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Morphological and molecular characterization of *Fusarium* spp pathogenic to pecan tree in Brazil

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ABSTRACT. The occurrence of *Fusarium* spp associated with pecan tree (*Carya illinoinensis*) diseases in Brazil has been observed in recent laboratory analyses in Rio Grande do Sul State. Thus, in this study, we i) obtained *Fusarium* isolates from plants with disease symptoms; ii) tested the pathogenicity of these *Fusarium* isolates to pecan; iii) characterized and grouped *Fusarium* isolates that were pathogenic to the pecan tree based on morphological characteristics; iv) identified *Fusarium* spp to the species complex level through *TEF-1*α sequencing; and v) compared the identification methods used in the study. Fifteen isolates collected from the inflorescences, roots, and

seeds of symptomatic plants (leaf necrosis or root rot) were used for pathogenicity tests. Morphological characterization was conducted using only pathogenic isolates, for a total of 11 isolates, based on the mycelial growth rate, sporulation, colony pigmentation, and conidial length and width variables. Pathogenic isolates were grouped based on morphological characteristics, and molecular characterization was performed by sequencing *TEF-1*α genes. Pathogenic isolates belonging to the *Fusarium chlamydosporum* species complex, *Fusarium graminearum* species complex, *Fusarium proliferatum*, and *Fusarium oxysporum* were identified based on the *TEF-1*α region. Morphological characteristics were used to effectively differentiate isolates and group the isolates according to genetic similarity, particularly conidial width, which emerged as a key morphological descriptor in this study.

Key words: Pathogenicity; Morphological characters; *TEF-1*α; Unweighted pair group method with arithmetic mean

INTRODUCTION

The pecan tree [*Carya illinoinensis* (Wangenh.) K. Koch] belongs to the botanical family Juglandaceae and includes the genera *Carya* and *Juglans*, which are best known in Southern Brazil for their production of edible nuts and valuable timber (Marchiori, 1997). In Southern Brazil, the area dedicated to pecan production has increased, particularly because this species produces nuts between 5 and 6 years of age. This growth has been accompanied by an increased incidence of diseases caused by fungi, bacteria, or other microorganisms, which are not well understood. Therefore, many of the pathogens causing disease in pecan trees in Brazil have not been identified and reported.

Some fungi, such as those causing scab disease (*Cladosporium caryigenum*), which is the main disease that attacks young tissues, anthracnose (*Colletotrichum gloesporioides*), which causes depressed lesions in fruits, mold (*Cephalothecium roseum*), powdery mildew (*Microsphaera penicillata*), phytophthora rot (*Phytophthora cactorum*), and leaf blight, whose etiology remains unclear, have been described by Ortiz and Camargo (2005).

A new type of rot root disease has been identified in Rio Grande do Sul State of Brazil, which is associated to *Fusarium* genus fungi. This disease particularly attacks seedlings in the nursery by causing wilt and foliar necrosis associated with root rot, but also occurs in the field where plants exhibit reduced development. The intensity of attack in the nursery is approximately 10%; in severe cases, seedling death occurs.

According to Taylor et al. (2000), the most effective method for identifying fungi is based on the phylogenetic species concept via phylogenetic analysis of variable characteristics of nucleic acids. However, Leslie and Summerell (2006) reported that although DNA sequences are the most frequently used characteristics for identifying and delimiting phylogenetic species, other sufficiently informative markers, including morphological characteristics, can be used to delineate phylogenetic species.

The region most commonly used for the molecular identification of fungi is the internal transcribed spacer (ITS) in the ribosomal RNA gene structure, which is divided into the 18S, 5.8S, and 28S regions, in addition to *ITS1* and *ITS2*. Waalwijk et al. (1996) found

sequencing the *ITS* region is effective for identifying some species of *Fusarium*, but that the presence of 2 non-homologous copies within the *ITS2* region causes divergent results. The translation elongation factor 1α (*TEF-1*α) region is highly conserved, involved in translation processes, and has recently been widely examined in intra- and inter-specific variation studies and phylogenetic analyses of a wide variety of eukaryotic groups, including fungi from the *Fusarium* genus (O'Donnell et al., 1998).

The aims of this study were the following: i) obtain *Fusarium* isolates from plants with symptoms of disease; ii) test the pathogenicity of these *Fusarium* isolates to pecan; iii) characterize and group *Fusarium* isolates that are pathogenic to the pecan tree based on morphological characteristics; iv) identify *Fusarium* spp to the species complex level through *TEF-1*α sequencing; and v) compare the identification methods used in the study.

