7739. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *C. truncatum*, *D. ueckerae* and *D. longicolla*.

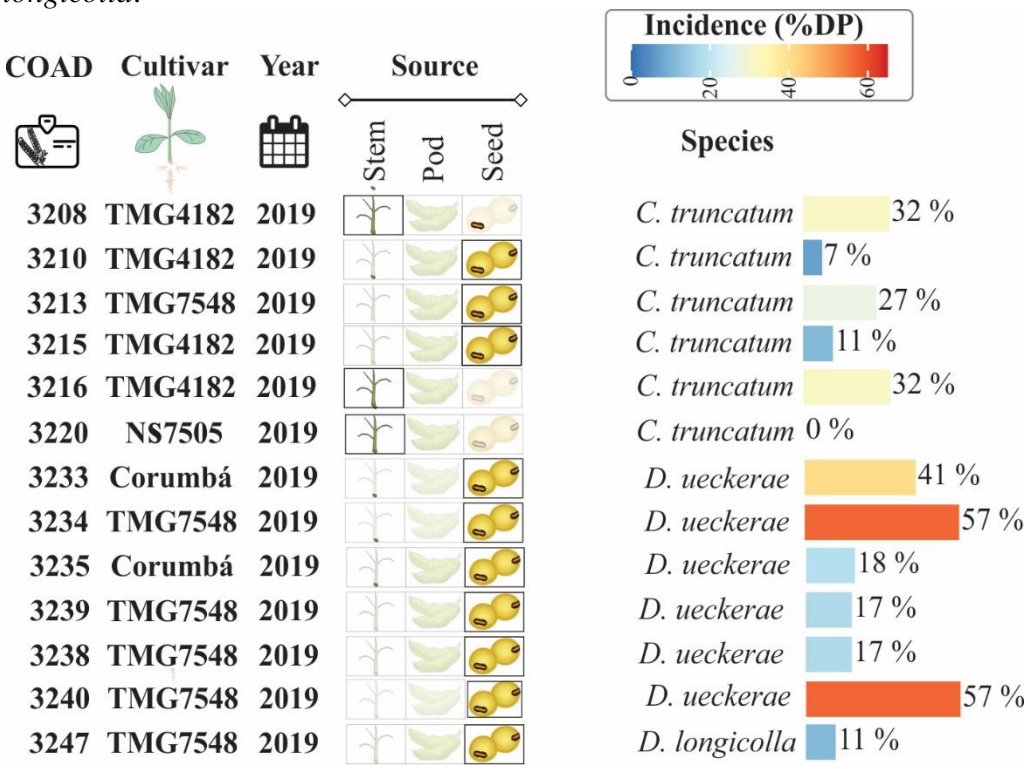


Figure. 13: Pathogenicity test on cultivar TMG4182. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *C. truncatum*, *D. ueckerae* and *D. longicolla*.

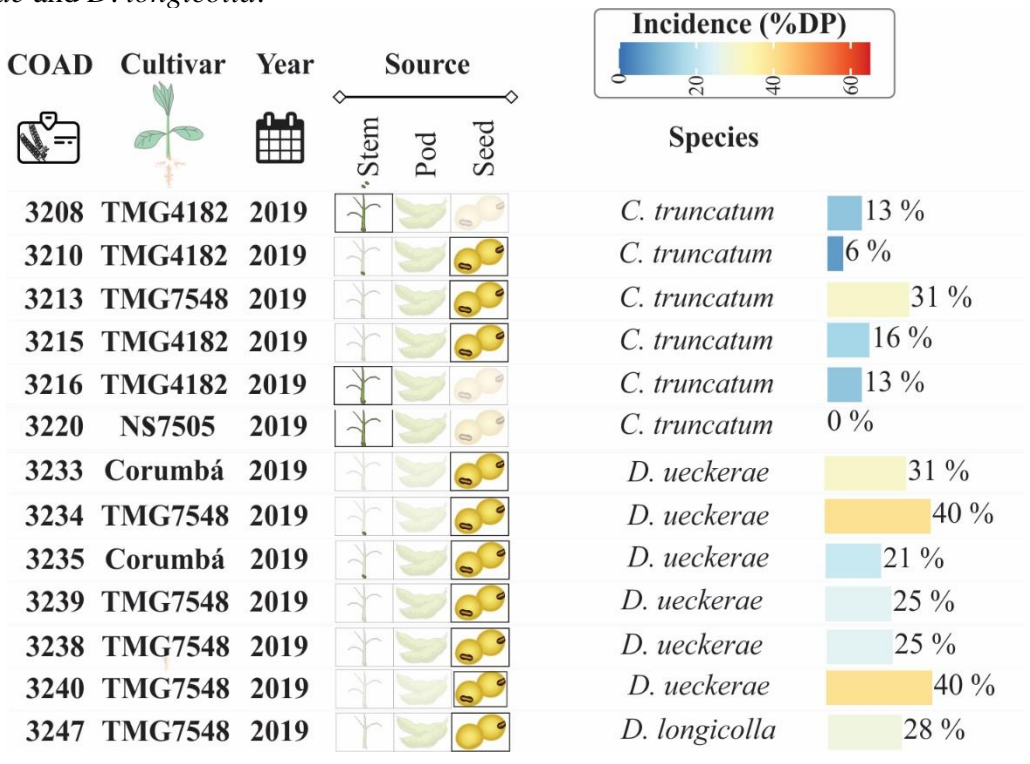
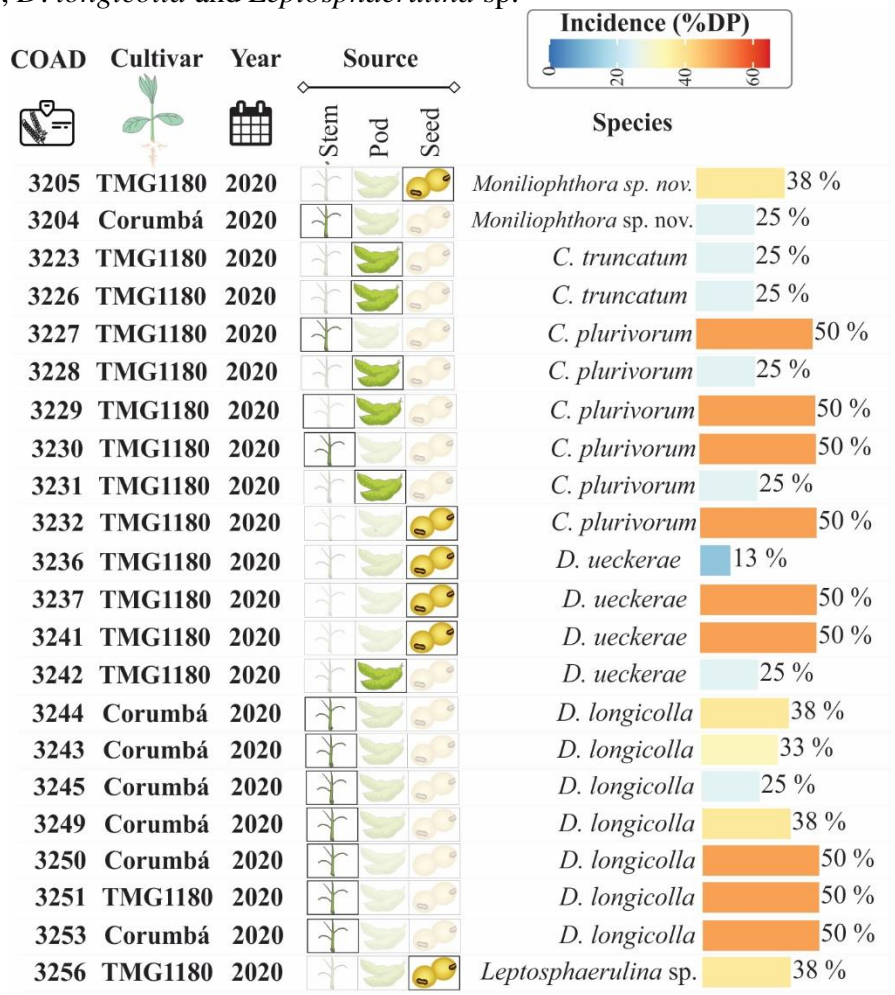


Figure 14: Pathogenicity test and visualization of symptoms on soybean cultivar TMG4182 with 21 days after inoculation. (a-f) *C. truncatum*: (a) COAD3208, (b) COAD3210, (c) COAD3213, (d) COAD3215, (e) COAD3216, (f) COAD3220. (g-k) *D. ueckerae*: (g) COAD3233, (h) COAD3234, (i) COAD3235, (j) COAD3238, (k) COAD3240. (l) Cross section of the stem to visualize the internal necrosis caused by the isolate COAD3247 of *D. longicolla*.

