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Characterization of *Fusarium graminearum* from *Acacia* and *Eucalyptus* using β-tubulin and histone gene sequences

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Abstract: During routine surveys of diseased *Acacia mearnsii* and *Eucalyptus grandis* in South Africa, isolates of an unknown, nonsporulating fungus with a red mycelium were collected. Symptoms associated with the disease included branch dieback and stem cankers on both hosts. None of the isolates of the fungus produced spores, making identification using morphological characteristics impossible. An attempt was thus made to identify the isolates using DNA sequence data for the β-tubulin and histone genes. Using this approach, the fungus was tentatively identified as *Fusarium graminearum*. Sequences were then compared to those for isolates of *F. graminearum* from cereal hosts. The relative pathogenicity of *F. graminearum* to *A. mearnsii* and an *E. grandis* clone was determined in pathogenicity trials. Pathogenicity tests were conducted by inoculating 18-mo-old *A. mearnsii* trees and 12-mo-old *E. grandis* clone trees under field conditions. All the isolates tested produced significant lesions on both the *A. mearnsii* and *E. grandis* clones. The isolates collected from *A. mearnsii* and *E. grandis* were further compared to other *F. graminearum* isolates using ß-tubulin and histone gene sequence. In these comparisons the isolates collected from *A. mearnsii* and *E. grandis* consistently grouped with *F. graminearum* isolates. The occurrence of *F. graminearum* on *A. mearnsii* and *E. grandis* is intriguing, and as far as we are aware, this is the first report of the fungus associated with a disease of a woody host.

Key Words: black wattle, disease, forestry, South Africa

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INTRODUCTION

The genus *Fusarium* contains many well-known plant pathogens and has a cosmopolitan distribution with a large host range. It is especially known for the diseases it causes on cereals and the fact that many species produce mycotoxins (Nelson et al 1981, 1983, Marasas et al 1984). The best known example of a *Fusarium* disease in the forestry environment is that of pitch canker disease of *Pinus* spp. (Hepting and Roth 1946, Dwinell et al 1981) caused by *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas f.sp. *pini* Correll et al [Correll et al 1992 = *Gibberella fujikuroi* mating population H (Britz et al 1999, Steenkamp et al 1999)], also known by the name *F. circinatum* Nirenberg & O'Donnell (Nirenberg and O'Donnell 1998, Anonymous 1999, O'Donnell et al 2000b).

A number of *Fusarium* spp. have been reported from diseased *A. mearnsii* trees in South Africa and elsewhere (Stephens and Goldschmidt 1938, Zeijlemaker 1968, Bakshi 1976, Lee 1993). Most reports are from nurseries where species such as *F. oxysporum* Schlecht. and *F. solani* (Mart.) Sacc. cause damping-off of young seedlings (Bakshi 1976, Lee 1993, Lenné 1992). *Fusarium* spp. have also been reported from other commercially grown plantation *Acacia* spp. In Malaysia, an unidentified *Fusarium* sp. is reported to be associated with leaf spot and lesions on *A. mangium* Willd. (Lee 1993). In South Africa, an unknown species was isolated from trees with a serious wilt disease in the 1930s (Stephens and Goldschmidt 1938). *Fusarium oxysporum* has also regularly been isolated from trees suffering from a disease known as "black butt" (Zeijlemaker 1968).

South Africa has nearly 1.5 million hectares of plantations of exotic forest trees, of which *Acacia mearnsii* de Wild. encompasses about 7% and *Euca-*

lyptus spp. approximately 40% (Anonymous 1998). Interest in the diseases affecting *A. mearnsii* gained prominence in 1988 with the outbreak of a new wilt disease, known as Ceratocystis wilt (Morris et al 1993). Subsequently, intensive disease surveys have been conducted in an effort to identify all pathogens infecting these trees (Roux and Wingfield 1997). During these surveys, an unidentified and non-sporulating fungus, with a red mycelium in culture, was regularly isolated from stem cankers (Roux and Wingfield 1997). This fungus was also isolated from apparently healthy plant material in a study of the endophytes occurring in non-symptomatic *A. mearnsii* tissue (Roux, unpubl). Based on superficial morphological and cultural characteristics, this fungus was tentatively identified as a species of *Fusarium*.