MATERIAL AND METHODS

Preparation and purification of *Fusarium* spp isolates

Symptomatic samples were collected from pecan orchards in different municipalities of the State of Rio Grande do Sul (RS), Brazil. The intensity of attack of the disease was approximately 10% in the nursery. All collection points were georeferenced using a global positioning system as shown in Table 1.

Table 1. Coordinates of the collection locations of *Fusarium* spp isolates derived from pecan tree orchards in RS.

Municipality	Date	Coordinates (DMS)	Altitude (m)
Cachoeira do Sul	November 2010	29°59'45"S-52°55'22"W	114
Mata	December 2010	29°31'3"S-48°27'39"W	208
Anta Gorda	January 2011	28°53'54.7"S-52°01'59.9"W	514
Santa Maria	November 2010	29°43'13.0"S-53°43'1.9"W	88

DMS = degrees minutes and seconds coordinates.

Isolates were collected from municipalities in the State of Rio Grande do Sul, Brazil from October 2010 to August 2011 and were derived from different plant components presenting symptoms such as leaf necrosis, shoot wilt, and root rot. F_1, F_6, F_7, F_8, and F_{15} were isolated from inflorescences in the municipality of Santa Maria; F_3 and F_{14} were isolated from inflorescences in the municipality of Anta Gorda; F_5 was obtained from roots in the municipality of Cachoeira do Sul; F_9 and F_{11} were isolated from roots in Mata; F_{17} was obtained from roots in Santa Maria; and F_{19}, F_{20}, F_{21}, and F_{23} were isolated from seeds in the municipality of Anta Gorda. All samples were collected and stored in paper bags until being taken to the laboratory for pathogen analysis and identification.

Plant materials (inflorescences, roots, and seeds) collected in the field were placed in a humid chamber for 3 days for subsequent identification. After pathogen detection, plant tissues were surface-sterilized and the pathogen structures were transferred to Petri dishes containing potato dextrose agar (PDA) culture media using a histology needle as described by Alfenas and Maffia (2007). After 10 days of growth, the cultured isolates were purified by single-conidial purification and stored on Synthetischer Nährstoffarmer agar (SNA) culture media for subsequent use.

Pathogenicity test

The following procedures were performed for all the isolates collected, for a total of 15 isolates. a) Inoculation of *Fusarium* spp in maize kernels: maize kernels were soaked in water and incubated at room temperature for 8 h, after which excess water was discarded and approximately 80 g kernels were placed in 100-mL glass flasks to be autoclaved twice for 40 min at an interval of 24 h. After cooling, 5 5-mm-diameter PDA culture medium discs containing pathogen mycelia were transferred to each vial. The flasks were incubated at 24°C for 14 days with 12-h light/dark cycles according to a method adapted from Klingelfuss et al. (2007). Five PDA culture medium discs not containing pathogens were used as controls. b) Inoculation: plastic trays (approximate capacity 7 L) with holes in the bottom were filled approximately halfway with commercial substrate (composition: *Sphagnum* peat, expanded vermiculite, dolomitic limestone, agricultural gypsum, and NPK fertilizer; 101 kg/m³ density). Next, 80 cm³ inoculated maize kernels was mixed with the substrate, which was then moistened with sterile distilled water. Subsequently, 20 seeds derived from pecan tree seedlings produced by seeds (without cultivar identification), divided into 4 sets of replicates, were sown in each treatment substrate. The seeds were manually sanded at the opposite end from the embryo to overcome dormancy. The trays were maintained in a temperature-controlled room at 25°C ± 2°C with a 12-h photoperiod for 90 days, after which the percentage of disease incidence (presence of symptoms like leaf necrosis, choke disease, root rot) was assessed for each isolate, with subsequent pathogen re-isolation on PDA and carnation leaf agar (CLA) media toward fulfilling Koch's postulates. All isolates were replicated on Synthetischer Nährstoffarmer agar (SNA) media for storage. The next procedures were conducted using only the isolates that had been identified as pathogenic to pecan.

