Mycologia, 96(6), 2004, pp. 1294–1305.
© 2004 by The Mycological Society of America, Lawrence, KS 66044-8897

Cephalosporium maydis is a distinct species in the *Gaeumannomyces-Harpophora* species complex

Amgad A. Saleh[1]
John F. Leslie[2]

Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas 66506-5502

Abstract: *Cephalosporium maydis* is an important plant pathogen whose phylogenetic position relative to other fungi has not been established clearly. We compared strains of *C. maydis,* strains from several other plant-pathogenic *Cephalosporium* spp. and several possible relatives within the *Gaeumannomyces-Harpophora* species complex, to which *C. maydis* has been suggested to belong based on previous preliminary DNA sequence analyses. DNA sequences of the nuclear genes encoding the rDNA ITS region, β-tubulin, histone H3, and *MAT-2* support the hypothesis that *C. maydis* is a distinct taxon within the *Gaeumannomyces-Harpophora* species complex. Based on amplified fragment length polymorphism (AFLP) profiles, *C. maydis* also is distinct from the other tested species of *Cephalosporium, Phialophora* sensu lato and members of *Gaeumannomyces-Harpophora* species complex, which supports its classification as *Harpophora maydis*. Oligonucleotide primers for *H. maydis* were developed that can be used in a PCR diagnostic protocol to rapidly and reliably detect and identify this pathogen. These diagnostic PCR primers will aid the detection of *H. maydis* in diseased maize because this fungus can be difficult to detect and isolate, and the movement of authentic cultures may be limited by quarantine restrictions.

Key words: AFLP, β-tubulin, Corn, *Harpophora maydis,* Histone H3, *Phialophora* sensu lato, maize, mating type, rDNA ITS

INTRODUCTION

Late wilt of maize, caused by the fungus *Cephalosporium maydis* Samra, Sabet & Hingorani (Samra et al 1962, 1963), is one of the most important fungal diseases in Egypt. This disease also has been reported from India (Payak et al 1970, Ward and Bateman 1999) and Hungary (Pecsi and Nemeth 1998). *C. maydis* reproduces asexually, and no perfect state has been identified. Saleh et al (2003) and Zeller et al (2000) showed that the pathogen is clonal in Egypt and that the Egyptian population contains four lineages, three of which are widely distributed throughout the country.

C. maydis originally was described based on growth characters and the morphology of hyphae, conidia and conidiophores. Domsch and Gams (1972) suggested that the conidial state of *C. maydis* was a *Phialophora* (the anamorph of *Gaeumannomyces* Arx & D. Olivier) and that spore production in *C. maydis* was typical of that genus (Ward and Bateman 1999). Most members of the genus *Cephalosporium* were transferred to the genus *Acremonium,* a genus of hyaline hyphomycetes with aculeate (spine-like) phialides unrelated to either *Phialophora* or *Harpophora,* when Gams (1971) reintroduced *Acremonium.* Gams (2000) introduced *Harpophora* as a new genus (contains anamorphs of *Gaeumannomyces* and *Magnaporthe*) that is distinct from *Phialophora. Harpophora* spp. are characterized by fast-growing, thin colonies with sickle-shape conidia. Older hyphae are heavily pigmented, younger hyphae are nearly hyaline and phialides are intermediate in pigmentation relative to the older and younger hyphae. When he introduced *Harpophora,* Gams (2000) also introduced the new combination *Harpophora maydis* (Samra, Sabet and Hingorani) Gams as a replacement for *Cephalosporium maydis.*

Ward and Bateman (1999) used RFLP hybridization and portions of the rDNA repeat sequence to associate *C. maydis* with the *Gaeumannomyces* species complex, but their results were insufficient to determine whether *C. maydis* was a distinct taxon at the species level and whether *C. maydis* should be reclassified. The distinguishing morphological characters available for *C. maydis* are limited, and applying them to identify the species is not easy, so we used DNA sequence-based approaches to help differentiate this species, as has been done with numerous other fungal taxa.

Our objectives in this study were: (i) to determine

Accepted for publication June 28, 2004.

[1] Permanent address: Agricultural Genetic Engineering Research Institute, Agricultural Research Center, 9 Gamaa Street, Giza, Egypt.

[2] Corresponding author. Department of Plant Pathology, 4002 Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas 66506-5502. Phone: 785-532-1363. Fax: 785-532-2414. E-mail: jfl@plantpath.ksu.edu

TABLE I. Total number of amplified bands and polymorphic bands in the four AFLP profiles evaluated (EAA/MCA, EAA/MCC, EAA/MCG, and EAA/MGA)

Species	Number of strains	Number of bands in the four AFLP profiles	Number of polymorphic bands (within species)
C. maydis	17	68	25
C. gramineum	6	118	29
A. strictum	10	129	93
G. cylindrosporus	15	103	92
G. graminis var. *avenae*	8	63	42
G. graminis var. *tritici*	16	61	49
G. graminis var. *graminis*[a]	9		
Group 1	4	70	32
Group 2	2	71	44

[a] Based on the AFLP data, *G. graminis* var. *graminis* strains divided into five groups. Group 1 contained four strains; group 2 contained two strains; and groups 3, 4, and 5 (not shown) each contained only a single strain.

the relatedness of *C. maydis* to strains representing the *Gaeumannomyces-Harpophora* species complex and other *Cephalosporium* species, (ii) to assess the integrity of the species examined and their genetic relationships and (iii) to develop a rapid PCR method to detect *C. maydis*. Our working hypothesis is that *C. maydis* is a distinct species in the *Gaeumannomyces-Harpophora* species complex and that new molecular diagnostics are needed to rapidly and reliably identify this species.

MATERIALS AND METHODS

Fungal strains.—We examined 44 strains from the *Acremonium-Cephalosporium* species complex and 48 strains from *Gaeumannomyces-Phialophora* species complex (TABLE I). The strains of *C. maydis* represent the four clonal lineages found in Egypt (Saleh et al 2003). The other species of *Cephalosporium* examined include: (i) *Acremonium diospyri* (Crandall) W. Gams (syn., *Cephalosporium diospyri* Crandall), the causal agent of American persimmon wilt (Halls 1990); (ii) *Cephalosporium gramineum* Nisikado & Ikata in Nisikado et al (*Hymenula cerealis* Ellis & Everh.), the causal agent of Cephalosporium stripe of winter wheat (Bockus and Claassen 1985); and (iii) *Acremonium strictum* W. Gams (syn., *Cephalosporium acremonium* Auct. non Corda), the causal agent of stalk rot and black bundle diseases of maize or Acremonium wilt of sorghum (Bandyopadhyay et al 1987, Hanlin et al 1978). Three varieties of *Gaeumannomyces graminis* (anamorph *Harpophora* spp.) (Sacc.) Arx & Olivier were examined: (i) *G. graminis* var. *tritici* J. Walker (GGT), the causal agent of take-all of wheat and barley; (ii) *G. graminis* var. *avenae* (E.M. Turner) Dennis (GGA), the causal agent of take-all disease of oats and which also can infect barley and cause take-all patch disease of bentgrass (Dernoeden and O'Neill 1983, Couch 1995); and (iii) *G. graminis* var. *graminis* (GGG), which has a wider host range than either GGT or GGA, is a weak pathogen of wheat (Bryan et al 1995) and causes Bermudagrass decline (Elliott 1991), take-all root rot of St. Augustine grass (Elliott et al 1993, Wilkinson and Pedersen 1993), crown sheath rot of rice (Walker 1981) and root rot of centipede grass (Wilkinson 1994). We also examined *Gaeumannomyces cylindrosporus* D. Hornby, D. Slope, R. Gutteridge & Sivanesan [anamorph *Harpophora graminicola* (Deacon) W. Gams], associated with root discoloration of *Poa pratensis* (= Kentucky bluegrass) (Jackson and Landschoot 1986). Two field strains of *Fusarium verticillioides* (Sacc.) Nirenberg, causal agent of stalk and root rot of maize (Leslie et al 1990) were used as outgroup.