Research into the fungal diseases affecting plantation *Eucalyptus* spp. has received a high priority for many years. Intensive breeding programs have been established in response to disease outbreaks caused by fungi such as *Cryphonectria cubensis* (Bruner) Hodges (Hodges et al 1986, Wingfield et al 1989, Conradie et al 1990) and *Mycosphaerella* spp. (Doidge et al 1953, Park and Keane 1982, Crous and Wingfield 1995, Crous 1999). Few *Fusarium* diseases of *Eucalyptus* are known, with the most serious problems experienced in India. For example, *F. solani* causes a vascular wilt of *Eucalyptus* spp. through infection of the roots (Kumar and Nath 1988), while *F. oxysporum* causes stem cankers and root rot of young seedlings in Indian nurseries (Sharma et al 1984). During routine analysis of diseased *E. grandis* Hill ex. Maid. material in South Africa a non-sporulating red mycelial fungus, similar to the one isolated from *A. mearnsii* was also collected.

The objective of this study was to identify and test the pathogenicity of the non-sporulating red mycelial fungus that is regularly isolated from *A. mearnsii* and *E. grandis*. Initially we attempted to induce isolates of the fungus to sporulate and to identify it using conventional morphological characters. Subsequently, we attempted its identification by comparing the Internal Transcribed Spacer region (ITS) DNA sequences with those in public domain databases such as GenBank. After identification of the fungus as a *Fusarium* sp., isolates were compared with other *Fusarium* spp. in the GenBank database using ß-tubulin sequence data. Once identified as *F. graminearum*, histone *H3* and ß-tubulin gene sequences were compared with those of other strains of this species, previously isolated from cereals (Aoki and O'Donnell 1999, O'Donnell et al 1998, Steenkamp et al 1999).

MATERIALS AND METHODS

Symptoms and isolation.—The non-sporulating red colored fungus was isolated from stem and branch cankers on *A. mearnsii* growing in plantations and from diseased *E. grandis* seedlings from a commercial forestry nursery. Isolations from *A. mearnsii* were also made from basal cankers associated with black butt disease (Zeijlemaker 1971), blister and mottle lesions associated with Ceratocystis wilt (Morris et al 1993) and mechanical wounds on stems and branches. Isolates from *E. grandis* were collected from diseased seedlings sent to the diagnostic clinic of the Tree Pathology Co-operative Program (TPCP), South Africa. All isolations were made in a similar fashion from the leading edges of lesions or from the surface of lesions onto 2% malt extract agar (MEA) (20 g/L Biolab Malt Extract, 15 g/L Biolab Agar, Merck Pty. Ltd., Halfway House, South Africa). All isolates used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and the culture collection of the South African Medical Research Council (MRC), Cape Town, South Africa (TABLE I). All isolates have been stored under the same conditions. Cultures have also been deposited in PREM [Collection of fungi at the Plant Protection Research Institute (PPRI), Pretoria, South Africa] and the culture collection of the Fusarium Research Center, Pennsylvania State University, University Park, Pennsylvania.

Morphological characteristics.—No fruiting structures were found on any of the material collected from the field or in any of the original MEA grown cultures. Only abundant red mycelium was found growing in the lesions of diseased trees. For this study we examined 10 isolates from *A. mearnsii* and 3 isolates from *E. grandis*. To induce sporulation, isolates were incubated under continuous near UV light, continuous darkness, continuous white light and alternating white/near UV light cycles, as well as at different temperatures and on different growth media. Growth media tested included MEA, PDA (2% potato dextrose agar, Difco Laboratories, Michigan, USA) and water agar to which sterilized *A. mearnsii* twigs or *Eucalyptus* leaves had been added. Isolates were also transferred to carnation leaf agar (CLA) (Nelson et al 1983) in a further attempt to induce sporulation. Isolates were tested for more than a year to induce sporulation and temperature treatments for all techniques ranged from 4 C to 37 C.