Figure. 15: Pathogenicity test on cultivar TMG238. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *C. truncatum*, *C. plurivorum*, *D. ueckerae*, *D. longicolla* and *Leptosphaerulina* sp.

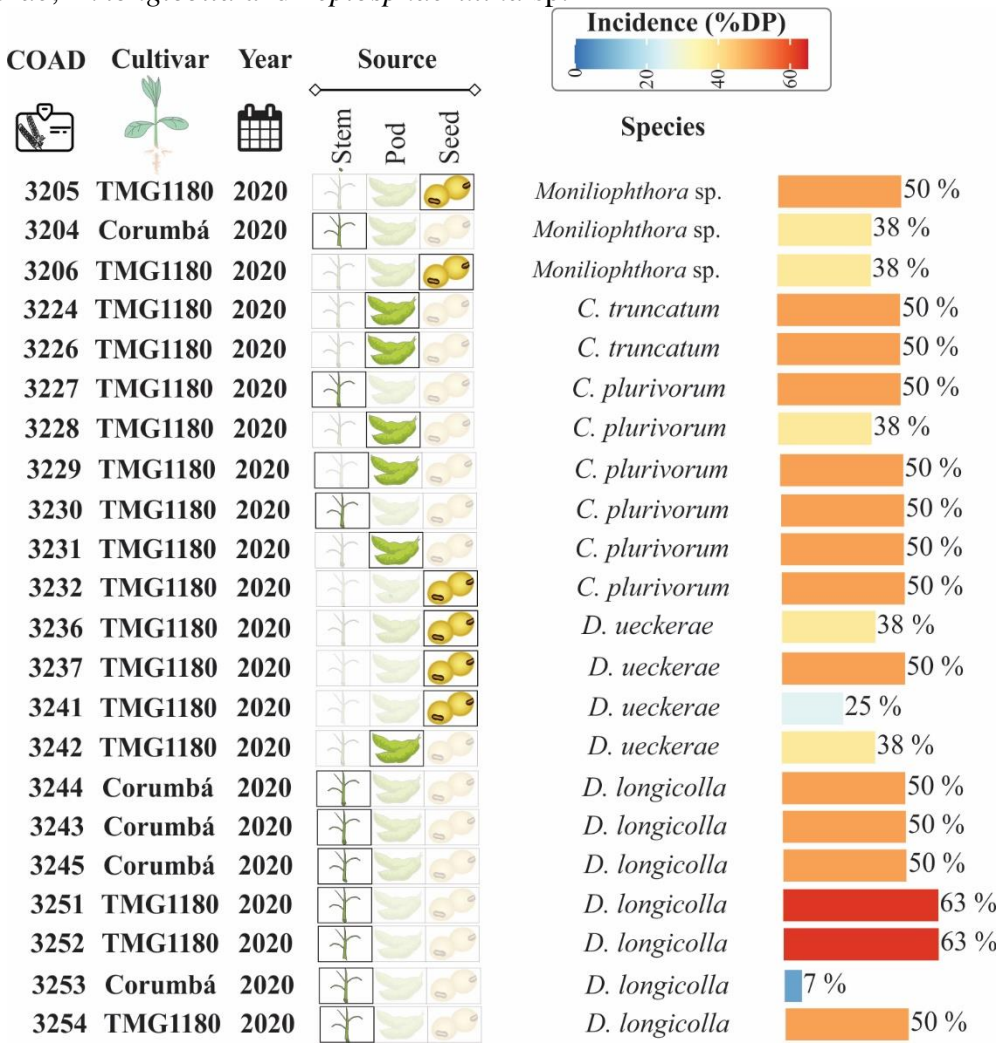


The %DP varied from 25% (COAD3245) to 50% (COAD3250, COAD3251 and COAD3253) on cultivar TMG238 with the isolates of *D. longicolla* (Fig 15). The isolate COAD3256 of *Leptosphaerulina* sp. were pathogenic to cultivar TMG238 with 38% of dead plants (Fig 15, Fig 16 l).

The isolates of *Moniliophthora* sp. COAD3205, COAD3204 and COAD3206 were pathogenic to cultivar NS7901 (Fig 18 a, b), with the %DP varying from 38% (COAD3204 and COAD3206) to 50% (COAD3205) (Fig 17). *C. truncatum* COAD3224 and COAD3226 were pathogenic to cultivar NS7901 (Fig 18 c, d), with the %DP of 50% to both isolates (Fig 17). The isolates of *C. plurivorum* COAD3227, COAD3228, COAD3229, COAD3230, COAD3231 and COAD3232 were pathogenic to NS7901 (Fig 18 e-i) and %DP was 38% to isolate COAD3228 and 50% to the others (Fig 17).

Figure 16: Pathogenicity test and visualization of symptoms with cross section of stem on soybean cultivar TMG238 with 21 days after inoculation. (a-b) *Moniliophthora* sp.: (a) COAD3205, (b) COAD3204. (c, d) *C. truncatum*: (c) COAD3223, (d) COAD3226. (e-h) *C. plurivorum*: (e) COAD3227, (f) COAD3229, (g) COAD3230, (h) COAD3232. (I, j) *D. ueckerae*: (i) COAD3237, (j) COAD3241. (k) *D. longicolla* COAD3245. (l) *Leptosphaerulina* sp. COAD3256.