DNA isolation.—Fungal cultures were grown in complete medium (CM) broth (Correll et al 1987) and incubated on an orbital shaker (150 rpm) at least 3 d at room temperature (25–28 C). The mycelia were harvested, ground to a powder with liquid nitrogen in a mortar and pestle and stored at −70 C until DNA was extracted. Fungal DNA was isolated by a CTAB method (Murray and Thompson 1980) as modified by Kerényi et al (1999).

AFLP reactions and data analysis.—AFLP reactions (Vos et al 1995) were performed as described by Saleh et al (2003) in a PTC-2000 Thermal Cycler (MJ Research Inc., Watertown, Massachusetts). The AFLP primers used in this study were *Eco*RI primer (5′-AGACTGCGTACCAATTC-3′) followed by two base pairs (AA), abbreviated as EAA, and the *Mse*I primer (5′-GATGAGTCCTGAGTAA-3′) followed by two base pairs (CA, CC, CG, or GA), abbreviated as MCA, MCC, MCG, and MGA.

AFLP fingerprints were scored manually as "1" for the presence of a band and "0" for the absence of a band, assuming that bands with the same molecular size in different individuals were homologous. The Unweighted Pair Grouping by Mathematical Averages (UPGMA) subroutine of PAUP* 4.0b10 (Swofford 2000) was used to construct phylograms (phenograms) and to estimate the genetic similarity among fungal strains of each species.

Conversion of AFLP markers into diagnostic PCR markers for C. maydis.—AFLP bands that differentiate the lineages of

TABLE II. PCR primers for nuclear gene fragments sequenced in this study

Name	Nucleotide sequence[a]	Gene	Reference
ITS3	5′-GCATCGATGAAGAACGCAGC-3′	rDNA-ITS	White et al (1990)
ITS4	5′-TCCTCCGCTTATTGATATGC-3′		
ITS5	5′-GGAAGTAAAGTCGTAACAAGG-3′		
H3-1a	5′-ACTAAGCAGACCGCCCGCAGG-3′	Histone H3	Steenkamp et al (1999)
H3-1b	5′-GCGGGCGAGCTGGATGTCCTT-3′		
T1	5′-AACATGCGTGAGATTGTAAGT-3′	β-tubulin	O'Donnell and Cigelnik (1997)
T2	5′-TAGTGACCCTTGGCCCAGTTG-3′		
T21	5′-GGTTTGCCAGAAAGCAGCACC-3′		
ChHMG1	5′-AAGGCNCCNCGYCCNATGAAC-3′	*MAT-2*	Arie et al (1997)
ChHMG2	5′-CTNGGNGTGTAYTTGTAATTNGG-3′		
NcHMG1	5′-CCYCGYCCYCCYAAYGCNTAYAT-3′		
NcHMG2	5′-CGNGGRTTRTARCGRTARTNRGG-3′		
CMaflp11	5′-TTTCCTGCGGTGCCAA-3′	Unknown	This study
CMaflp12	5′-TAATGCGGTTAGCCACTC-3′		

[a] Abbreviations: Y = C or T; N = A, T, C, or G.

C. maydis were cut from the polyacrylamide gels and transferred to 1.5 mL microfuge tubes containing 8 μl of H_2O. These tubes were incubated at 37 C for 1 h (or overnight at 4 C), and the resulting DNA suspension was used as template DNA for PCR reactions. PCR was performed in a total volume of 20 μl in the presence of 27 ng of each primer (EAA and MXX) and 200 μM deoxynuleoside triphosphates (New England Biolabs, Beverly, Massachusetts) in 1× NH_4 buffer (Bioline USA Inc., Springfield, New Jersey), 1.5 mM $MgCl_2$, and 0.2 U of Biolase® DNA polymerase (Bioline). The PCR cycling program used to re-amplify these bands was the same as that used for the original AFLP amplification reactions (Saleh et al 2003). Two μl of the PCR reaction products were separated in 1.5% agarose in 1× TAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.0). PCR products were purified with the Wizard DNA Clean Up kit (Promega, Madison, Wisconsin) to remove unincorporated nucleotides, proteins and other impurities. The DNA concentration of the final purified PCR products was determined with an ethidium bromide method (Sambrook et al 1989) by estimating DNA concentrations relative to *Hind*III-digested phage λ DNA of known concentration. The purified DNA products were sequenced with ABI Prism® BigDye® Terminator Ready Cycle Sequencing Kits (Applied Biosystems, Foster City, California). Sequencing reactions were run on an ABI Prism® 3700 DNA Analyzer at the Kansas State University DNA sequencing facility.

Specific PCR primers were designed based on the sequences of the AFLP fragments. PCR reactions, to test the utility of the specific primers, were performed in a total volume of 25 μL containing 0.5 μM of each primer, 200 μM deoxynucleoside triphosphates, 1× NH_4 buffer, 2.5 mM $MgCl_2$, 1 U of Biolase® DNA polymerase, and 25 ng of fungal genomic DNA. The PCR program was one cycle of 94 C for 3 min, followed by 35 cycles of 94 C for 30 s, annealing temperature (depending on the melting temperature of the primers) for 30 s, and 72 C for 1 min, followed by a final extension at 72 C for 5 min. PCR products were separated on 1.2% agarose gels to assess amplification and reaction specificity.