DNA isolation and PCR reactions.—In the molecular characterization of the red mycelial fungus, two isolates from *A. mearnsii* (CMW6425, CMW6427), two isolates from *E. grandis* (CMW6496, CMW6424), six isolates of *F. graminearum* Schwabe (Aoki and O'Donnell 1999 = *F. graminearum* group 2 sensu Francis and Burgess 1977) from maize (*Zea mays* L) and wheat (*Triticum astivum* L) and two isolates of *F. pseudograminearum* Aoki & O'Donnell (Aoki and O'Donnell 1999 = *F. graminearum* group 1 sensu Francis and Burgess 1977) from maize and wheat were used (TABLE I). Three isolates of *F. crookwellense* Burgess, Nelson et Toussoun [Nelson et al 1983, also known as *F. cerealis* (Cooke) Sacc. according to Nirenberg 1990] were also included (TABLE I). All isolates were grown on 2% MEA plates at 25 C until these were covered with mycelium. Mycelial tissue was scraped directly from the surface of the agar and transferred to 1.5 mL Eppendorf tubes. DNA was isolated

TABLE I. List of *Fusarium* species used in molecular comparisons and pathogenicity studies

Isolate number[a]	Origin	Host	PREM[b] numbers
Fusarium graminearum			
CMW6496	Seven Oaks, South Africa	*Eucalyptus grandis*	57204
CMW6424	Seven Oaks, South Africa	*Eucalyptus grandis*	57200
CMW963	Seven Oaks, South Africa	*Eucalyptus grandis*	—
CMW6425	Pietermaritzburg, South Africa	*Acacia mearnsii*	57201
CMW4490	Pietermaritzburg, South Africa	*Acacia mearnsii*	—
CMW6427	Pietermaritzburg, South Africa	*Acacia mearnsii*	57203
CMW6426	Pietermaritzburg, South Africa	*Acacia mearnsii*	57202
F. pseudograminearum			
MRC4517	Bethlehem, South Africa	*Triticum aestivum*	57249
MRC4977	Caledon, South Africa	*Triticum aestivum*	
NRRL28334	Swellendam, South Africa	*Medicago truncatula*	
F. graminearum			
MRC4712	Winterton, South Africa	*Triticum aestivum*	57250
MRC4927	Swellendam, South Africa	*Triticum aestivum*	57251
MRC5049	George, South Africa	*Triticum aestivum*	
MRC1106	Lusikisiki, South Africa	*Zea mays*	
MRC1113	Butterworth, South Africa	*Zea mays*	57245
MRC1115	Butterworth, South Africa	*Zea mays*	57246
NRRL28303	Tsukuba, Ibaraki, Japan	*Triticum aestivum*	
F. crookwellense (syn. *F. cerealis*)			
MRC2878	Michigan, USA	Soil	57247
MRC3926	Bethlehem, South Africa	*Triticum aestivum*	
MRC4643	South Africa	Potato	57248
NRRL13721	Poland	Unknown	

[a] CMW numbers refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. MRC numbers refers to the culture collection of the Medical Research Council, Tygerberg, Cape Town, South Africa. NRRL numbers are sequences obtained from Genbank and deposited by Aoki and O'Donnell (1999a).

[b] PREM numbers refer to culture collection of the collection of fungi at the Plant Protection Research Institute (PPRI), Pretoria, South Africa.

from the mycelium using a modified version of the method described by Raeder and Broda (1985). The DNA was resuspended in 100 µL sterile water and stored at −20 C until used.

ITS regions and 5.8S gene.—Two isolates (CMW6425, CMW6427) were used in the molecular comparison of the Internal Transcribed Spacer Regions (ITS) and 5.8S gene of the ribosomal RNA operon. These areas were polymerase chain reaction (PCR) amplified with primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-'3) and ITS 4 (5'-TCCTCCGCTTATTGATATGC-'3) (White et al 1990). The PCR reaction mixture included 0.04 U/µL Expand® (Roche Diagnostics GmbH, Mannheim, Germany, High Fidelity PCR system), 0.2 mM dNTPs, 10× Buffer, 1 mM $MgCl_2$ supplied by the manufacturer, 0.75 mM of each primer and ~2ng/µL of DNA. PCR reaction conditions were as follows: an initial denaturation step at 96 C for 1 min, followed by 35 cycles of primer annealing at 55 C for 30 s, chain elongation at 72 C for 1 min and denaturation at 92 C for 1 min. A final chain elongation step was included at 72 C for 5 min.

β-tubulin and histone genes.—For amplification and sequencing of the ß-tubulin gene, forward primer Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and reverse primer Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson 1995) were used. The PCR reaction mixture included 0.4 U/µL Expand®, 0.2 mM dNTP's, 10× Buffer, 1 mM $MgCl_2$ supplied by the manufacturer, 0.75 mM of each primer and ~2 ng/µL of DNA. PCR cycling conditions were as follows: an initial denaturation step at 94 C for 1 min, followed by 40 cycles consisting of 94 C for 1 min, primer annealing at 51 C for 30 s and chain elongation at 72 C for 1 min. An additional chain elongation step at 72 C for 1 min was performed.