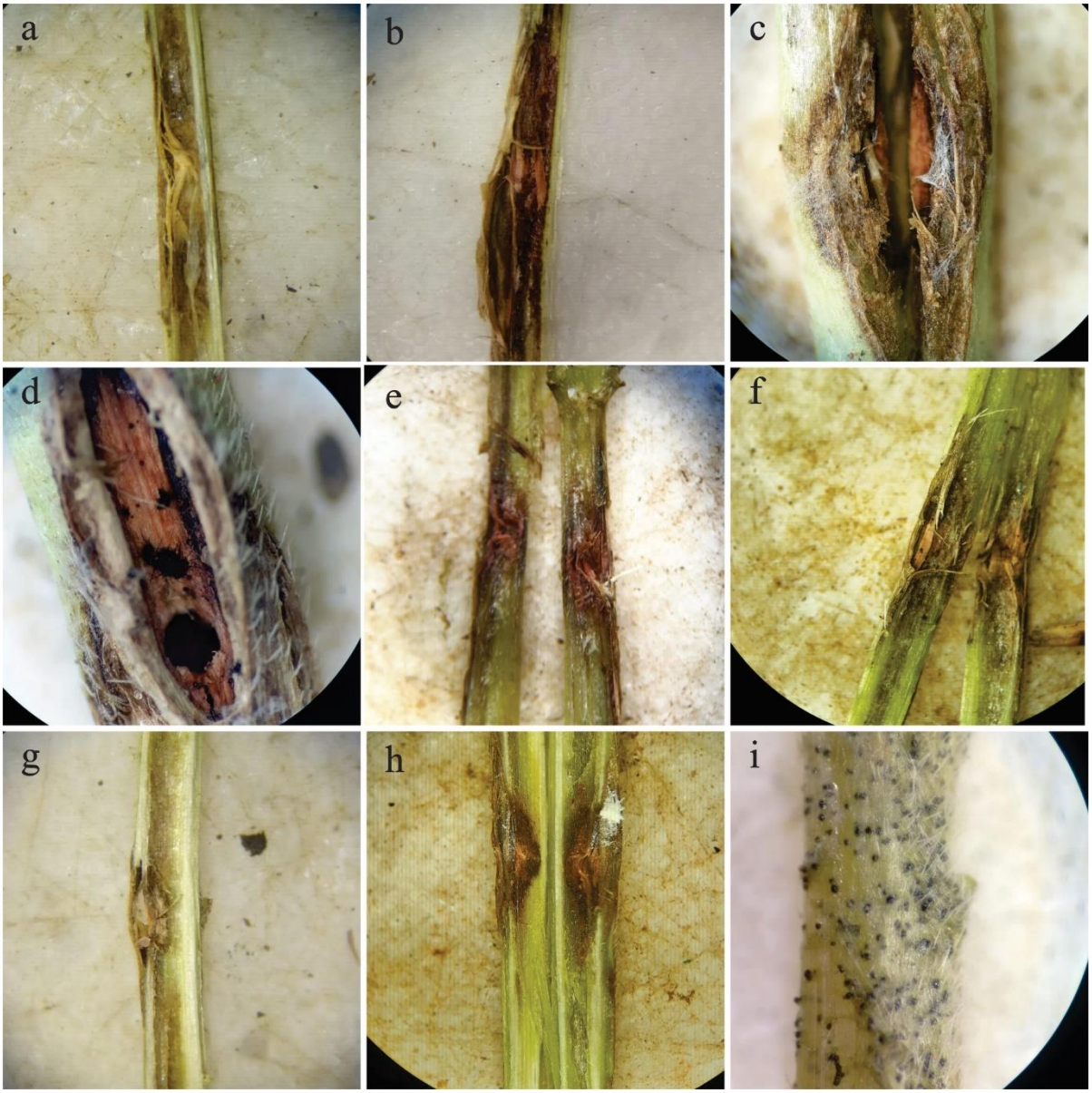
Figure. 17: Pathogenicity test on cultivar NS7901. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *C. truncatum*, *C. plurivorum*, *D. ueckerae*, *D. longicolla* and *Leptosphaerulina* sp.



The isolates of *D. ueckerae* COAD3236, COAD3237, COAD3241 and COAD3242 were pathogenic to cultivar NS7901 (Fig 19 g-k) and %DP varied from 25% (COAD3241) to 50% (COAD3237) (Fig 17). The isolates COAD3244, COAD3243, COAD3245, COAD3251, COAD3252, COAD3253 and COAD3254 of *D. longicolla* were pathogenic to cultivar NS7901 (Fig 19) and %DP varied from 7% (COAD3253) to 63% (COAD3251 and COAD3252) (Fig 17). The isolate COAD3252 formed pycnidia linearly on stem of dead plants, with the production of conidia (Fig 19 h, i).

The isolate COAD3206 of *Moniliophthora* sp. was not pathogenic with any dead plant or necrosis with more than 1 cm to cultivar DESAFIO (Fig 20, Fig 21 a). The isolates COAD3221, COAD3222 and COAD3224 of *C. truncatum* were pathogenic to cultivar DESAFIO (Fig 21 b, c) and %DP varied from 13% (COAD3222) to 28% (COAD3224) (Fig 20).

Figure 18: Pathogenicity test and visualization of symptoms with cross section of stem on soybean cultivar NS7901 with 21 days after inoculation. (a-b) *Moniliophthora* sp.: (a) COAD3205, (b) COAD3204. (c, d) *C. truncatum*: (c) COAD3224, (d) COAD3226. (e-i) *C. plurivorum*: (e) COAD3227, (f) COAD3228, (g) COAD3229, (h) COAD3230, (i) COAD3231 with sporulation on dead tissue.



The isolates COAD3248, COAD3252 and COAD3254 of *D. longicolla* were pathogenic to cultivar DESAFIO (Fig 21 d-f) and %DP varied from 40% (COAD3254) to 50% (COAD3252) (Fig 20). The isolates COAD3221, COAD3222, COAD3223 and COAD3225 of *C. truncatum* were pathogenic to cultivar BRAGG (Fig 23 a-d) and %DP varied from 13% (COAD3223) to 50% (COAD3225) (Fig 22). The isolates COAD3245, COAD3248, COAD3249 and COAD3250 of *D. longicolla* were pathogenic to BRAGG (Fig 23 e, f) and %DP varied from 38% (COAD3248) to 50% (COAD3245 and COAD3249) (Fig 22).

Figure 19: Pathogenicity test and visualization of symptoms with cross section of stem on soybean cultivar NS7901 with 21 days after inoculation. (a-d) *D. ueckerae*: (a) COAD3236, (b) COAD3237, (c) COAD3241, (d) COAD3242. (e-i) *D. longicolla*: (e) COAD3243, (f) COAD3244, (g) COAD3251, (h) COAD3251 with sporulation on dead tissue, (i) COAD3252.