PCR amplification and DNA sequencing.—We amplified portions of four nuclear genes (TABLES II and III). Amplification reactions for each locus were performed in a total volume of 10 μL containing 0.25 μM of each primer and 200 μM deoxynucleoside triphosphates, 1× NH_4 buffer, 2.5 mM $MgCl_2$, 0.2 U of Biolase® DNA polymerase, and 10 ng of fungal genomic DNA. Cycling conditions for primer pairs ITS3 + ITS4; and ITS4 + ITS5 (White et al 1990) were 94 C for 3 min and then 94 C for 1 min, 52 C for 1 min and 72 C for 1 min (35 cycles), followed by a 5 min extension at 72 C. Cycling conditions for primer pairs T1 + T2; and T1 + T21 (O'Donnnell and Cigelnik 1997) were 94 C for 3 min and then 94 C for 1 min, 63 C for 1 min, and 72 C for 1 min (35 cycles), followed by a 5 min extension at 72 C. Cycling conditions for primers H3-1a and H3-1b (Steenkamp et al 1999) were 94 C for 3 min and then 94 C for 1 min, 68 C for 1 min, and 72 C for 1 min (35 cycles), followed by a 5 min extension at 72 C. Cycling conditions for degenerate primers NcHMG1 and NcHMG2, and ChHMG1 and ChHMG2 (Arie et al 1997) were 94 C for 2 min and then 94 C for 1 min, 55 C for 1 min, and 72 C for 1 min (30 cycles), followed by a 5 min extension at 72 C. For diagnostic purposes, the cycling conditions for primers CMaflp11 and CMaflp 12 were 94 C for 3 min and then 94 C for 1 min, 67 C for 30 s, and 72 C for 45 s (35 cycles), followed by a 5 min extension at 72 C. After PCR, amplified DNA was quantified on agarose gels, purified with the Wizard PCR purification system (Promega), and sequenced as described above.

Phylogenetic analysis.—DNA sequences were edited and aligned with the Clustal W algorithm (Thompson et al 1994) as implemented in the program BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Final alignments were optimized visually. Intron/exon junctions in the β-tubulin and histone H3 sequences were identified by

TABLE III. GenBank accession numbers and TreeBank alignment numbers for sequences used in this study

Species (KSU/ATCC)[a]	β-tubulin	rDNA ITS	Histone H3	*MAT-2*	*C. maydis* diagnostic
C. maydis					
KSU 10793/MYA-3357 (Lineage I)	AY435435	AY428788	AY435458	NA	AY435487
KSU 10800/MYA-3358 (Linage II)	AY435436	AY428787	AY435459	NA	AY435488
KSU 14435/MYA-3359 (Lineage III)	AY435437	AY428786	AY435460	AY435482	AY435489
KSU 10792/MYA-3356 (Lineage IV)	AY435438	AY428785	AY435461	NA	AY435490
C. gramineum					
KSU 14436/MYA-3360	NA	AY428791	AY435462	NA	NA
KSU 14437/MYA-3361	NA	AY428792	NA	NA	NA
A. diospyri					
KSU 14438/MYA-3362	AY435439	AY428793	AY435463	NA	NA
A. strictum					
KSU 5144/MYA-3363	AY435440	AY428789	AY435464	NA	NA
KSU 5147/MYA-3384	AY435441	AY428790	AY435465	NA	NA
F. verticillioides					
KSU 4773	AY435443	AY428795	AY435467	NA	NA
KSU 5146	AY435442	AY428794	AY435466	NA	NA
G. cylindrosporus					
KSU 14447/MYA-3385	AY435445	AY428772	AY435469	NA	NA
KSU 14448/MYA-3386	AY435444	AY428771	AY435468	AY435483	NA
KSU 14913	AY435446	AY428773	AY435470	NA	NA
KSU 14914	AY435447	AY428774	AY435471	NA	NA
KSU 14916	AY435448	AY428775	AY435472	NA	NA
KSU 14919	AY435449	AY428776	AY435473	NA	NA
G. g. avenae					
KSU 14439/MYA-3367	AY435450	AY428777	AY435474	AY435484	NA
KSU 14440/MYA-3368	AY435451	AY428778	AY435474	NA	NA
G. g. tritici					
KSU 14441/MYA-3369	AY435452	AY428783	AY435476	AY435485	NA
KSU 14449/MYA-3370	AY435453	AY428774	AY435477	NA	NA
G. g. graminis					
KSU 14445/MYA-3373	AY435454	AY428779	AY435478	AY435486	NA
KSU 14442/MYA-3371	AY435455	AY428780	AY435479	NA	NA
KSU 14443/MYA-3372	AY435456	AY428781	AY435480	NA	NA
KSU 14446/MYA-3374	AY435457	AY428782	AY435481	NA	NA
Alignment	SN1643	SN1646	SN1647	SN1649	

NA—No amplification

[a] Strain numbers from the collections at the Department of Plant Pathology, Kansas State University (Manhattan, Kansas), and the American Type Culture Collection (Manasas, Virginia).

aligning these sequences with the known *F. verticillioides* sequences of β-tubulin (GenBank accession number U34413) and histone H3 (GenBank accession nnumber AF150859) genes (O'Donnnell and Cigelnik 1997, Steenkamp et al 1999). Phylogenetic analyses of aligned DNA sequences were performed with PAUP* version 4.0b10 (Swofford 2000). The heuristic search option was used to infer maximum parsimony trees. Clade stability was assessed by 1000 bootstrap replications (Felsenstein 1985, Hillis and Bull 1993) calculated from PAUP trees. Other measures, including tree length, consistency index (CI) and retention index (RI), were calculated with PAUP* 4.0b10 (Swofford 2000). Phylogenies were inferred from each of the three genes individually and then for the combined data for the three genes.

RESULTS

Comparison of AFLPs from C. maydis *and related fungal species.*—We used four AFLP primer pairs to assess the relatedness of *C. maydis* to the other fungal spe-

TABLE IV. Pairwise average genetic similarity within and between strains of species entities used in this study based on AFLPs. Number of polymorphic bands used to generate these values was 411

	Average of genetic similarity								
	CM	CG	AS	AD	GC	GGA	GGG	GGT	FV
CM[a] (27)[b]	0.90								
CG (6)	0.04	0.94							
AS (10)	0.01	0.11	0.69						
AD (1)	0.0	0.0	0.01	—[c]					
GC (15)	0.01	0.01	0.02	0.04	0.60				
GGA (8)	0.03	0.04	0.02	0.06	0.03	0.74			
GGG (9)	0.02	0.01	0.05	0.08	0.02	0.01	0.15		
GGT (16)	0.04	0.0	0.01	0.18	0.0	0.18	0.02	0.85	
FV (1)	0.0	0.01	0.06	0.0	0.07	0.0	0.02	0.0	—[c]

[a] The letters in the strain name indicate the species name: (AD) *A. diospyri,* (AS) *A. strictum,* (CG) *C. gramineum,* (CM) *C. maydis,* (GC) *G. cylindrosporus,* (GGA) *G. graminis* var. *avenae,* (GGG) *G. graminis* var. *graminis,* (GGT) *G. graminis* var. *tritici,* and (FV) *F. verticillioides.*

[b] Number of strains.

[c] Not calculated since only a single strain was analyzed.

cies. Each species had a distinctive AFLP profile. All strains from the same species shared > 11% of the bands (i.e., monomorphic bands, presumed to be species specific), while strains from different species had no bands common to all isolates (TABLE I). The three varieties of *G. graminis* each had distinct AFLP profiles that were no more similar to one another than they were to other species examined. GGG strains were the most diverse and produced five different AFLP patterns that shared almost no bands, even with each other. Overall, the highest percentage of shared bands (75%) occurred within *C. gramineum* (TABLE I).