For the amplification of the histone *H3* gene, primers H3–1a (5'-ACTAAGCAGACCGCCCGCAGG-3') and H3–1b (5'-GCGGGCGAGCTGGATGTCCTT-3') (Glass and Donaldson 1995) were used. Each PCR mixture contained 0.05 U/µL *Taq* DNA polymerase, 1× reaction buffer, 0.2 mM dNTP's, 1 mM $MgCl_2$, 0.75 µM of each primer and ~2 ng/µL of DNA. PCR cycling conditions were as follows: an initial denaturation step of 96 C for 2 min followed by 30 cycles of 92 C for 1 min, 60 C for 1 min and 72 C for 1 min. A final elongation step of 72 C for 5 min was performed. All PCR reactions were performed on a Hybaid

Touchdown Thermocycler (Hybaid Ltd., Teddington, Middlesex, UK).

DNA sequencing.—All PCR products were visualized in agarose gels stained with ethidium bromide under UV illumination. PCR products were purified using the QIAquick PCR purification kit (QIAGEN, GmbH, Hilden, Germany) and sequenced in both directions using the Big Dye Cycle Sequencing kit with Amplitaq® DNA polymerase, FS (Perkin-Elmer, Warrington, UK), according to the manufacturer's protocol, on an ABI PRISM® 377 DNA Autosequencer (Perkin-Elmer). For sequencing the ITS regions, primers ITS1 and ITS4 were used. Primers H3–1a and H3–1b were used for sequencing the histone H3 gene. The amplified portion of the β-tubulin gene was sequenced using the primers Bt2a and Bt2b.

All sequences were analysed with Sequence Navigator version 1.0.1.® (Perkin Elmer Applied Biosystems, Inc., Foster City California, USA). ITS DNA sequences were then compared against the NCBI (National Centre for Biotechnology Information) nucleotide database using BLAST (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/). From this, the possible identity of the unidentified fungi included in this study was determined. β-tubulin and histone DNA sequences were manually aligned by the insertion of gaps and analysed using PAUP* 4.0 (Swofford 1998). Gaps were treated as a "fifth character" (newstate). Analysis was done using parsimony with trees generated by heuristic searches with simple addition and Tree Bisection Reconnection (TBR) branch swapping and MULPARS effective. Bootstrap values of the branch points were generated using a 1000 replicates (Felsenstein 1988). The β-tubulin and histone sequence data sets were also tested for combinability. For this purpose partition homogeneity tests were performed as previously described (O'Donnell et al 2000b, Swofford 1998). *Fusarium pseudograminearum* was used as an outgroup and was treated as a monophyletic sister group to the ingroup.

Pathogenicity tests.—Inoculation experiments on *A. mearnsii* were conducted at the Bloemendal Field Experiment Station [South African Wattle Growers Union (SAWGU) and the Institute for Commercial Forestry Research (ICFR)] (29°32′93″S, 30°27′33″E). During Jan of 1998, three strains (CMW6425, CMW4490, CMW6427) of the unidentified fungus from *A. mearnsii* and two strains (CMW6496, CMW963) from *E. grandis,* were randomly selected and each was inoculated into twenty 18-mo-old *A. mearnsii* trees. Twenty trees were inoculated with sterile agar plugs for the controls. The entire experiment was repeated in Feb 1998. The same isolates were also inoculated into 12-mo-old coppice stems of a *E. grandis* clone (ZG14) near Kwambonambi (28°38′63″S, 32°11′66″E) in the KwaZulu-Natal Province. This experiment was also repeated once.

Inoculum was prepared by growing the isolates on 3.9% potato dextrose agar (PDA) (39 g of Merck PDA per liter of water). Actively growing isolates were inoculated by removing a 9-mm-diam bark plug from the trees with a cork borer. Mycelial plugs of equal dimension were placed into each wound with the mycelium facing inwards. For the controls, trees were inoculated with sterile agar plugs. All inoculation wounds were covered with masking tape to prevent desiccation. Lesions were measured after 6 wk and statistical differences in lesion length were determined using a one way ANOVA ($P = 0.05$). Isolations were made directly from the lesions and also from material placed in moisture chambers to fulfill Koch's postulates.