Morphological characterization of pathogenic isolates

Morphological characterization was done only with the 11 *Fusarium* spp isolates identified as being pathogenic to the pecan tree. The procedures were based on the method described by Michereff et al. (2003), wherein each isolate was evaluated using the following 4 variables. Mycelial growth rate was assessed by transferring 6-mm-diameter PDA medium discs derived from 7-day-old colonies into other PDA medium plates, including 4 replicates for each isolate, and assessing colony growth daily until the 7th day of incubation by averaging the colony's diameter in diametrically opposite directions. Sporulation was evaluated at day 10 of incubation by adding 20 mL sterile distilled water to each plate used for evaluating mycelial growth, scraping the colonies and sieving through a double layer of gauze to estimate the conidial concentration (conidia/mL) using a Neubauer chamber. Colony pigmentation was based on observation and the colony and aerial mycelium pigmentation was recorded. The colors were defined as described by Nelson et al. (1983). Conidial length and width were assessed from a sample from a 2-mL fungal conidia suspension in sterile distilled water, which was pipetted onto a microscope slide. Conidia were measured using a light microscope with a micrometer at 40X magnification. Thirty conidia were measured for each isolate. Isolates were also transferred onto CLA media (Nelson et al., 1983) to detect the presence of chlamydospores (Leslie and Summerell, 2006) in case they were present but had not been observed on the PDA media.

Molecular characterization

This procedure was also conducted using only the 11 pathogenic *Fusarium* spp isolates. Pathogen DNA extraction was performed according to the cetyltrimethylammonium bromide method described by Doyle and Doyle (1990). Extracted genomic DNA samples were analyzed by polymerase chain reaction (PCR) to amplify the *TEF-1*α region using the EF1-T (ATGGGTAAGGARGACAAGAC) and EF1-1567R (ACHGTRCCRATACCACCRATCTT) primers (Rehner and Buckley, 2005).

The reaction contained approximately 30 ng DNA, 10X buffer, 2.5 μM each dNTP, 20 nM $MgCl_2$, 25 pmol each primer, and 5 U *Taq* polymerase enzyme; the reaction mixture was brought to volume using ultrapure water. Reactions were performed in an MJ Research, Inc. PTC - 100 MT thermocycler (Quebec, Canada) under the following thermal conditions: 94°C for 2 min; 30 cycles at 94°C for 45 s, 55°C for 30 s, and 72°C for 35 s; and 72°C for 10 min. PCR products were stored at 4°C upon completion of the reaction. A negative control without DNA was included in the PCR amplifications. Amplified fragments and controls were separated by 1.2% agarose gel electrophoresis in 1X TBE buffer (10.8 g Tris base, 5.5 g boric acid, 4 mL 0.5 M ethylenediaminetetraacetic acid, and 4 mL distilled water) containing ethidium bromide and bands were visualized under ultraviolet light.

The PCR products were purified using 13% PEG 8000 and the sequences generated were 900-1200 base pairs. Sequencing was performed on a Mega BACE 500 Sequencer (Amersham Biosciences; Amersham, UK). Sequenced fragments were analyzed using the BioEdit software (Hall, 1999). Nucleotide sequences were compared with those previously deposited in GenBank regarding the pathogens isolated. The GenBank sequences with the highest scores (more than 98% of similarity and coverage) were selected and aligned with sequences determined by sequencing using the ClustalW algorithm, and phylogenetic analysis was conducted using the neighbor-joining statistical method with 1000 replicates on the MEGA software version 4 (Tamura et al., 2007). The model was selected using the FindModel software, which establishes the best model based on a file with the sequences chosen aligned using BioEdit (Hall, 1999). The similarity of nucleotide sequences between isolates was calculated using the BLAST procedure (http://blast.ncbi.nlm.nih.gov). All isolate sequences were submitted to GenBank and the accession codes are shown on the dendrograms.

Data analysis

Quantitative data regarding morphological characterization were submitted to a multivariate analysis technique for cluster analysis of the isolates. These analyses were performed using the GENES software (version 2009.7.0). The array of standardized Euclidean distances (D2) was calculated as a dissimilarity measure and used to cluster the isolates according to the unweighted pair group method with arithmetic mean (UPGMA) method (Cruz, 2008).

The sequence obtained for each isolate was aligned with the sequences available in the database using BLAST, and the sequences with the highest coverage (higher than 98%) and degree of similarity (higher than 98%) were selected for each isolate. Subsequently, sequences chosen using the software were aligned with isolate sequences using the BioEdit software (Hall, 1999).