Pairwise average genetic similarities within and between species were analyzed on the basis of AFLP profiles, generated from two primer pairs (EAA/MCC and EAA/MCG), for the 93 strains used in this study (TABLE IV). Four hundred eleven polymorphic bands were generated by these two primer pairs. The highest average similarity was seen within *C. gramineum* (0.94), while the lowest was within GGG (0.15). The average genetic similarity between strains from different species generally was very low (TABLE IV). The highest average genetic similarity was between GGA and GGT and between GGT and AD (0.18). The uniqueness of the clade for each species was supported with a bootstrap value ≥88% (FIG. 1). The distinctness of the four lineages within *C. maydis* was supported with bootstrap values ≥69%. The clade that included strains from *C. maydis* and *Gaeumannomyces-Harpophora* species complex was supported with a bootstrap value of 89%. The strains of GGT grouped into two clusters, one contained Kansas strains while the other contained a strain (KSU14449) from Oregon. When the strains of GGA and GGT were analyzed together, the KSU14449 strain formed a separate clade in the GGA cluster. The strains of GGG divided into five groups, two of which were supported with bootstrap values of 94 and 88%, while the other three groups were each represented by a single isolate. The first group contained four strains isolated from Bermudagrass from Florida, and the second contained two strains, one from Florida and the other from Missouri. The third, fourth and fifth groups each contained only one strain, isolated from rice, soybean and St. Augustine grass, respectively.

Comparison of DNA sequences from C. maydis *and related fungal species.*—AFLP data were used to select representative strains from each species from which portions of four unrelated nuclear genes (rDNA, β-tubulin, histone H3 and *MAT-2*) were amplified and sequenced (TABLES III and V). The exon sequences of these genes were alignable across all species. Alignments of the intron sequences were not obvious for the β-tubulin and histone H3 genes. However, ITS1 and ITS2 were alignable across all the species. Thus, intron sequences of the β-tubulin and the histone H3 genes were excluded from the DNA alignments, and the presence/absence of introns was used as a fifth character state in the alignments.

rDNA ITS.—Amplification of the rDNA-ITS regions yielded fragments ranging in length from 528 to 576 bp (TABLES III and V), with a total of 25 variable sites throughout the exon across all isolates. No nucleotide variation in the rDNA exons was detected within a species. The total number of variable nucleotide sites across the entire region was 295, with 271 of these sites being phylogenetically informative. A

neighbor-joining analysis based on rDNA ITS sequences identified two major clades. *C. gramineum* was used as outgroup for this analysis because it was the most distant taxon. The first clade received 100% bootstrap support and contained strains identified as *C. maydis* and *Gaeumannomyces* spp. The second clade, which received 99% bootstrap support, contained strains belonging to two other species in the *Acremonium-Cephalosporium* complex and *F. verticillioides*. The clade containing the GGA strains and isolate KSU14449 received 99% bootstrap support. Each fungal species clade had 100% bootstrap support. The maximum parsimony analysis produced 18 equally parsimonious trees (tree length = 520 steps, CI = 0.81, and RI = 0.91) that differed from one another only in the branching order within the clade containing the *G. graminis* strains. These most parsimonious trees were similar in topology to that of the neighbor-joining trees.

β-tubulin.—Amplification of the β-tubulin region yielded fragments ranging from 564 to 819 bp in length (TABLES III and V). The exon sequences of the β-tubulin gene were alignable across all the species, with 71 variable sites in the exon, 61 of which were phylogenetically informative. The β-tubulin region from *C. gramineum* strains did not amplify with the T1, T2, and T21 primers under the tested PCR reaction conditions. A neighbor-joining analysis based on partial β-tubulin gene sequences gave almost exactly the same results as those obtained with the rDNA sequences. *C. maydis* again was located in the *Gaeumannomyces-Harpophora* species complex with 94% bootstrap support. The bootstrap support of the clade containing the remaining strains in the *Acremonium-Cephalosporium* complex and *F. verticillioides* was relatively low (58%). The maximum parsimony analysis resulted in 12 most parsimonious trees (tree length = 114 steps, CI = 0.71 and RI = 0.88) that differed from one another only in the branching order within the clade containing the *G. graminis* strains. These most parsimonious trees were similar in topology to the neighbor-joining tree produced from the β-tubulin sequences.

Histone H3.—The amplification of the histone region yielded fragments ranging from 398 to 525 bp in length (TABLES III and V). The histone H3 gene sequence was the most variable of those examined. As with the β-tubulin gene, the histone H3 exon sequences were alignable but not the introns. The total number of variable nucleotide sites in the exon regions was 82, with 63 being phylogenetically informative sites. Again *C. gramineum* was the most genetically distant of the tested species and was used as outgroup. Branching orders observed in the neighbor-joining and maximum parsimony trees were not as strongly supported by the bootstrap analysis as they were for the other loci examined, although the clade containing strains of *Gaeumannomyces* sp. and *C. maydis* received 69% bootstrap support.

Mating type.—The NcHMG degenerate primers amplified DNA fragments ranging from 214 to 216 bp in length from one strain each of *C. maydis, G. cylindrosporus* and three varieties of *G. graminis*. No amplification was detected when the ChHMG primers were used. The GGA and GGT fragment sequences were identical. The pairwise comparisons of nucleotide sequence similarity for this region between *C. maydis* and *G. cylindrosporus,* GGA/GGT and GGG were 75, 79 and 76%, respectively. When the *MAT-2* DNA sequences were translated into amino acid sequences, they aligned very well with *MAT-2* sequences (Treebase accession number SN1649) from other Ascomycetes in the GenBank database (e.g., *Magnaporthe grisea* accession number BAC65094). Based on these alignments the *MAT-2* HMG box of *C. maydis* and its allied species have a novel intron that has not yet been reported from any of the other Ascomycete *MAT-2* sequences available in GenBank.

Combined analysis of rDNA ITS, β-tubulin, and histone H3.—The maximum parsimony phylogeny inferred from the combined datasets (806 steps) is shorter than the sum of the tree lengths for each of the individual datasets (840 steps). These data sets consequently can be combined (Farris et al 1994). Again *C. maydis* was in the same clade (received 100% bootstrap support) as the *Gaeumannomyces-Harpophora* strains (FIG. 2). The clade containing strains from GGA and GGT received 86% bootstrap support. Maximum parsimony analysis of the combined datasets produced two most parsimonious trees (tree length = 806 steps, CI = 0.77 and RI = 0.88) and differed from one another in the branching order within the clade containing *G. graminis* strains (FIG. 2). The topology of the trees from the maximum parsimony and neighbor-joining analyses were the same.