RESULTS

Morphological characters.—Identification using classical morphological techniques was impossible for the fungus associated with disease of *A. mearnsii* and *E. grandis* trees. None of the isolates could be induced to sporulate in culture despite the various techniques used.

Molecular characterization.—Using primers ITS1 and ITS4, PCR reactions generated a fragment of ~600 base pairs (bp) from the two unknown isolates (CMW6425, CMW6427). Comparison of their ITS and 5.8S gene sequences to the NCBI database indicated that they are members of the genus *Fusarium.* The *A. mearnsii* isolates shared greatest similarity with *Fusarium* spp. in the Section *Discolor.* The ITS and 5.8S regions of the two unidentified strains were 97% homologous to *F. graminearum* (GenBank U34578), *F. lunulosporum* (GenBank U85537), *F. flocciferum* (GenBank U85536), *F. culmorum* (GenBank AF006342), *F. crookwellense* (GenBank AF006340) and *F. sambucinum* (GenBank U38278).

Comparison of the sequenced portion of the β-tubulin gene of isolates CMW6425 and CMW7427 to the NCBI database showed a 99.9% homology to *F. graminearum* (GenBank AF107855–AF107876). This portion of the β-tubulin gene in isolates CMW4490 and CMW6425 was also 99% homologous to *F. lunulosporum* (GenBank U85571), *F. flocciferum* (GenBank U85570) and *F. crookwellense* (GenBank U85568). Isolates CMW4490 and CMW6425 further showed 98% and 97% homology to *F. pseudograminearum* (GenBank AF107864) and *F. culmorum* (GenBank U85569), respectively.

Having determined that the non-sporulating isolates probably represented a *Fusarium* sp., and most likely *F. graminearum,* a larger group of isolates (TABLE I) was considered. Amplification of the portion of the β-tubulin gene with primers Bt2a and Bt2b generated a ~350 bp fragment. This primer set amplifies a portion of the *F. graminearum* β-tubulin gene that includes only one intron. The other three introns do not seem to exist in this fungus. Analysis of the sequenced portion of the ~500 bp histone *H3* gene fragment indicated that it contained more variable characters than the sequenced portion of the β-tubulin gene. The histone phylogeny was based on 30 variable characters of which 29 were parsimony informative. The tubulin tree, on the other hand, was