RESULTS

The *Fusarium* spp isolates F_1, F_3, F_5, F_6, F_7, F_8, F_9, F_{11}, F_{17}, F_{19}, and F_{20} were pathogenic to pecan seedlings, causing the following symptoms: choke disease (F_1, F_3, F_5, F_7, F_8, F_9, and F_{17} isolates), leaf necrosis associated with root rot (F_1, F_6, F_7, F_9, F_{11}, F_{19}, and F_{20} isolates), shoot wilt associated with root rot (F_1, F_3, F_5, F_7, F_8, F_9, and F_{17} isolates), and even plant death, which was diagnosed upon inoculation with F_{11}. Some isolates also produced pre-emergence damping-off (F_7 and F_9). Symptoms appeared approximately 60 days after emergence (Figure 1). Isolates F_{14}, F_{15}, F_{21}, and F_{23} produced no symptoms in seedlings and were therefore considered to be non-pathogenic.

Figure 1. Symptoms obtained by inoculation of *Fusarium* spp on *Carya illinoinensis*. Choke disease (**A**); root rot (**B**); leaf necrosis associated with root rot (**C**); damping-off (**D**).

Regarding the daily mycelial growth rate (MGR), F_3, F_{19}, and F_{20} were the fastest-growing isolates and reached confluence within the plate's maximum diameter (90 mm) within 7 days, whereas isolate F_{17} exhibited the lowest MGR (Table 2). Some isolates appeared to cluster when taking into account their MGRs; these isolates included F_8, F_9, and F_{11}, which exhibited very close MGR values, as well as F_1, F_5, and F_7. These results indicate the usefulness of this variable for differentiating and grouping the isolates. Regarding colony pigmentation, 5 different combinations of colony pigmentation and 4 different combinations of aerial mycelium color were observed, thus effectively differentiating colony characteristics because these colors are easily visualized.

Table 2. Characteristics of the colonies and macroconidia of *Fusarium* spp. pathogenic to *Carya illinoinensis* grown on PDA media.

Isolates	Diameter	MGR	Sporulation (x10^6	Macroconidia[1]			Colony pigmentation on PDA[1]		Chlamydospore[2,3]
	(mm)	(mm/day)	spores/mL)[1]	Length (µm)	Width (µm)	No. of septa	Colony	Aerial mycelium	
F_1	90.00	13.13	2.87	46.25 (43.0-49.5)	3.26 (3.05-3.4)	5	Violet	Light pink	+
F_3	90.00	18.57	-	-	-	-	Violet	Dark yellow	-
F_5	90.00	13.08	2.64	47.00 (44.5-49.5)	3.20 (2.95-3.4)	5	Violet	Light pink	+
F_6	87.70	12.53	1.46	45.25 (43.25-47.25)	3.18 (3.05-3.3)	4	Violet	Light pink	+
F_7	90.00	13.11	2.94	46.50 (43.0-50.0)	3.21 (3.08-3.4)	5	Violet	Light pink	+
F_8	56.79	8.11	2.60	27.15 (24.2-30.1)	2.88 (2.6-2.95)	3 or 5	Violet	Light pink	-
F_9	60.15	8.59	2.63	27.30 (25.5-29.1)	2.75 (2.55-2.9)	3 or 5	Violet	Light pink	-
F_{11}	60.66	8.67	2.70	28.90 (26.8-31.0)	2.62 (2.5-2.75)	3 or 5	Violet	Light pink	-
F_{17}	50.12	7.16	1.20	30.85 (28.9-32.8)	3.38 (3.25-3.5)	3-4	Light pink	White	+
F_{19}	90.00	18.70	-	-	-	-	Violet	Dark yellow	-
F_{20}	90.00	15.59	-	-	-	-	Violet	Dark yellow	-

90 mm is the maximum dimension of the plate; Diameter = colony diameter at day 7; MGR = daily mycelial growth rate (mm/day) calculated using the colony diameter at the final assessment/day of final assessment. [1]Potato-dextrose-agar culture medium, [2]Carnation leaf agar medium, [3]Presence (+) and absence (-). Values in parentheses of macroconidia length and width represent the minimum and maximum value observed, respectively.