Diagnostic PCR primers for C. maydis.—Of the 25 AFLP bands polymorphic between *C. maydis* lineages, 11 were cut from the polyacrylamide gel, purified and amplified. Six of these bands were amplified and sequenced directly. PCR with the primer pair CMaflp11 + CMaflp12:), derived from AFLP fragment EAA/MCG-5, amplifies a 300-bp sequence unique to *C. maydis* (TABLE III, FIG. 3). Amplification at different annealing temperatures (55, 58, 60, 65 and 67 C) with numerous strains of *C. maydis* (Saleh et al 2003), as well as with all the *C. maydis* strains used in this study, produced the same results. The

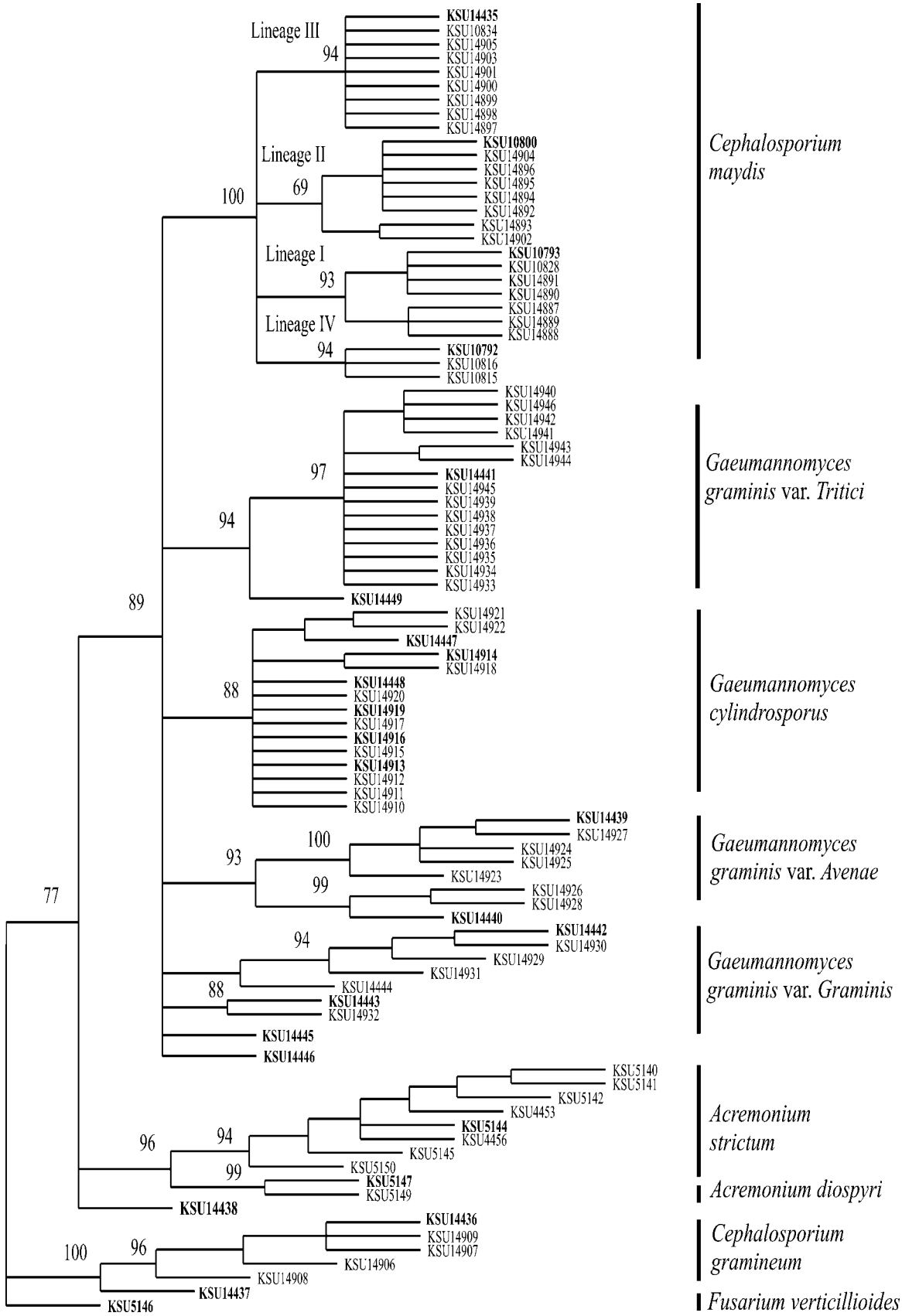

FIG. 1. UPGMA tree based on AFLP fingerprints generated from two primer pairs (EAA/MCC and EAA/MCG) for 93 fungal strains used in this study. Percent values on the branches of the tree generated with 1000 bootstrap replicates.

TABLE V. Representative fungal strains used in the phylogenetic studies and the size in bp of the PCR fragments generated, for the rDNA-ITS, β-tubulin, and histone H3 coding regions

Species	Strain number[a]	rDNA-ITS	β-tubulin	Histone H3
G. cylindrosporus	KSU 14448/MYA-3366	576	819	525
	KSU 14447/MYA-3365	576	815	525
	KSU 14913, KSU 14914	576	817	517
	KSU 14916	576	816	527
	KSU 14919	576	817	523
G. graminis var. *avenae*	KSU 14439/MYA-3367	541	779	479
	KSU 14440/MYA-3368	549	779	479
G. graminis var. *graminis*	KSU 14445/MYA-3373	538	771	470
	KSU 14442/MYA-3371	539	776	473
	KSU 14443/MYA-3372	537	784	460
	KSU 14446/MYA-3374	537	776	478
G. graminis var. *tritici*	KSU 14441/MYA-3369	540	779	479
	KSU 14449/MYA-3370	543	778	479
C. maydis lineages I and III	KSU 14435/MYA-3359, KSU 10793/MYA-3357	556	759	488
C. maydis lineage II	KSU 10800/MYA-3358	557	759	488
C. maydis lineage IV	KSU 10792/MYA-3356	555	579	488
A. stricum	KSU 5144/MYA-3363, KSU 5147/MYA-3364	560	644	398
A. diospyri	KSU 14438/MYA-3362	363	591	446
F. verticillioides	KSU 5146, KSU4773	528	564	479
C. gramineum	KSU 14436/MYA-3360, KSU 14437/MYA-3361	561	NA	523
	Range	528–576	564–819	398–525

[a] Strain numbers are those from the Department of Plant Pathology, Kansas State University and the American Type Culture Collection.

NA—no data, did not amplify.

clearest results were obtained with an annealing temperature of 67 C and 1.5 mM $MgCl_2$ concentration, because only the unique *C. maydis* band was amplified (FIG. 3). These primers and conditions did not result in the amplification of similar sized DNA fragments from *F. verticillioides* or from any of the other tested members of either the *Gaeumannomyces-Harpophora* or the *Acremonium-Cephalosporium* species complexes. When the DNA sequence of the PCR product unique to *C. maydis* was searched in GenBank, there was no significant match with any other DNA or protein sequences in the database.