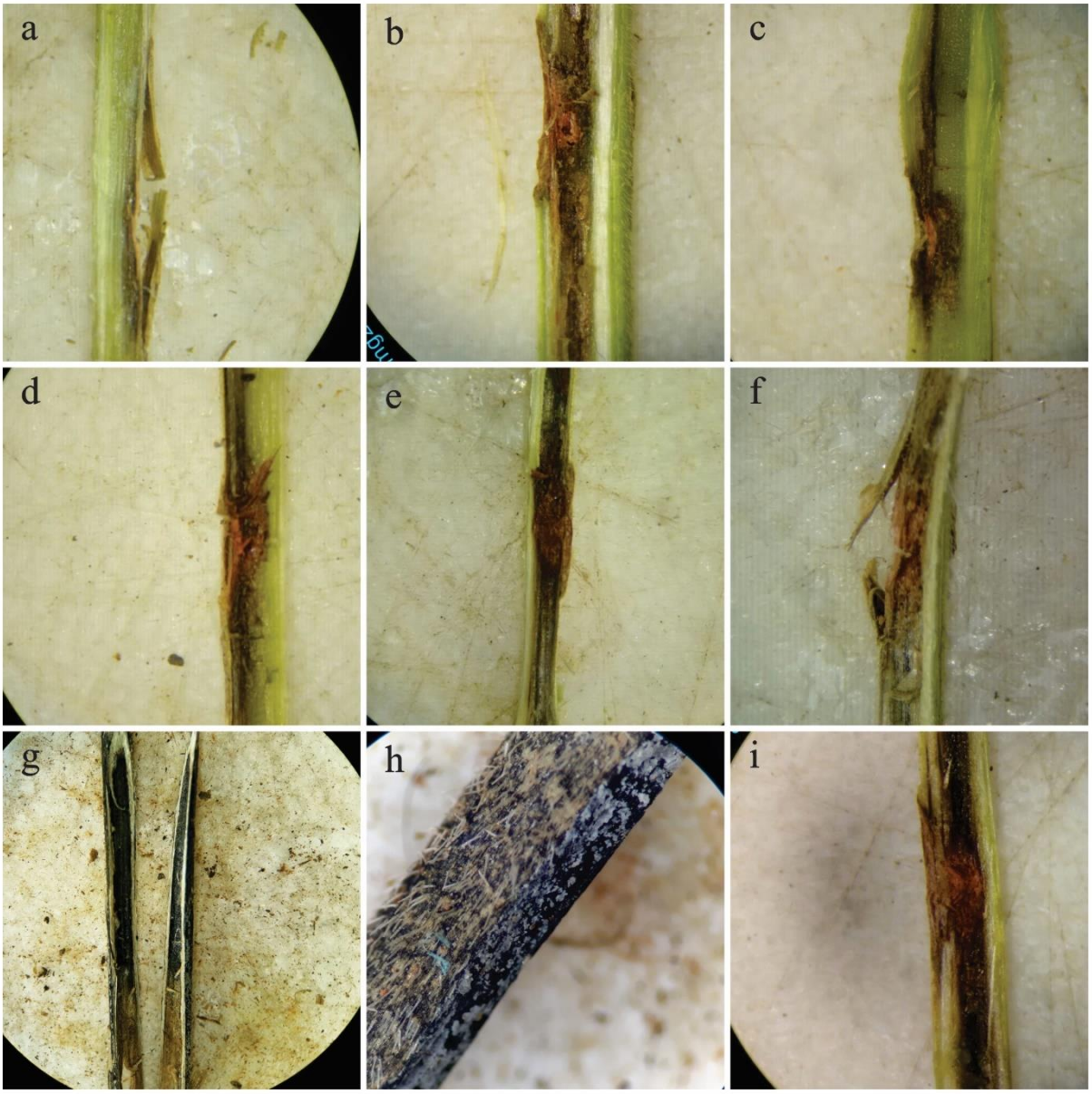


Figure. 20: Pathogenicity test on cultivar DESAFIO. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *Moniliophthora* sp., *C. truncatum* and *D. longicolla*.

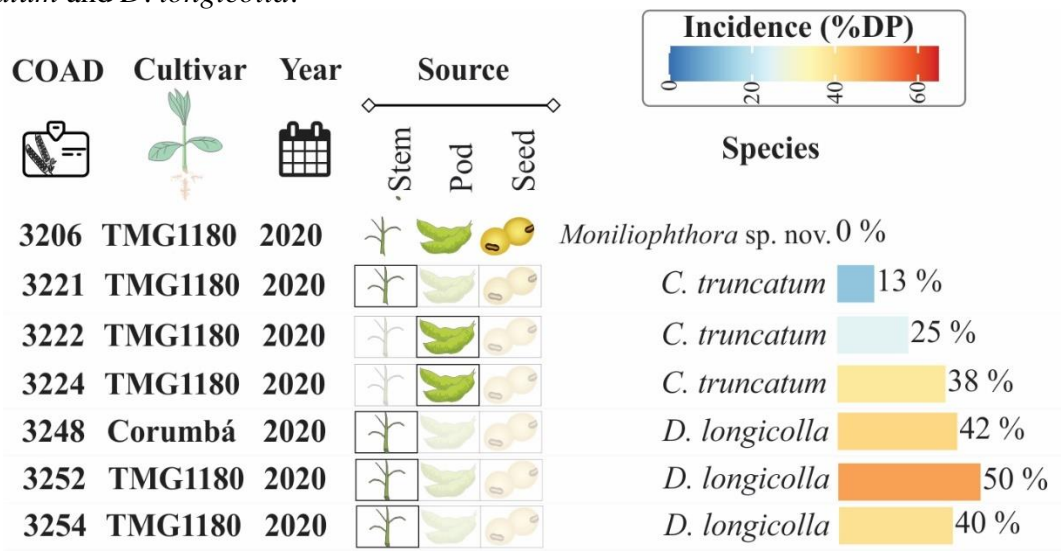


Figure 21: Pathogenicity test and visualization of symptoms with cross section of stem on soybean cultivar DESAFIO with 21 days after inoculation. (a) *Moniliophthora* sp. (b, c) *C. truncatum*: (b) COAD3221, (c) COAD3224 with sporulation on dead tissue. (d-f) *D. longicolla*: (d) COAD3248, (e) COAD3252, (f) COAD3254.

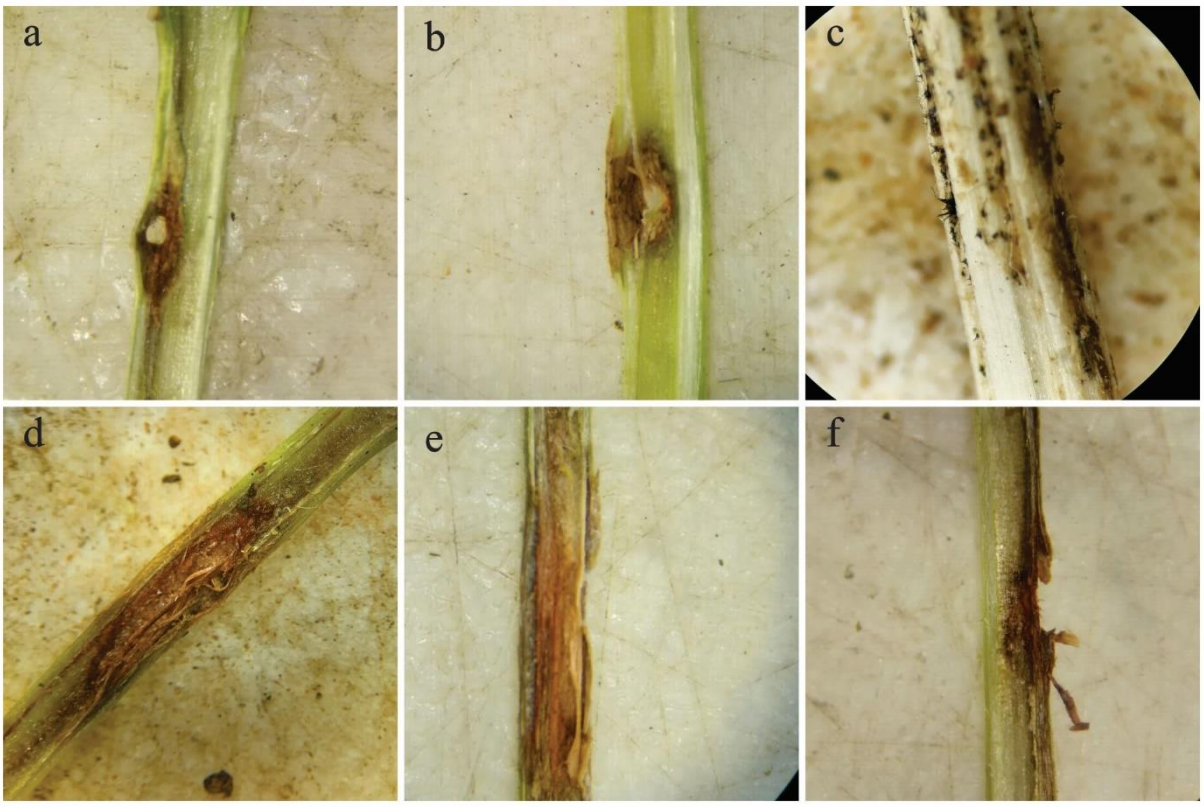


Figure. 22: Pathogenicity test on cultivar BRAGG. On the left origin of the isolates and on the right pathogenicity organized in bar graph according to isolates of *C. truncatum* and *D. longicolla*.