Sporulation ranged from 1.20 x 10^6 (F_{17}) to 2.94 x 10^6 spores/mL (F_7), the dimensions of macroconidia ranged in length from 27.13 (F_8) to 47.0 µm (F_5) and in width from 2.62 (F_{11}) to 3.38 µm (F_{17}), and the number of septa ranged from 3 to 5 (Table 2). The F_3, F_{19}, and F_{20} isolates exhibited no sporulation (macroconidia) on the media tested, which is a key characteristic used for grouping isolates. The formation of chlamydospores, noted in isolates F_1, F_5, F_6, F_7, and F_{17}, is common in some species of *Fusarium*, including *Fusarium chlamydosporum* Wollenweber & Reinking and *Fusarium oxysporum* Schlechtendahl emend. Snyder & Hansen (Gerlach and Nirenberg, 1982).

We established whether differences in morphological characteristics (colony diameter, sporulation, macroconidial width, and length) represented valid characters for grouping isolates by using the dendrogram generated via the UPGMA clustering method (Figure 2A). The formation of 3 clusters was noted: the first was formed by isolates F_1, F_7, F_5, and F_6; the second was formed by F_9, F_{11}, and F_8; and the third was formed by F_{17} alone. Isolates F_3, F_{19}, and F_{20} were not included in this dendrogram because they exhibited no sporulation and only the colony diameter parameter was available for assessment. The lowest dissimilarities were observed between isolates F_1 and F_7, followed by F_9 and F_{11}, and clustering between these pairs of isolates showed dissimilarities below 10%.

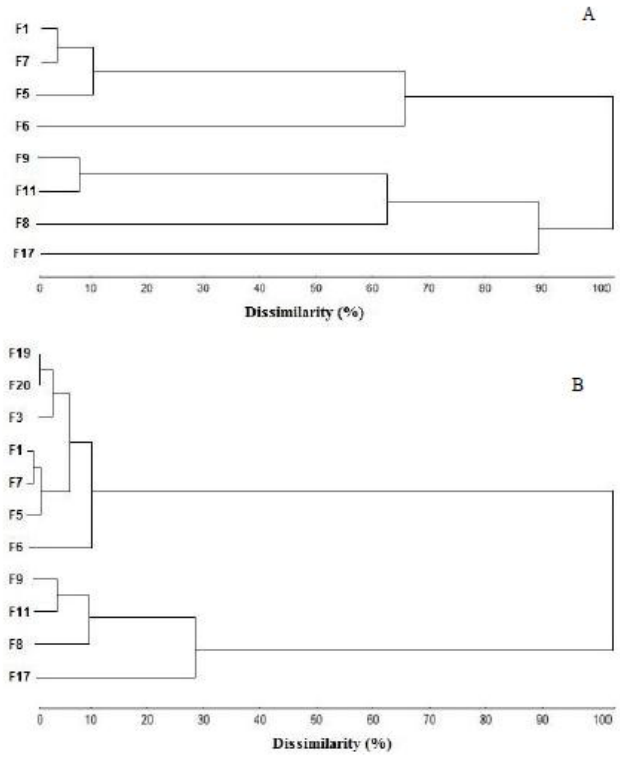

Figure 2. Dendrogram showing the percentage of dissimilarity between *Fusarium* spp isolates pathogenic to *Carya illinoinensis*. The dendrogram was designed using the unweighted pair group with arithmetic mean method based on a Euclidean distance matrix analysis using the 4 morphological characteristics evaluated: colony diameter, sporulation, and conidial length and width (**A**) as well as using only colony diameter (**B**).

Clustering analysis was performed using only the colony diameter variable to include isolates that did not exhibit sporulation (F_3, F_{19}, and F_{20}), as shown in Figure 2B. Based on colony diameter, the isolates included were grouped on the same branch with a dissimilarity below 10%. F_{19} and F_{20} were considered to be identical because the dissimilarity was null. Multivariate analysis related to Figure 2A also provided the relative contributions of each of the characteristics to divergence; conidial width was responsible for 99.98% of divergence. This result indicates that the differentiation of the *Fusarium* spp isolates into groups formed in the dendrogram was due nearly entirely to the conidial width characteristic.

Figure 3 shows the phylogenetic dendrogram constructed based on the *TEF-1α* sequences, which should be more confinable for identification of *Fusarium* species or species complex compared to other gene regions. The F_{17} isolate was in the first branch of the dendrogram grouped with the *F. oxysporum* sequences. This isolate also showed greater dissimilarity from other isolates when morphological characteristics were used for clustering (Figure 2A and B). Therefore, molecular identification confirmed that F_{17} was different at the species level from the other isolates.