DISCUSSION

The primary objective of our study was to determine the genetic relatedness of *C. maydis* to several morphologically similar fungi and to confirm its identity as a distinct taxon. AFLPs have been used to group strains into species in *Fusarium* (Marasas et al 2001, Zeller et al 2003), with strains in biologically distinct species sharing no more than 40% of the bands in a profile. AFLPs usually are most useful for studying genetic diversity at or below species level and often provide little, if any, useful information about genetic relatedness between taxa above species level other than that they are different (e.g., Rehmany et al 2000, Marasas et al 2001, Zeller et al 2003). *C. maydis* shared no AFLP bands with any of the other species examined, suggesting that the *C. maydis* strains belong to a single distinct species. The GGG strains had the lowest within-group average genetic similarity (0.15) and produced very different AFLP profiles, suggesting that this variety of *G. graminis* probably contains several cryptic species. When the strains from GGA and GGT were analyzed together, the KSU14449 strain was related more closely to the GGA isolates than it was to the other GGT isolates. Isolates of *G. graminis* identified as GGT on the basis of ascospore length—isolates capable of infecting oats and isolates that are genetically close to GGA—have been reported from Australia (Bryan et al 1995, 1999). Bryan et al (1999) suggested that these strains of *G. graminis* represent either a third cereal-attacking variety or are intervarietal hybrids between strains of GGA and GGT. Our limited data support the hybrid hypothesis in which outcrosses can occur between strains of GGA and GGT. *C. gramineum, A.*

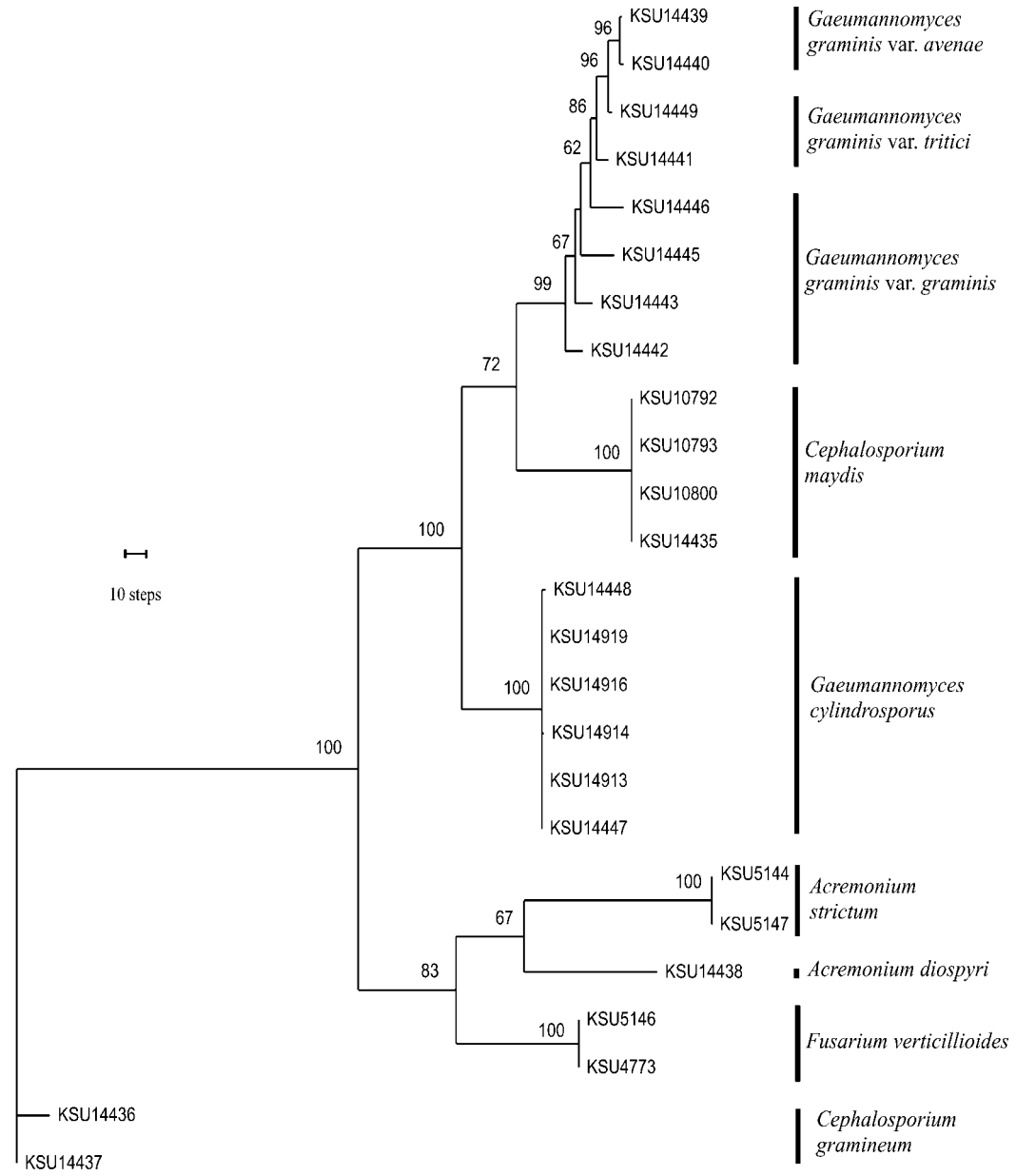

FIG. 2. One of the most parsimonious trees inferred from sequences of rDNA-ITS, β-tubulin and histone H3. The percent bootstrap values obtained from 1000 replications are indicated above the branches. Tree length is 806 steps, CI = 0.77 and RI = 0.88. *C. gramineum* served as outgroup.

strictum (*C. acremonium*) and *G. cylindrosporus* had no bands in common with any of the other species examined and should all be considered distinct species.

The analysis of three sequenced genes is consistent with the conclusions of the AFLP study. The similarity of the β-tubulin, rDNA ITS and histone H3 sequences was almost 100% among the strains representing the four lineages of *C. maydis*. The only difference in the nucleotide sequences of the three genes between the four lineages occurred at two single nucleotide insertions in the ITS-1 region of the rDNA sequence. The lineage II strains had both these inserted nucleotides, whereas lineage IV strains had neither. Strains of lineages I and III had one or the other of these nucleotide insertions. Based on DNA sequences already deposited at GenBank (Ward and Bateman 1999), the Egyptian strains previously included in GenBank belong to lineage II and the Indian strain represented there belongs to lineage IV. Moreover, *C. maydis* rDNA-ITS sequences were not the same as those from *G. graminis* var. *maydis* Yao et al (GenBank accession number AY120939), which causes take-all disease of maize (Yao et al 1993).

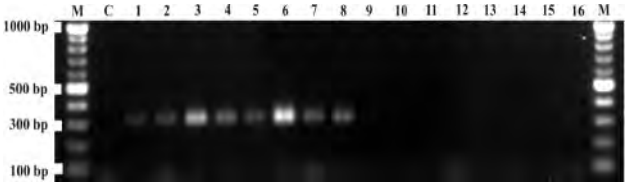

FIG. 3. PCR amplification products obtained with the cmaflp11 and cmaflp12 primers to differentiate between *H. maydis* strains from other related fungi. Lanes: M, 100 bp DNA Ladder marker (Invitrogen, Carlsbad, California); C, negative control that contains everything in the reaction mix except DNA; 1–8, *H. maydis* (includes representatives of all four lineages); 9, *C. gramineum*; 10, *A. strictum*; 11, *A. diospyri*; 12, *G. cylindrosporus*; 13, *G. graminis* var. *avenae*; 14, *G. graminis* var. *graminis*; 15, *G. graminis* var. *tritici*; and 16, *F. verticillioides*.