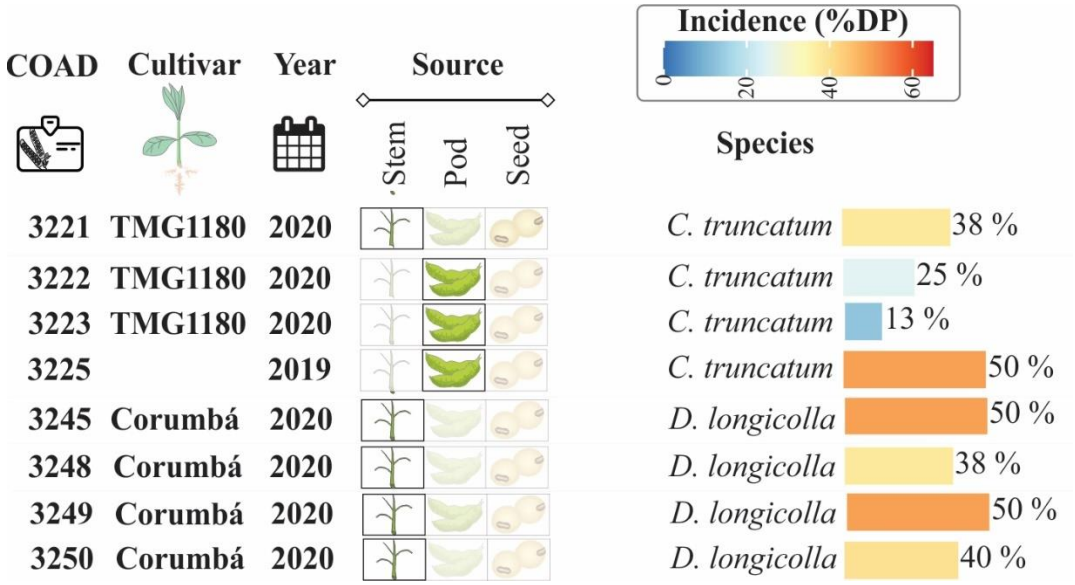
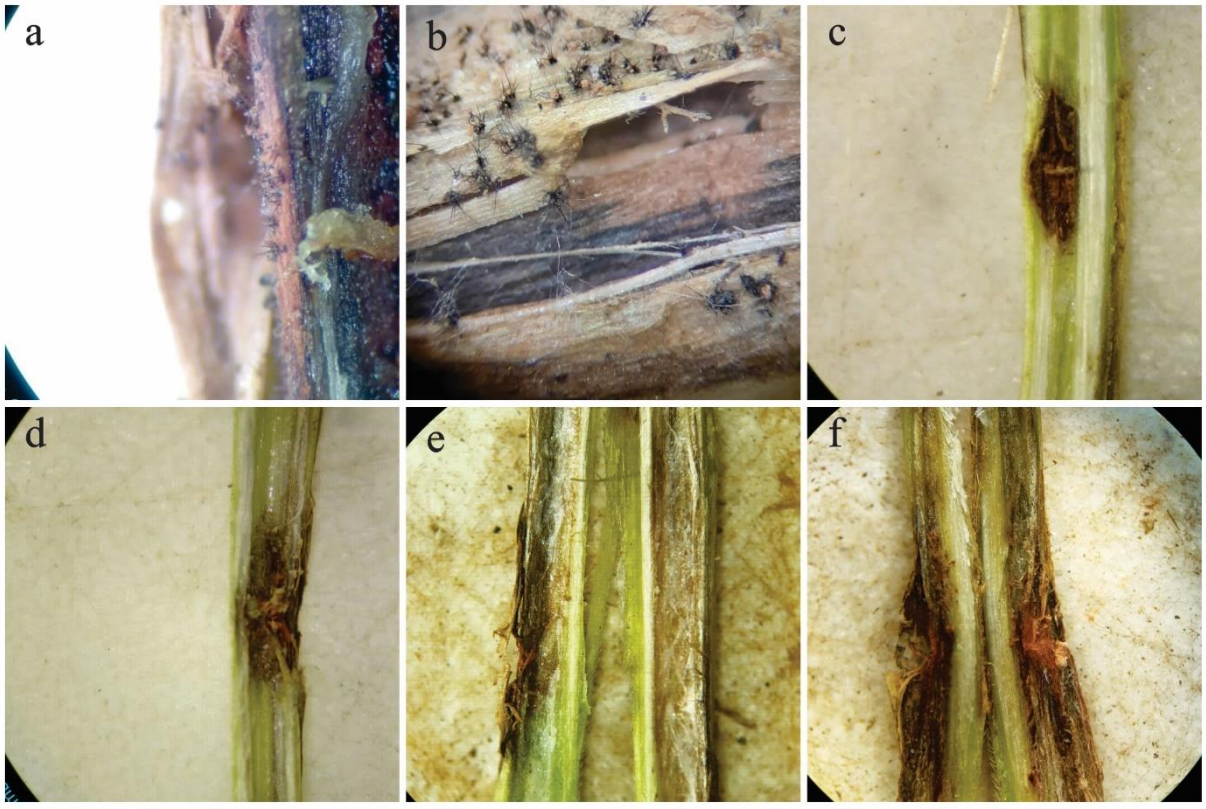


Figure 23: Pathogenicity test and visualization of symptoms with cross section of stem on soybean cultivar BRAGG with 21 days after inoculation. (a-d) *C. truncatum*: (a) COAD3221, (b) COAD3222 with sporulation on dead tissue, (c) COAD3223, (d) COAD3225. (e, f) *D. longicolla*: (e) COAD3245, (f) COAD3249.



4. DISCUSSION

In Brazil there are 10 species belonging to the genus *Diaporthe* causing disease in soybean *D. aspalathi*, *D. caulivora*, *D. citri*, *D. endophytica*, *D. infertilis*, *D. longicolla*, *D. phaseolorum*, *D. meridionalis*, *D. sojae* and *D. ueckerae* (FARR & ROSSMAN, 2021). *D. longicolla* is the main pathogen associated with Phomopsis seed decay, one of the most destructive diseases of soybean, and also to pod and stem blight disease (BRUMMER et al., 2018; HOSSEINI et al., 2020). As found in this work, this species can be isolated from stems and pods, as well as seeds, from diseased plants. HOSSEINI et al. (2020) worked with species belonging to the genus *Diaporthe* associated with soybean seeds in Europe, and found that *D. longicolla* is predominant, causing the same symptoms described in our study, and during the pathogenicity tests, carried out by conidia suspension, differing from our tests, *D. longicolla* isolates were the most aggressive, a result similar to those described here, since many isolates presented 50% DP and the highest index was obtained by isolates COAD3251 and COAD3252 on cultivar NS7901. In Brazil, the stem and pod blight are mainly associated with *D. sojae* (ALMEIDA et al., 2005), showing that there is still a need for studies on this disease in Brazil, since many isolates previously reported as *D. phaseolorum* var. *sojae*, are currently classified as *D. longicolla* (ZHANG et al., 1998; DIVILOV, 2014; HOSSEINI et al., 2020) and the correct etiology of the agent of this disease in Brazil needs to be further investigated. The variation in aggressiveness of *D. longicolla* isolates as a function of cultivars has already been reported by TORMEN (2014) when working with isolates identified as *D. phaseolorum* var. *sojae* and the cultivars SYN1080RR and SYN1180RR.

UDAYANGA et al. (2015) reassessed *Diaporthe* species associated with soybean and other crops and described the species *D. ueckerae*, mainly associated with *Cucumis melo*, but one isolate from soybean seeds was identified as belonging to that species from Brazil, but details of its pathogenicity were not made and only one isolate was obtained from soybean. We obtained isolates of *D. ueckerae* mainly from seeds and one isolate obtained from the pod. THOMPSON et al. (2015) described the species *D. miriciae* but which in 2016 was synonymous with *D. ueckerae* by GAO et al. (2016). PETROVIC et al. (2020) found this species associated with seed decay disease, showing that several species belonging to the genus *Diaporthe* are associated with this disease as well *D. longicolla*, but only isolates from the seed were obtained. The variation in aggressiveness of *D. ueckerae* isolates as a function of soybean cultivars has not been reported yet, therefore, we are the first to report this.

Although *Colletotrichum truncatum* is more commonly associated with soybean anthracnose, other species of *Colletotrichum* are also involved, and in Brazil there is still the association of *C. cliviicola*, *C. dematium*, *C. gloeosporioides*, *C. lindemuthianum*, *C. plurivorum* and *C. sojae*, in addition to these species, there is also the association of *C. chlorophyti*, *C. coccodes*, *C. destructivum*, *C. glycines*, *C. incanum*, *C. phaseolorum* and *C. trifolii* in other countries (FARR & Rossman, 2021), indicating that the etiology of soybean anthracnose requires in-depth studies.