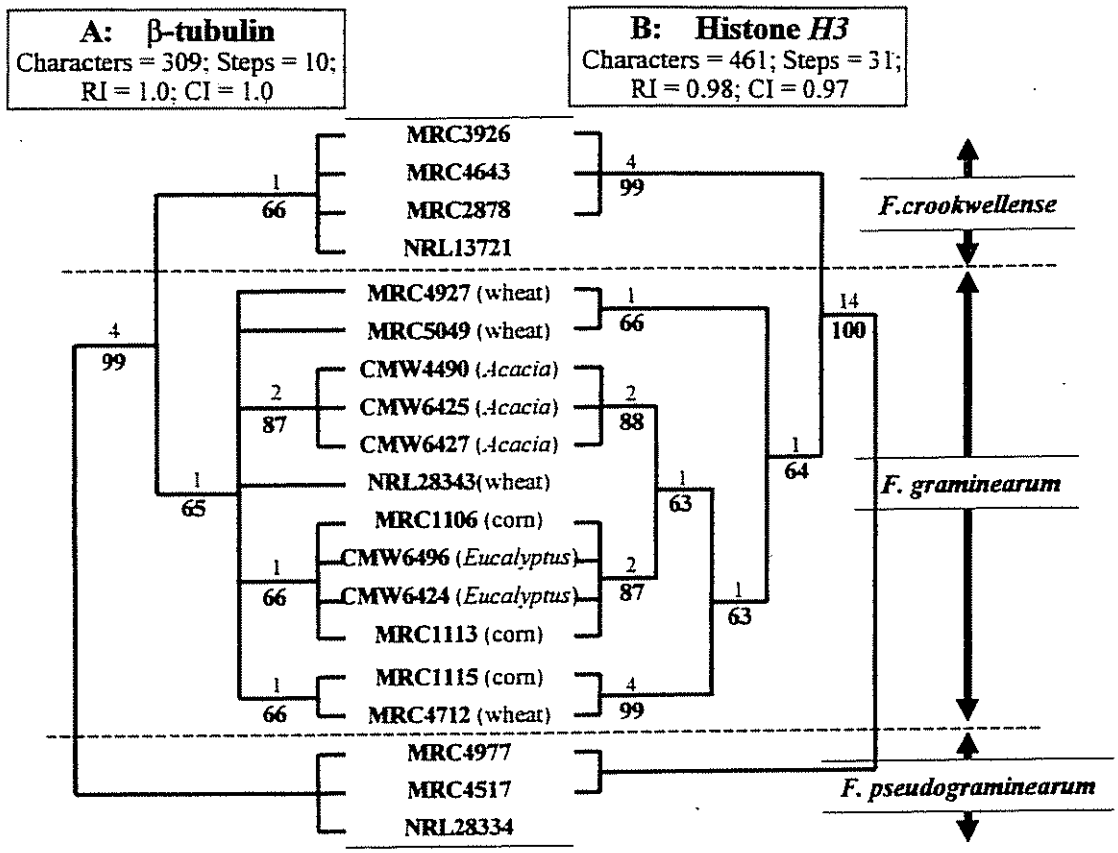

FIG. 1. Cladograms generated from (A) β-tubulin and (B) histone *H3* gene sequences using PAUP*4.0. Branch lengths greater than zero are indicated above the internodes. Bootstrap values based on 1000 replications (Felsenstein 1988) are indicated below internodes in bold letters. *Fusarium pseudograminearum* was used as outgroup. The values for the Consistency Index (CI), Retention Index (RI) and Homoplasy Index (HI) are indicated in the boxes.

based on only 9 parsimony informative characters. All sequences obtained in this study have been deposited in GenBank [NCBI (National Centre for Biotechnology Information] (AF314469–AF314500).

Phylogenetic analyses of the β-tubulin and histone H3 gene sequence data generated a single most parsimonious tree for both genes. The β-tubulin and histone H3 gene trees had similar topologies and the partition homogeneity test indicated these data sets are combinable (P = 1) (TreeBASE SN608–1738, SN608–1739). Analysis of single and combined data sets indicated that the fungal isolates collected from *Acacia* and *Eucalyptus* are closely related to each other and cluster with *F. graminearum* isolates from cereal hosts in South Africa (FIG.1). The only difference between the two gene trees is the fact that the histone gene provides better resolution of the deeper branches within the *F. graminearum* clade.

TABLE II. Lesions produced by *Fusarium graminearum* isolates on *Acacia mearnsii*

Isolate number	Lesion length (mm)	
	Jan[a]	Feb[a]
CMW6427	38.1 a	34.5 b
CMW6425	35.4 a	37.8 b
CMW4490	30.8 ab	37.8 b
CMW6426	21.7 c	28.8 bc
CMW963	24.4 bc	44.8 a
CMW6496	19.8 c	27.7 bc
Control	10 d	10 d

[a] Each value represents an average of 20 measurements. Coefficient of variance (CV) = 29.52% (Jan). CV = 25.93% (Feb). Values followed by different letters differ significantly at $P = 0.05$.

Pathogenicity tests.—Lesions were produced by all the isolates tested in this study (TABLES II, III). The largest lesions for the Jan inoculations on *A. mearnsii* were an average of 38.1 mm in length (CMW6427) and for the Feb inoculations 37.8 mm (CMW4490 and CMW6425). Isolate CMW6426 produced the smallest lesions in both experiments (21.7 mm and 28.8 mm). Lesions were characterized by a black discoloration of the outer bark surface and the formation of sunken cankers, spreading from the point of

TABLE III. Lesions produced by *F. graminearum* on *Eucalyptus grandis*

Isolate number	Lesion length (mm)	
	Jan[a]	Feb[a]
CMW6425	84.9 a	96.4 a
CMW963	61.8 b	87.85 a
CMW6427	55.6 bc	52 bc
CMW4490	45.4 c	36.6 c
CMW6496	39.2 cd	40.5 c
Control	30.1 e	30.1 f

[a] Each value represents an average of 20 measurements. CV = 35.8% (Jan). CV = 47.3% (Feb). Values followed by different letters differ significantly at $P = 0.05$.

inoculation. Extensive discoloration of the xylem spreading from the inoculation point was also observed. No lesions were produced in the control inoculations and all these inoculation wounds were rapidly covered by callus tissue. Lesion lengths for all isolates also varied significantly from the control inoculations ($P = 0.05$). For the *E. grandis* inoculations (TABLE III), isolate CMW6425 from *A. mearnsii* produced the largest lesions in both inoculations. Control inoculations on *E. grandis* also produced lesions for some of the trees. Lesion lengths for isolates, however, still differed significantly from those of the controls. Isolations from the lesions resulted in the red fungus. It was obtained from both moisture chambers and direct isolations from the wood. No fruiting bodies were obtained, only the abundant red mycelium was produced in culture.