Although *C. maydis* is a vascular wilt pathogen, and hence unlike the other pathogens in the *Gaeumannomyces-Harpophora* species complex, which are root-infecting pathogens, it is clearly a soilborne disease. The AFLP profiles and DNA sequences reported here clearly place *C. maydis* in the *Gaeumannomyces-Harpophora* species complex, a conclusion consistent with previous studies (Domsch and Gams 1972, Gams 2000, Walker 1981, Ward and Bateman 1999). Thus, *Cephalosporium maydis* Samra, Sabet & Hingorani (in *Phytopathology* 53:404–405, 1963) is recognized as *Harpophora maydis* (Samra, Sabet & Hingorani) Gams (in *Studies in Mycology* 45:192), because its morphological and cultural characters resemble those of *Harpophora* (Domsch and Gams 1972, Gams 2000, Walker 1981), and the molecular characters place it firmly within the *Gaeumannomyces-Harpophora* species complex.

Gaeumannomyces-Harpophora *complex*.—Morphological characters used to delimit species of *Gaeumannomyces* include hyphopodia (structures of attachment and penetration produced by epiphytic hyphae on the host), perithecia, asci and ascospores (Walker 1981). We included two species of *Gaeumannomyces* in this study, *G. cylindrosporus* and *G. graminis*. The three varieties of *G. graminis* cannot be distinguished by their *Harpophora* anamorphs. GGA and GGT have simple hyphopodia, whereas GGG has lobed hyphopodia both on plants and in axenic culture. Nucleotide sequence variation in the exon regions of the rDNA, β-tubulin and histone H3 sequences from the *G. cylindrosporus* strains was very low, while the nucleotide variation of similar sequences from the *G. graminis* strains were much more heterogeneous.

The *G. graminis* strains formed a strongly supported clade (FIG. 2) in which the GGA and GGT strains were closer to each other than either set of strains was to the strains representing GGG. Although the GGG strains formed a monophyletic clade in our study, differences in both nucleotide sequence and AFLP profile were sufficient to prevent any conclusions as to the number of species into which this taxon eventually might be resolved. These results are consistent with previous studies based on morphology and host range (Walker 1981, Bryan et al 1999), RFLP hybridization and rDNA-ITS sequences (Bryan et al 1995, Ward and Akrofi 1994, Fouly et al 1997, Ward and Bateman 1999) and RAPD profiles (Fouly et al 1996).

Both isolates of *A. strictum* (KSU 5144 and KSU 5147) were isolated from sorghum in Egypt. The DNA sequences of the three genes we examined were identical for these two strains. The rDNA ITS sequence of KSU 5144 was ~91% similarity to that of the *A. strictum* type strain CBS 346.70 (GenBank accession number AY138845). The sequences from the strains of *A. strictum* we examined were more similar (>95%) to a strain of *Nectria mauritiicola* (NRRL 20420; GenBank accession number AJ557830) that was identified morphologically as *A. strictum* by Novicki et al (2003). They suggested that *A. strictum* either is polyphyletic or a genetically diverse taxon, a conclusion supported by our analysis of the two strains described here.

C. gramineum is the causal agent of Cephalosporium stripe of winter wheat as well as many other graminaceous plants (Bockus 1992), and its generic position is known to be in need of correction (Bruehl 1963, Farr et al 1989). We could not amplify DNA fragments from this fungus with the β-tubulin or *MAT-2* primers we used. For the rDNA-ITS region, there were 25 polymorphic sites in the exon region for the entire set of species examined. *C. gramineum* differed from *H. maydis* at 14 of these sites, with 10 of these site differences unique to *C. gramineum*. With respect to the histone H-3 region, the introns within the *C. gramineum* sequence are positioned differently from those in any of the other species examined. Similarly, of the 82 polymorphic sites for the entire set of species in the histone H3 exon, *C. gramineum* differed from *H. maydis* at 43 sites, 13 of which were unique to *C. gramineum*. Thus, *C. gramineum* appears to be distantly related to both the *Acremonium-Cephalosporium* and the *Gaeumannomyces-Harpophora* species complexes fungi. Indeed, *F. verticillioides* is more closely related to the other members of the *Acremonium-Cephalosporium* species complex than is *C. gramineum* (FIG. 2). The closest match with *C. gramineum* rDNA-ITS sequence in the EMBL and GenBank databases was to *Rhynchosporium secalis* (96%) (Goodwin 2002). Thus, additional

work is needed to determine the evolutionary position of this species.

PCR primers for identifying H. maydis.—PCR primers designed on the basis of AFLP markers potentially can be used for rapid identification and detection of *H. maydis*. This diagnostic PCR-based method is quick and easy to apply. Such primers are of particular importance because *H. maydis* is not widely distributed yet and both pure cultures and infected materials are subjected to plant quarantines and other restrictions in movement. These primers can be synthesized locally and used for diagnosis even if authenticated cultures of the fungus are not available for comparative analyses.

In conclusion, *H. maydis* belongs in the *Gaeumannomyces-Harpophora* species complex even though the vascular wilt it causes is quite different from the root diseases caused by other pathogenic species in this species complex. The species-specific primers we developed can be used to rapidly identify this pathogen when it is found in new locations. We found that *C. gramineum* clearly falls outside either the *Acremonium-Cephalosporium* or *Gaeumannomyces-Harpophora* species complexes and that the evolutionary position and nomenclatural status of this species require further investigation and reconsideration. Within *Gaeumannomyces graminis,* the two varieties *avenae* and *tritici* could be the same species and GGG may need to be divided into several species.

ACKNOWLEDGMENTS

This work was financially supported in part by the Kansas Agricultural Experimentation Station, and ATUT collaborative research grant No. 58-314-7-057 through the U.S. Agency for International Development (Cairo, Egypt). Mr. Saleh was supported by a fellowship from the Institute of International Education. We thank Bill Bockus for providing us with cultures of *Cephalosporium gramineum* and *Gaeumannomyces graminis* var. *tritici*; Ned Tisserat for providing us with cultures of *Acremonium diospyri, Gaeumannomyces cylindrosporus, Gaeumannomyces graminis* var. *avenae, Gaeumannomyces graminis* var. *graminis* and *Gaeumannomyces graminis* var. *tritici*; Amy Beyer and Brook van Scoyoc for technical assistance; and Walter Gams and Kurt Zeller for critically reading the manuscript. Contribution 04-179-J from the Kansas Agricultural Experimentation Station, Manhattan.

LITERATURE CITED

Arie T, Christiansen SK, Yoder OC, Turgeon BG. 1997. Efficient cloning of Ascomycete mating type genes by PCR amplification of the conserved *MAT* HMG box. Fungal Genet Biol 21:118–130.

Bandyopadhyay R, Mughogho LK, Satyanarayana MV. 1987. Systemic infection of sorghum by *Acremonium strictum* and its transmission through seed. Plant Dis 71:647–650.

Bockus WW. 1992. *Cephalosporium*. In: Singleton LL, Mihail JD, Rush CM, eds. Methods for research in soilborne phytopathogenic fungi. St. Paul, Minnesota: APS Press. p 100–102.