ROGÉRIO et al. (2017) conducted a study with a total of 51 isolates belonging to the genus *Colletotrichum*, obtained between 1992 and 2007 in the South, Southeast and Midwest regions of Brazil. Although they observed the formation of 3 phylogenetic groups, identified as haplotypes, only the species *C. truncutam* was observed and concluded that this species would be the only one to cause anthracnose on soybean in Brazil. However, BARBIERI et al. (2017) found *C. cliviicola* causing anthracnose on soybean and BOUFLEUR et al. (2020) found *C. musicola*. DAMM et al. (2019) described *C. plurivorum* and *C. sojae* as associated with soybean in Brazil, but did not indicate a relationship with anthracnose or other soybean disease, pathogenicity tests have not been conducted. DIAS et al. (2019) described haplotypes of *C. truncatum* with RAPD, and found a variation in the reactions of different cultivars from a germplasm bank, and indicate resistance in some materials and a variable incidence of *C. truncatum* on cotyledons and stems of different cultivars, but none of these accessions (cultivars) were used in our study. We observed in our evaluations only the resistance of cultivars TMG4182 and M7739 to the isolate COAD3220 belonging to the species *C. truncatum*. The variability in the pathogenicity tests of isolates belonging to *C. truncatum* can be explained by possible multiple recent introduction events of different populations in Brazil (ROGÉRIO et al., 2019).

CASTRO (2018) indicated that *C. plurivorum* isolates show a slower mycelial growth than *C. truncatum*, a result not observed with our isolates. In addition, they observed less aggressiveness compared to the isolates of *C. truncatum* on the cultivar AMS Tibagi, in our work *C. plurivorum* was more aggressive than *C. truncatum* on the cultivar TMG238, where isolates of the first came to cause 50% DP while isolates of *C. truncatum* caused 25% DP. On cultivar NS7901, the isolates of the two species caused similar rates of dead plants. CASTRO (2018) also observed that there is variation in the resistance of soybean cultivars for different isolates of *C. plurivorum*, a result also observed in our study. ZAW et al. (2020).

The genus *Leptosphaerulina* was proposed by Daniel McAlpine in 1902, having as type *L. australis*, and described as a pathogen of *Prunus melanocarpa* and currently has 64 species

officially recognized according to the official nomenclatural repositories Mycobank and Index Fungorum (MCALPINE, 1902; MYCOBANK, 2020; INDEX FUNGORUM, 2020). The species belonging to the genus are found in several countries causing diseases in a wide range of hosts, often associated with leaf spots, such as *L. crassiasca* on *Arachis hypogaea*, *L. australis* on *Vigna sinensis*, and *L. trifolii* on *Arachis hypogaea* and *Lablab purpureus* (SIMMONDS, 1966; LENNÉ, 1990; PANDE & RAO, 1998; CHEN et al., 2015). In Brazil, only 4 species are described in association with plant diseases, namely *L. briosiana* on *Medicago sativa*, *L. conyzicola* on *Conyza canadensis*, *L. crassiasca* on *Arachis glabrata*, *A. hypogaea* and *Medicago sativa*, and *L. trifolii* on *Sorghum halepense*, *Trifolium repens* and *T. vesiculosum* (MENDES et al., 1998; DUARTE et al., 2016; VICTORIA et al., 2020).

Only two species are reported on soybean, associated with leaf spots, *L. briosiana* occurring only in the USA, and *L. trifolii* being reported in Brunei Darussalam, Fiji, India, Malaysia, Taiwan and Zimbabwe (WHITESIDE, 1966; TURNER, 1971; FIRMAN, 1972; PEREGRINE et al., 1982; GRYBAUSKAS, 1986; PANDE & RAO, 1998; HSIEH et al., 2000). NECHET et al. (2008) found an unidentified species of this genus associated with leaf spots on soybeans in the Roraima state in Brazil, but the disease had a low incidence, identification was performed only at the gender level based on morphological characters and the isolates obtained were not pathogenic. Therefore, the isolate COAD3256 is the first report of a species belonging to the genus *Leptosphaerulina* associated with soybean diseases in Brazil with established pathogenicity.

The genus *Moniliophthora* was described by EVANS et al. (1978) being separated from the genus *Monilia* and *M. roreri* was proposed as the type of the genus and was described as an important pathogen of plants of the genus *Theobrama* and *Herrania* sp. Currently the genus is associated with diseases on different plants and the following species are currently reported on their respective hosts, *M. brasiliensis* on *Heteropterys acutifolia* (Malpighiaceae), *M. mayarum* on *Ceiba pentandra* (Malvaceae), *M. perniciosa* on *Allophylus edulis* (Sapindaceae), *Capsicum annuum*, *C. frutescens*, *Cestrum* sp., *Solanum gilo*, *S. lycocarpum*, *S. melongena*, *S. paniculatum*, *S. stipulaceum*, *S. cladotrichum*, *S. gemellum*, *S. grandiflorum*, *S. mauritianum*, *S. subumbellatum*, *S. swartzianum*; *S. melongena* (Solanaceae), *Herreria* sp. (Liliaceae), *Theobrama cacao*, *T. grandiflorum* (Malvaceae), *M. roreri* on *Herraria albiflora*, *H. nitida* (Liliaceae), *T. bicolor*, *T. cacao*, *T. gileri*, *T. grandiflorum*, *T. mammosum*, *T. speciosum* (Malvaceae), and *M. ticoi* on *Holocalyx balansae* (Fabaceae), *Myrcianthes pungens* (Myrtaceae) and *Pogonopus tubulosus* (Rubiaceae) (PHILLIPS-MORA et al., 2007; PATROCINIO et al., 2017; LISBOA et al., 2020; NIVEIRO et al., 2020).

In addition, the genus has species described only as saprophytes, these are *M. aurantiaca* founded on fallen twigs and other woody material in littoral forest (KROPP & ALBEE-SCOTT, 2012), *M.* canescens founded on dead fallen twig of broadleaved tree, *M marginate* on undetermined decaying woody stem and *M. nigrilineata* on dead twigs in forest (KEREKES & DESJARDIN, 2009), *M. conchata* found on fallen twig of *Trachalospermum asiaticum* and *M. conchata* var. *brevispora* on fallen twigs of a liana (ANTONÍN ET AL. 2014), and AIME & PHILLIPS-MORA (2005) found isolates belonging to an undetermined species such as an endophyte from *Bouteloua eriopoda*.

Therefore, our work reports the first time that the genus Moniliophthora is associated with a disease on soybean worldwide, in this case, mycelium of these isolates were found in pods and seeds of plants showing stem blight and pod rot and pathogenicity was verify through visualization of necrosis in different soybean cultivars in pathogenicity tests., this being the second host reported on Fabaceae family, previously only *M. ticoi* had been described causing disease on *Holocalyx balansae*, a tree with the common name Alecrim de Campinas, in Argentina (NIVEIRO et al., 2020). The COAD3204, COAD3205 and COAD3206 isolates show great genetic uniformity based only on the ITS region, with only 1 difference in the composition of nucleotides, but they show a difference in pathogenicity with isolate COAD3206 not being pathogenic on cultivar DESAFIO, while the other isolates were pathogenic. On the cultivar NS7901, all 3 isolates were pathogenic, with isolate COAD3205 showing greater aggressiveness.

Final considerations

In our study, plants with symptoms of anthracnose, stem and pod blight, and pod rot were collected. The pathogens found are already associated with these diseases in the literature. Although fungi associated with stem canker were not found, we found a diversity of pathogens associated with the collected material, indicating that there is a need for research related to soybean pathogens, as an example the diversity of species of the genus *Colletotrichum* still little explored, the first report of *Leptosphaerulina* sp. in Brazil and the possible new disease associated with the genus *Moniliophthora*.

The new pathogenic species of *Monilophthora* will be described and published according to the International Nomenclature Code for fungi, algae and plants. The *Leptosphaerulina* isolate will be sequenced the rpb2 and LSU gene regions to identify the species to which it belongs.

As a future perspective, in addition to taxonomy work, the collection may be used as a reference for other phylogenetic, phylogeographic studies, population studies and breeding programs.