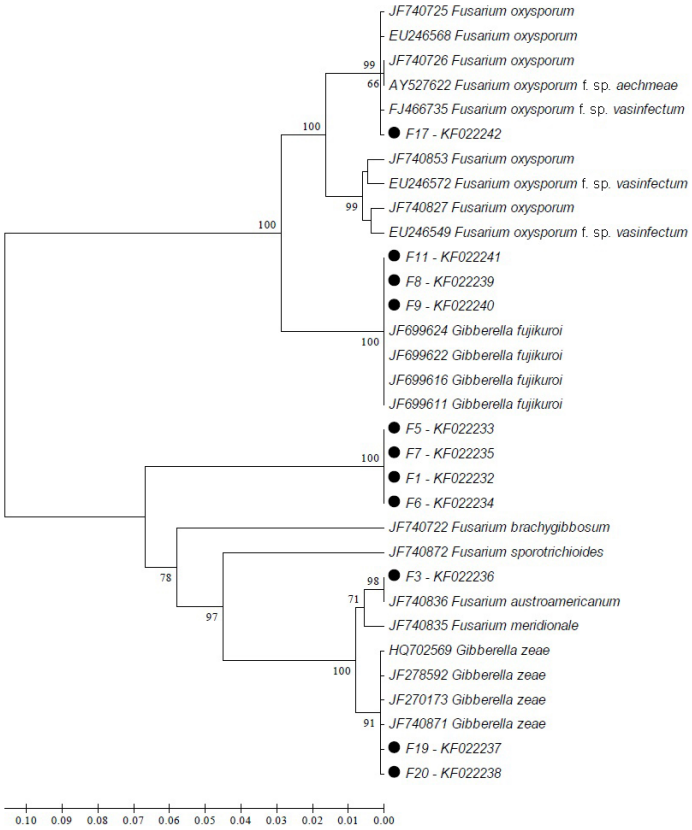

Figure 3. Phylogenetic dendrogram of the *Fusarium* spp isolates that were pathogenic to *Carya illinoinensis*, derived from sequences of the *TEF-1α* gene according to the neighbor-joining statistical method and based on 1000 bootstrap replicates. Evolutionary distances were calculated using the Jukes-Cantor model. The numbers associated with the branches represent the bootstrap numbers.

The F_8, F_9, and F_{11} isolates appeared in the second branch of the dendrogram grouped with the *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura (anamorph: *F. fujikuroi* Nirenberg) sequences, known as the *G. fujikuroi* species complex, to which *Fusarium proliferatum* also belongs (Gerlach and Nirenberg, 1982) (Figure 3). F_1, F_5, F_6, and F_7 were again clustered in the same clade, as occurred with clusters based on morphological characteristics, in combination with sequences of *F. brachygibbosum* Padwick and *Fusarium sporotrichioides* Sherbakoff, which belong to the same species complex as *F. chlamydosporum*. Thus, for these 4 isolates, identification was achieved at the species complex level. The lack of other accessions of the *F. chlamydosporum* species in the dendrogram resulted from the limited number of sequences deposited in GenBank for this region and for other species of *Fusarium*.

Finally, F_{19} and F_{20} were grouped with sequences of *Gibberella zeae.* These isolates (F_{19} and F_{20}) were closer to one another, indicating greater genetic proximity, which can be explained by their origin of collection. In contrast, F_3 was grouped into the same branch as *Fusarium austroamericanum* T. Aoki, Kistler, Geiser & O'Donnell when using the *TEF-1*α region. Isolate F_3 is likely *F. austroamericanum*, but sequence data from another informative locus would be desirable for a definitive identification. Therefore, sequencing of the *TEF-1*α region raised doubts regarding the identification of F_3 at the species level, supporting that this isolate belongs to the *F. graminearum* species complex.

DISCUSSION

Angelotti et al. (2006) performed morphological characterization of *F. graminearum* isolates associated with *Fusarium* head blight of wheat and triticale in the southern region of Brazil using some of the characteristics that were used in this study, including culture pigmentation on PDA medium, the dimensions of macroconidia on CLA medium, the number of macroconidial septa, and the presence of chlamydospores. The authors used these characteristics and molecular techniques as well as PCR, and they identified 20 of the isolates collected as *F. graminearum*. In taxonomic keys, including those developed by Gerlach and Nirenberg (1982) and Nelson et al. (1983), these same characteristics were used to identify *Fusarium* species. Leslie and Summerell (2006) highlighted some species of *Fusarium* that rarely produce macroconidia, including one species, *F. musarum* Logrieco & Marasas, in which macroconidia are entirely absent, which may be an important characteristic of cluster isolates. Kristensen et al. (2005) suggested that phylogenetic analyses of *Fusarium* spp should be developed based on isolates that are well-characterized morphologically and, preferably, accompanied by specific descriptions, including host, geographic origin, secondary metabolites produced, and pathogenicity. Leslie and Summerell (2006) advocate the use of the morphological basis of *Fusarium* spp but recognize the limitations of this approach and the need for improvement and changes.