———, Claassen MM. 1985. Effect of lime and sulfur application to low-pH soil on incidence of *Cephalosporium* stripe of winter wheat. Plant Dis 69:576–578.

Bruehl GW. 1963. *Hymenula cerealis,* the sporodochial stage of *Cephalosporium gramineum*. Phytopathology 53:205–208.

Bryan GT, Daniels MJ, Osbourn AE. 1995. Comparison of fungi within *Gaeumannomyces-Phialophora* complex by analysis of ribosomal DNA sequences. Appl Environ Microbiol 61:681–689.

———, Labourdette E, Melton ER, Nicholson P, Daniels MJ, Osbourn AE. 1999. DNA polymorphisms and host range in the take-all fungus, *Gaeumannomyces graminis*. Mycol Res 103:319–327.

Correll JC, Klittich CJR, Leslie JF. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640–1646.

Couch HB. 1995. Diseases of Turfgrasses. 3rd ed. Malabar, Florida: Krieger Publ. Co.

Dernoeden PH, O'Neill NR. 1983. Occurrence of *Gaeumannomyces* patch disease in Maryland and growth and pathogenicity of the causal agent. Plant Dis 67:528–532.

Domsch KH, Gams W. 1972. Fungi in agricultural soils. New York: Halsted Press.

Elliott ML. 1991. Determination of etiological agent of bermudagrass decline. Phytopathology 81:1380–1384.

———, Hagan AK, Mullen JM. 1993. Association of *Gaeumannomyces graminis* var. *graminis* with St. Augustine grass root rot disease. Plant Dis 77:206–209.

Farr DF, Bills GF, Chamuris GP, Rossman AY. 1989. Fungi on plants and plant products in the United States. St. Paul, Minnesota: APS Press.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution 39:783–791.

Fouly HM, Wilkinson HT, Domier LL. 1996. Use of random amplified polymorphic DNA (RAPD) for identification of *Gaeumannomyces* species. Soil Biol Biochem 28:703–710.

———, ———, Chen W. 1997. Restriction analysis of internal transcribed spacers and the small subunit gene of ribosomal DNA among four *Gaeumannomyces* species. Mycologia 89:590–597.

Gams W. 1971. *Cephalosporium*-artige Schimmelpilze (*Hyphomycetes*). Stuttgart, Germany: G. Fischer.

———. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Stud Mycol 45:187–199.

Goodwin SB. 2002. The barley scald pathogen *Rhynchosporium secalis* is closely related to the discomycetes *Tapesia* and *Pyrenopeziza*. Mycol Res 106:645–654.

Halls LK. 1990. *Diospyros virginiana* L. common persimmon. In: Burns RM, Honkala BH, eds. Silvics of North America. Vol. 2. Hardwoods. Washington, D.C.: U.S. Department of Agriculture, Forest Service. p 294–298.

Hanlin TR, Foudin LL, Berisford Y, Glover SU, Jones JP, Huang LH. 1978. Plant disease index for maize in the United States, Part I: host index. Agric Expt Sta, Univ of Georgia (Athens), Res Rept 277:1–62.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. System Biol 42:182–192.

Jackson N, Landschoot PJ. 1986. *Gaeumannomyces cylindrosporus* associated with diseased turfgrass in Rhode Island. Phytopathology 76:654.

Kerényi Z, Zeller KA, Hornok L, Leslie JF. 1999. Standardization of mating-type terminology in the *Gibberella fujikuroi* species complex. Appl Environ Microbiol 65: 4071–4076.

Leslie JF, Pearson CAS, Nelson PE, Toussoun TA. 1990. *Fusarium* species from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathology 80:343–350.

Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA, Leslie JF. 2001. *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia 93:1203–1210.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325.

Novicki TJ, Lafe K, Bui L, Bui U, Geise R, Marr K, Cookson B. 2003. Genetic diversity among clinical isolates of Acremonium strictum determined during an investigation of a fatal mycosis. J Clin Microbiol 41:2623–2628.

O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116.

Payak MM, Lal S, Lilaramani J, Renfro BL. 1970. *Cephalosporium maydis*—a new threat to maize in India. Ind Phytopathol 23:562–569.

Pecsi S, Nemeth L. 1998. Appearance of *Cephalosporium maydis* Samra, Sabet and Hingorani in Hungary. Facult Landbouw en Toegepaste Biolog Wetenschappen, Univer Gent 63:873–877.

Rehmany AP, Lynn JR, Tör M, Holub EB, Beynon JL. 2000. A comparison of *Peronospora parasitica* (downy mildew) isolates from *Arabidopsis thaliana* and *Brassica oleracea* using amplified fragment length polymorphism and internal transcribed spacer 1 sequence analysis. Fungal Genet Biol 30:95–103.

Saleh AA, Zeller KA, Ismael A-SM, Fahmy ZM, El-Assiuty EM, Leslie JF. 2003. Amplified fragment length polymorphism (AFLP) diversity in *Cephalosporium maydis* from Egypt. Phytopathology 93:853–859.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Samra AS, Sabet KA, Hingorani MK. 1962. A new wilt disease of maize in Egypt. Plant Dis Reptr 46:481–483.

———, ———, ———. 1963. Late wilt disease of maize caused by *Cephalosporium maydis*. Phytopathology 53: 402–406.

Steenkamp ET, Wingfield BW, Coutinho TA, Wingfield MJ, Marasas WFO. 1999. Differentiation of *Fusarium subglutinans* f. sp. *pini* by histone gene sequence data. Appl Environ Microbiol 65:3401–3406.

Swofford DL. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23:4407–4414.

Walker J. 1981. Taxonomy of take-all fungi and related genera and species. In: Asher MCJ, Shipton PL, eds. Biology and control of take-all. San Diego, California: Academic Press. p 15–74.

Ward E, Akrofi AY. 1994. Identification of fungi in the *Gaeumannomyces-Phialophora* complex by RFLPs of PCR-amplified ribosomal DNAs. Mycol Res 98:219–224.

———, Bateman GL. 1999. Comparison of *Gaeumannomyces*- and *Phialophora*-like fungal pathogens from maize and other plants using DNA methods. New Phytol 141: 323–331.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplified and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego, California: Academic Press. p 315–322.

Wilkinson HT. 1994. Root rot of centipede grass (*Eremochloa ophiuroides* [Munro] Hack.) caused by *Gaeumannomyces graminis* ([Sacc.] Aex & Olivier) var. *graminis*. Plant Dis 78:1220.

———, Pedersen D. 1993. *Gaeumannomyces graminis* var. *graminis* infecting St. Augustine grass in southern California. Plant Dis 77:536.

Yao JM. 1993. The discovery and classification of the corn take-all pathogen in China. Proc 6th Internat Cong Plant Pathol (Montreal, Canada):132.

Zeller KA, Jurgenson JE, El-Assiuty EM, Leslie JF. 2000. Isozyme and amplified fragment length polymorphisms from *Cephalosporium maydis* in Egypt. Phytoparasitica 28:121–130.

———, Summerell BA, Bullock S, Leslie JF. 2003. *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. Mycologia 95:943–954.