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6. SUPPLEMENTARY INFORMATION

Supplementary table 1. Host / substrate, locality, collector and GenBank accession numbers of strains used in the phylogenetic analyses.

Species	Country	Host	Collection number	GenBank accession number		
				ITS	TEF1	TUB2
C. brevisporum	Thailand	*Neoregalia sp.*	BCC 38876 T	JN050238		JN050244
C. cattleyicola	Belgium	*Cattleya sp.*	CBS 170.49 T	MG600758		MG601025
C. cattleyicola	Japan	*Cattleya sp.*	MAFF 238321	MG600759		MG601026
C. cliviicola	Clivia miniata	*Clivia miniata*	CBS 125375 T	MG600733		MG601000
C. cliviicola	South Africa	*Clivia sp.*	CBS 133705	MG600732		MG600999
C. musicola	Mexico	*Musa sp.*	CBS 132885 T	MG600736		MG601003
C. musicola	Mexico	*Musa sp.*	CBS 127557	MG600737		MG601004
C. orchidearum	China	*Cymbidium hookerianum*	CORCG3	HM585402		HM585416
C. orchidearum	Netherlands	*Dendrobium nobile*	CBS 135131 T	MG600738		MG601005
C. piperis	Malaysia	*Piper nigrum*	CPC 21195 T	MG600760		MG601027
C. plurivorum	Vietnam	*Coffea sp.*	CBS 125474 T	MG600718		MG600985
C. plurivorum	Benin	*Phaseolus lunatus*	CBS 903.69	MG600721		MG600988
C. plurivorum	Japan	*Abelmoschus esculentus*	MAFF 306008	MG600727		MG600994
C. plurivorum	Japan	*Amorphophallus rivieri*	MAFF 243073	MG600730		MG600997
C. plurivorum	China	*Arundina graminifolia*	CORCX9	HM585398		HM585423
C. plurivorum	China	*Camellia sinensis*	CGMCC 3.17358	KJ955215		KJ955361
C. plurivorum	China	*Capsicum annuum*	LJTJ30*	KP748221		KP823853
C. plurivorum	China	*Capsicum annuum*	LJTJ3	KP748193		KP823850
C. plurivorum	China	*Capsicum annuum*	LJTJ16	KP748207		KP823851
C. plurivorum	China	*Capsicum annuum*	LJTJ22	KP748213		KP823852
C. plurivorum	Japan	*Carica papaya*	MAFF 238697	MG600724		MG600991
C. plurivorum	Vietnam	*Coffea sp.*	CBS 125473	MG600717		MG600984
C. plurivorum	China	*Cymbidium hookerianum*	CORCG2	HM585397		HM585422
C. plurivorum	Japan	*Glycine max*	MAFF 238875	MG600725		MG600992
C. plurivorum	Brazil	*Glycine max.*	LFN0008	KT696336		KT696282
C. plurivorum	Brazil	*Gossypium sp.*	CBS 132443	MG600719		MG600986
C. plurivorum	Brazil	*Gossypium sp.*	CBS 132444	MG600720		MG600987
C. plurivorum	Japan	*Lycopersicon esculentum*	MAFF 306007	MG600728		MG600995
C. plurivorum	Brazil	*Mangifera indica*	CMM 3742	KC702980		KC992327
C. plurivorum	Brazil	*Mangifera indica*	CMM 3746	KC702981		KC992328
C. plurivorum	Japan	*Musa sp.*	MAFF 305790	MG600726		MG600993
C. plurivorum	Japan	*Oncidium sp.*	MAFF 238315	MG600729		MG600996

C. plurivorum	Japan	*Passiflora edulis*	MAFF 305974	MG600731	MG600998
C. plurivorum	Iran	*Phaseolus vulgaris*	UTFC 261	MG600722	MG600989
C. plurivorum	Iran	*Spathiphyllum wallisii*	UTFC 260	MG600723	MG600990
C. sojae	Serbia	*Glycine max*	CBS 182.81	MG600754	MG601021
C. sojae	USA	*Glycine max*	ATCC 62257 T	MG600749	MG601016
C. vittalense	India	*Calamus thwaitesii*	GUFCC 15503	JN390935	KC790892
C. vittalense	India	*Theobroma cacao*	CBS 181.82 T	MG600734	MG601001
C. lindemuthianum	United Kingdom	*Phaseolus vulgaris*	CBS15128 T	GU227800	GU228094
C. anthrisci	the Netherlands	*Anthriscus sylvestris*	CBS125334 T	GU227845	GU228139
C. anthrisci	Chile	*Persea american*	TR5	MN203633	MN207160
C. boninense	Japan	*Crinum asiaticum* var. *sinicum*	MAFF 305972 T	AB051400	
C. boninense	Japan	*Crinum asiaticum* var. *sinicum*	CBS 123756	AB051403	JQ005589
C. chlorophyti	India	*Chlorophytum sp.*	IMI103806 T	GU227894	GU228188
C. chlorophyti	Australia	*Stylosanthes hamata*	CBS 142.79	GU227895	GU228189
C. circinans	Serbia	*Allium cepa*	CBS22181 T	GU227855	GU228149
C. circinans	USA	*Allium cepa*	CBS 123.25	GU227856	GU228150
C. cocodes	Netherlands	*Solanum tuberoum*	CBS36975 T	HM171679	JX546873
C. cocodes	China	*Capsicum annuum*	NJ23	KY995384	KY995488
C. curcumae	India	*Curcuma longa*	IMI288937 T	GU227893	GU228187
C. curcumae	China	*Curcuma wenyujin*	TJ0709	MF278791	MF278795
C. dematium	France	*Eryngium campestre*	CBS12525 T	GU227819	GU228113
C. dematium		*Xanthium* sp.	CBS:125346	MH863542	GU228118
C. fructi	United States	*Malus sylvestris*	CBS34637 T	GU227844	GU228138
C. gloeosporioides	Italy	*Citrus sinensis*	CBS 112999 T	JQ005152	JQ005587
C. gloeosporioides	Italy	*Citrus sinensis*	IMI 356878	EU371022	JX010445
C. hippeastri	China	*Hippeastrum vittatum*	CBS 125376 T	NR_137082	JQ005665
C. lineola	Czech Republic	*Apiaceae sp.*	CBS125337 T	GU227829	GU228123
C. lineola	China	*Panax ginseng*	TYJ7301-7	MN685238	MN894874
C. liriopes	Mexico	*Lirope muscari*	CBS119444 T	GU227804	GU228098
C. liriopes	China	*Capsicum annuum*	LJLP03	KY995386	KY995486
C. phaseolorum	Japan	*Phaseolus radiatu*	CBS15736 T	GU227896	GU228190
C. phaseolorum	Japan	*Vigna sinensis*	CBS 158.36	GU227897	GU228191
C. rusci	Italy	*Ruscuss* sp.	CBS119206 T	GU227818	GU228112
C. spaethianum	Germany	*Hosta sieboldiana*	CBS16749 T	GU227807	GU228101
C. spaethianum	China	*Polygonatum sibiricum*	CLHJY4-3	MH453905	MH456884
C. truncatum	United States	*Phaseolus lunatus*	CBS15135 T	GU227862	GU228156
C. truncatum	Cuba	*Homo sapiens*	CBS 134304	KX355743	KX355747
C. truncatum	United States	*Glycine max*	CBS 182525	GU227866	GU228160
C. truncatum	Brazil	*Phaseolus lunatus*	CBS 71070	GU227864	GU228158