Rahjoo et al. (2008) used various morphophysiological characteristics to identify 191 isolates of *Fusarium* spp, including the morphology of colonies grown on PDA and the morphology of macroconidia, microconida, conidiogenous cells, and chlamydospores evaluated using cultures grown on Synthetischer Nährstoffarmer Agar and CLA media. The species of 187 isolates were identified using only morphology; however, in the study above, the authors confirmed their identification by sequencing the *TEF-1*α gene. Again, the importance of combining morphological and genetic characters for fungal identification was highlighted.

Regarding clustering by morphological characters, Michereff et al. (2003) also used the UPGMA method based on a Euclidean distance matrix analysis to assess the variability of

38 *Alternaria brassicicola* (Schwein.) Wiltshire isolates, using epidemiological (incubation period, severity, rate of disease progression, and area under the disease progression curve) and physiological (mycelial growth rate, sporulation, and conidial germination) variables in addition to sensitivity to the iprodione fungicide in clustering analysis. Teixeira et al. (2004) used the same technique to group and differentiate 10 *Acremonium strictum* W. Gams isolates obtained from maize seeds (*Zea mays* L.) with relative genetic distances assessed using 9 morphophysiological markers.

Recently, studies involving the identification of fungal isolates have attempted to combine identification by morphological and molecular characteristics. Andrade et al. (2007) conducted a study aimed at characterizing the morphological, cultural, and pathogenic variability of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. samples isolated from the papaya tree (*Carica papaya*). Subsequently, the isolates were molecularly identified by PCR using taxon-specific primers and PCR-restriction fragment length polymorphism analysis of the *ITS* region, combining morphological and molecular techniques.

The combination of sequencing results from different regions or different genes provides more reliable fungal species identification. As observed in the present study, species complexes that were morphologically differentiated were confirmed by *TEF-1*α sequencing.

Therefore, isolates that are pathogenic to the pecan tree were identified as belonging to the *F. chlamydosporum* species complex, *F. graminearum* species complex, *F. proliferatum*, and *F. oxysporum*. These pathogen species have not been previously reported for pecan trees in Brazil. *Fusarium chlamydosporum* can be considered to be a saprophyte (Gerlach and Nirenberg, 1982), although Nahar and Mushtaq (2006) found that *F. chlamydosporum* is associated with sunflower seeds and detected its pathogenicity causing symptoms of wilt and collar rot.

Members of the *F. graminearum* complex can infect wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), and other cereals worldwide, causing the *Fusarium* head blight (Boutigny et al., 2011). Pathogen-infected grains also show contamination with mycotoxins from the group of trichothecenes, including deoxynivalenol and nivalenol (Astolfi et al., 2011). This species complex is very important for the pecan tree, but also may be a source of fungal spread to most other crops grown in the study region, such as rice and cereals.

The *F. proliferatum* species complex members are pathogenic to several crops, are highly toxicogenic, and can produce mycotoxins, including fumonisins, fusaric acid, beauvericin, fusaproliferin, and moniliformin, according to Bottalico (1998). Finally, *F. oxysporum* has been observed worldwide given its intense attacks on several crops, including bananas, tomatoes, beans, potatoes, sugar cane, and passion fruit. This species has been detected in forest species by Krugner et al. (1970), who correlated the symptoms of wilting and apical drying in seedlings of *Pinus elliottii* var. *elliottii*, and in *Pinus taeda* (Grigoletti and Auer, 2006).

Therefore, the morphological characteristics of pathogenic isolates of *Fusarium* spp, particularly conidial width, were important for separating and clustering in this study, indicating the potential differences and similarities at the genetic level, which were confirmed by sequencing the *TEF-1*α region. Sequencing of the region used in this study provided key data with greater reliability for species complex identification and increased the information regarding this species.

In this study, we identified species groups of *Fusarium* in Brazil that are pathogenic to the pecan tree under controlled conditions.

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