DISCUSSION

In this study we have clearly shown that the unknown, nonsporulating, fungus associated with die-back and canker symptoms of *A. mearnsii* (Roux and Wingfield 1997) and *E. grandis* is *F. graminearum* [teleomorph: *Gibberella zeae* (Schw.) Petch]. This is an intriguing discovery as the fungus has, to the best of our knowledge, never previously been associated with a disease of a woody host. In contrast, *F. graminearum* is a well known mycotoxin-producing pathogen of maize and wheat (Vesonder and Hesseltine 1981, Hart et al 1982, Marasas et al 1987, 1988a, 1988b, Blaney and Dodman 1988, Ouellet and Seifert 1993, Desjardins and Hohn 1997).

Despite considerable effort, the fungus associated with disease symptoms on *A. mearnsii* and *E. grandis* could not be induced to sporulate. The reason for this is unclear, as no problems with sporulation have been experienced with other *Fusarium* spp. For positive identification of this red fungus, we had to resort to comparison of conserved genes published in a public domain database. Initially, we used ribosomal ITS and 5.8S gene sequences, but these regions were found to be too conserved for identification at the species level. We were, however, able to ascertain that our fungus represented a *Fusarium* sp. (Section *Discolor*). Comparison of the β-tubulin gene sequences suggested that the unknown species from *A. mearnsii* and *E. grandis* was most closely related to *F. graminearum*. Based on this preliminary identification it was possible to compare a larger set of isolates using β-tubulin and histone gene sequences to identify the fungus positively.

F. graminearum isolates from *A. mearnsii* formed a separate subclade within the larger *F. graminearum* group. This result is congruent with those of O'Donnell et al (2000a). Using our isolates in their phylogenetic study of *F. graminearum*, they also showed that the isolates from *Acacia* represent a separate lineage. They further showed that the *Acacia* isolates were more closely related to Chinese isolates of *F. graminearum* than to isolates from Africa or other continents (O'Donnell et al 2000a). The isolates from *E. grandis* grouped separately from the *A. mearnsii* isolates in a subclade with two isolates from corn.

The role of *F. graminearum* as a pathogen of *A. mearnsii* and *E. grandis* is not clear. On *A. mearnsii*, the fungus is generally isolated from canker and die-back symptoms attributed to other primary pathogens such as *Ceratocystis albofundus* Wingfield, De Beer & Morris. *Fusarium graminearum* has been isolated from the major *A. mearnsii* growing areas in the KwaZulu-Natal Midlands and the South Eastern Mpumalanga Province. This indicates a wide distribution in the commercial growing areas, where it was isolated from collar rot and stem cankers. Pathogenicity tests conducted in this study have further shown that *F. graminearum* can cause well defined lesions on trees. It is not clear why some of the control inoculations on *E. grandis* produced lesions. Isolations from these wounds did not result in the re-isolation of any *Fusarium* spp., unlike isolations from lesions on trees inoculated with test cultures.

This report of *F. graminearum* from diseased *A. mearnsii* and *E. grandis* is enigmatic. To our knowledge, it represents the first report of this fungus as a pathogen of woody hosts. The only other known reports of *F. graminearum* from woody hosts are from an orange twig in New Caledonia (O'Donnell et al 2000a) and from coffee (Booth 1971). However, the latter report clearly states that the accuracy of the reports of *F. graminearum* from hosts other than the Gramineae is cast in doubt. This is because *G. saubinetii* was for a long time incorrectly regarded as a

synonym of *G. zeae* (Booth 1971). Neither of the reports states involvement in any disease.

The importance of *F. graminearum* as a pathogen of *Acacia* and *Eucalyptus* hosts needs further evaluation. The issue of host range, however, can have even wider implications, since molecular characterization indicated that the *F. graminearum* isolates from *Eucalyptus* and corn are very closely related (Fig. 2). This suggests that isolates from corn could potentially cause disease on *Eucalyptus* spp. or vice versa. For this reason the importance of *F. graminearum* in diseases of these hosts must be considered further. Special attention should also be given to the host specificity of *F. graminearum* isolates associated with these cereal and woody hosts.

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