C. truncatum	Burkina Faso	*Vigna unguiculata*	CBS50697	GU227871		GU228165
C. truncatum	Brazil	*Cyperus rotundus*	CBS 71170	GU227892		GU228186
C. verruculosum	Zimbabwe	*Crotalaria juncea*	IMI45525 T	GU227806		GU228100
D. aspalathi	South Africa	*Aspalathus linearis*	CBS 117169 T	KC343036	KC343762	KC344004
D. aspalathi	South Africa	*Aspalathus linearis*	CBS 117168	KC343035	KC343761	KC344003
D. aspalathi	South Africa	*Aspalathus linearis*	CBS 117500	KC343037	KC343763	KC344005
D. batatas	USA	*Ipomoea batatas*	CBS 122.21 T	KC343040	KC343766	KC344008
D. caulivora	Croatia	*Glycine max*	CBS 127268 T	KC343045	KC343771	KC344013
D. caulivora	Canada	*Glycine soja*	CBS 178.55	KC343046	KC343772	KC344014
D. corylina	China	*Corylus sp.*	CBS 121124 T	KC343004	KC343730	KC343972
D. crotalariae	USA	*Crotalaria spectabilis*	CBS 162.33 T	KC343056	KC343782	KC344024
D. eres	Germany	*Ulmus sp.*	AR5193 T	KJ210529	KJ210550	KJ420799
D. eres	USA	*Ulmus sp.*	CBS 138598	KJ210521	KJ210545	KJ420787
D. longicolla	USA	*Glycine max*	ATCC 60325 T	KJ590728	KJ590767	KJ610883
D. longicolla	USA	*Glycine max*	FAU644	KJ590730	KJ590769	KJ610885
D. melonis	Indonesia	*Glycine soja*	CBS 507.78 T	KC343141	KC343867	KC344109
D. melonis	USA	*Cucumis melo*	FAU640	KJ590702	KJ590741	KJ610858
D. miriciae	Australia	*Helianthus annuus*	BRIP 54736j T	KJ197282	KJ197244	KJ197262
D. miriciae	Australia	*Glycine max*	BRIP 55662c	KJ197283	KJ197245	KJ197263
D. passifloricola	Malaysia	*Passiflora foetida*	CBS 141329 T	KX228292		KX228387
D. phaseolorum	USA	*Aster exilis*	CBS 116020	KC343176	KC343902	KC344144
D. phaseolorum	USA	*Phaseolus vulgaris*	AR4203 T	KJ590738	KJ590739	KJ610893
D. sojae	USA	*Glycine max*	FAU635 T	KJ590719	KJ590762	KJ610875
D. sojae	USA	*Caperonia palustris*	CBS 116019	KC343175	KC343901	KC344143
D. tectoendophyta	Thailand	*Tectona grandis*	MFLUCC 13–0471 T	KU712439	KU749367	KU743986
D. thunbergiicola	Thailand	*Thunbergia laurifolia*	MFLUCC 12–0033 T	KP715097	KP715098	
D. ueckerae	USA	*Cucumis melo*	FAU656 T	KJ590726	KJ590747	KJ610881
D. ueckerae	Brazil	*Glycine max*	LGMF947	KC343203	KC343929	KC344171
D. unshiuensis	China	*Citrus* sp.	ZJUD52 T	KJ490587	KJ490466	KJ490408
D. unshiuensis	China	*Fortunella margarita*	ZJUD50	KJ490585	KJ490464	KJ490406
D. woodii	Western Australia	*Lupinus* sp.	CBS 558.93	KC343244	KC343970	KC344212
Diaporthe citri	USA	*Citrus* sp.	CBS 135422 T	KC843311	KC843071	KC843187
Diaporthe citri	USA	*Citrus* sp.	AR4469	KC843321	KC843081	KC843197
Leptosphaerulina americana	USA	*Trifolium pratense*	CBS 213.55	GU237799		
L. arachidicola	—	*Arachis hypogaea*	CBS 275.59	GU237820		
L. australis	Indonesia	*Eugenia aromatica*	CBS 317.83	GU237829		
L. briosiana	The Netherlands	*Medicago sativa*	CBS 533.66 T	EU167575		
L. briosiana	USA	*Medicago sativa*	CBS 301.54	MN973594		

L. chartarum	South Africa	*Galenia procumbens*	CBS 329.86 T	MN973604
L. gaeumannii	—	—	CBS 233.53	MN972987
L. gaeumannii	Ecuador	Soil	CBS 100575	MN972985
L. gaeumannii	Switzerland	Lawn	CBS 311.51 T	MN973601
L. obtusispora	The Netherlands	Soil	CBS 939.69	GU237911
L. obtusispora	The Netherlands	*Lonicera periclymenum*	CBS 569.94 T	MN973602
L. saccharicola	The Netherlands	Lawn	CBS 234.58	MN973603
L. saccharicola	—	*Agrostis* sp.	CBS 298.54	MN972981
L. saccharicola	Kenya	*Protea* sp.	CBS 116307	MN972983
L. saccharicola	—	*Rosa* sp.	CBS 297.54	MN972984
L. saccharicola	—	Soil and air contaminant	CBS 299.54	MN972986
L. saccharicola	USA	*Lycopersicon* sp.	CBS 138146	MN972982
L. saccharicola	—	Soil and air contaminant	CBS 300.54	MN973600
L. sisyrinchiicola	Thailand	*Saccharum officinarum*	ICMP 19875 T	KF670717
L. trifolii	New Zealand	*Sisyrinchium* sp.	CBS 121688 T	MN973605
L. trifolii	The Netherlands	*Trifolium s*p.	CBS 235.58	GU237806
L. trifolii	USA	*Trifolium repens*	CBS 215.55	MN973598
Neodidymelliopsis moricola	Russia	*Morus alba*	MFLUCC 17-1063 T	KY684939
Phoma herbarum	USA	*Polytrichum juniperinum*	CBS 127589 T	KT389539
P. herbarum	The Netherlands	*Rosa multiflora*	CBS 615.75	FJ427022
Phomatodes aubrietiae	The Netherlands	*Aubrietia* sp.	CBS 627.97 T	GU237895
P. aubrietiae	The Netherlands	*Aubrietia hybrida*	CBS 383.67	GU237854
Ascochyta clinopodiicola	Italy	*Clinopodium nepeta*	MFLUCC 18-0344 T	MH017431
A. phacae	Switzerland	*Phaca alpina*	CBS 184.55 T	KT389475
Chaetocalathus liliputianus	Puerto Rico		MCA 485	AY916682
Chaetocalathus sp.	Ecuador		MCA 2538	AY916686
Crinipellis scabella			CBS 243.53 T	MH857177
C. pallidipilus	Republic of Korea		BRNM 751595 T	KF380833
Marasmius sp.	Guyana		MCA 1708	AY916720
Marasmius sp.	Cameroon		MCA 7492	MG717368
Marasmius rotula	USA		PBM2563	DQ182506
Moniliophthora aurantiaca	American Samoa	woody debris	UTC253824T	JN692482
M. brasiliensis	Brazil	*Heteropterys acutifolia*	UB2053	AY317137
M. canescens	Malaysia	dead fallen twig of broad-leaved tree	DED 7518	FJ167668
M. mayarum	Belize	Dead tree root, possibly *Ceiba pentandra*	DJL BZ511T	MT162718
M. perniciosa	Guyana	*Theobroma cacao*	CBS 193.77	MH861049
M.a perniciosa	Ecuador	*Theobroma cacao*	MCA 2520	AY916743
M. roreri	Mexico	*Theobroma cacao*	MCA 2953	DQ222925
M. roreri	Belize	*Theobroma cacao*	MCA 2954	DQ222927
Moniliophthora sp.	USA	*Bouteloua eriopoda*	MCA 2500	AY916754

Moniliophthora sp.	USA	*Bouteloua eriopoda*	MCA 2501	MT162719
M. ticoi	Bolivia	*Myrcianthes pungens*	NY00511157T	MT162721
M. ticoi	Argentina	*Pogonopus tubulosus*	Niveiro 2249	MT162720
M. conchata	Korea	Fallen twigs of a liana	BRNM 751596 T	KF380834
Moniliophthora sp.	Papua New Guinea	Twigs	n97404	FJ167639
Tetrapyrgos nigripes	USA		MCA 6925	MG717370