Mycologia, 103(4), 2011, pp. 806–819. DOI: 10.3852/10-155

New species of *Fusarium* associated with dieback of *Spartina alterniflora* in Atlantic salt marshes

Wade H. Elmer[1]
Robert E. Marra

Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven, Connecticut 06504

Abstract: Sudden vegetation dieback (SVD) is the loss of smooth cordgrass (*Spartina alterniflora*) along intertidal creeks in salt marshes of the Atlantic and Gulf states. The underlying cause of SVD remains unclear, but earlier work suggested a contributing role for *Fusarium* spp. in Louisiana. This report investigated whether these or other *Fusarium* species were associated with *S. alterniflora* dieback in mid- to north-Atlantic states. Isolations from seven SVD sites yielded 192 isolates of *Fusarium* spp., with more than 75% isolated from aboveground tissue. Most isolates (88%) fell into two undescribed morphospecies (MS) distinguished from each other by macroconidial shape, phialide ontogeny and growth rates. Pathogenicity tests on wound-inoculated *S. alterniflora* stems and seedling roots revealed that isolates in MS1 were more virulent than those in MS2 but no single isolate caused plant mortality. No matches to known species of *Fusarium* were revealed by DNA sequence queries of translation elongation factor 1-α (*tef1*) sequences. A phylogenetic analysis of partial sequences of three genes, β-tubulin (β-*tub*), calmodulin *(cal)* and *tef1*, was conducted on representative isolates from MS1 (n = 20) and MS2 (n = 18); it provided strong evidence that the MS1 isolates form a clade that represents a heretofore undescribed species, which we designate *Fusarium palustre* sp. nov. Isolates from the more variable MS2 clustered with the *F. incarnatum-equiseti* species complex as *F.* cf. *incarnatum*. Although a strong association exists between both species and declining *S. alterniflora* in SVD sites, neither appears to play a primary causal role in SVD. However, our findings suggest that *F. palustre* might play an important secondary role in the ecological disruption of the salt marshes.

Key words: *Fusarium palustre*, morphology, pathology, phylogeny, smooth cordgrass, sudden vegetation dieback

Submitted 14 May 2010; accepted for publication 29 Dec 2010.
[1] Corresponding author. E-mail: wade.elmer@ct.gov

INTRODUCTION

Salt marshes are immeasurably valuable to humans and are among the most productive ecosystems on the planet, producing in excess of 3 kg dry matter/m^2/y (Bertness 2007). Salt marshes protect coastal communities from storm surges and wave erosion, absorb excess nitrogen released via sewage and fertilizer runoff into rivers and detoxify hydrocarbon-contaminated runoff from industrial areas. These unique ecosystems provide habitat and shelter for many hundreds of species of shellfish, finfish, migratory and sedentary birds, and other marine animals. Of interest, despite this species richness, only a few plant species dominate the intertidal marshes of the Atlantic salt marsh ecosystem. Of these the dominant plant species is smooth cordgrass (*Spartina Spartina alterniflora* Loisel.).

In 2002 wetland ecologists in New England noticed that large areas of marshes along tidal creeks had become barren, leaving areas of remnant peat (Alber et al. 2008). In the absence of any obvious causal agents, the condition reported in Connecticut, Delaware, Georgia, Maine, Massachusetts, New Hampshire, New York, South Carolina and Virginia, was termed Sudden Vegetation Dieback (SVD) (Alber et al. 2008). SVD has been associated with drought, plant pathogens, heavy metal toxicity, wrack deposition, fire, pollutants, erosion from ice rafting, and herbivory of geese, crabs and snails (Holdredge et al. 2009, Silliman et al. 2005, Smith 2009), but none of these, either singly or in combination, can be attributed to the rapid vegetation loss in all SVD sites. Examination of aerial photographs suggested that in many New England SVD sites the dieback occurred years before its discovery (Smith 2009), perhaps in some cases coincident with the widespread dieback of salt marshes that occurred along Louisiana's coast in 2000, when more than 150 000 Ha suddenly perished (McKee et al. 2004). While the phenomenon of SVD continues to occur, its causes remain elusive.

Plant pathogens had been suspected in a salt marsh dieback that occurred in the UK in the 1950s, in which more than 200 ha of *Spartina townsendii* Groves were denuded (Goodman 1959, Goodman et al. 1959). However, of more than 20 fungal species that were isolated from dead rhizomes onto nonselective media, none was shown to be pathogenic, resulting in the conclusion that a pathogen was not involved. By contrast Useman and Schneider (2005) reported that

TABLE I. Origin of *Spartina alterniflora* plants and *Fusarium* colonies obtained from SVD sites and healthy sites

Marsh, city, state	*Fusarium* isolates	*F. palustre*[a]	*F.* cf. *incarnatum*[b]	*F. solani*[a]	*F. oxysporum*	*F. proliferatum*
Tom's Creek tributary, Madison, CT	62	44	14	2	2	0
Prime Hook Natural Wildlife Preserve, Milton, DE	32	8	18	3	1	2
Sapelo Island, GA	15	2	12	1	0	0
Drakes Island, Wells, ME	18	17	1	0	0	0
Lieutenant Island, Cape Cod, Wellfleet, MA	12	12	0	0	0	0
Great Sippewissett Marsh, Sippewisset, MA	10	7	2	1	0	0
Flax Pond, Old Field, NY	9	3	2	1	1	2
Upper Phillips Creek Marsh, Nassawadox, VA	34	11	16	4	2	1
Totals	192	104	65	12	6	5

[a] Morphospecies originally were based on specific phenotypes on carnation leaf agar and potato dextrose agar.
[b] Species that resemble *F. incarnatum*.

declining *S. alterniflora* plants on the perimeter of SVD sites in Louisiana had symptoms of black leaf spots and internal stem rots. Isolates of *Fusarium proliferatum*, *F. fujikuroi*, along with an undescribed species of *Fusarium*, all belonging to the *Gibberella fujikuroi* species complex (Elmer unpubl), were associated with declining plants in Louisiana. Although Koch's postulates were satisfied, the *Fusarium* pathogens were suggested in this case to be endophytes opportunistically contributing to the decline of plants that already were stressed.

In an effort to determine whether pathogenic *Fusarium* species also might be associated with affected *S. alterniflora* in SVD sites of the mid- and north-Atlantic coasts, the objectives of this study were threefold: (i) to identify the *Fusarium* species associated with *S. alterniflora* plants in SVD sites ranging from the mid-Atlantic and into northern coastal New England, (ii) to determine which of these species may be pathogenic on *S. alterniflora* and (iii) to determine with morphological and molecular analyses the phylogenetic placement of isolates.

MATERIALS AND METHODS

Origin of isolates.—*Spartina alterniflora* plants either were sampled by the authors or obtained from colleagues (TABLE I). Plants were removed from the perimeter of an SVD site and hand-carried to the laboratory or transported via postal mail. Plants from Flax Pond, Old Village, New York, were from a marsh undergoing submergence, not from an SVD site. Plants were rinsed in tap water and examined for discolored tissue or lesions on roots, stems and leaves. Pieces of tissue (0.5 cm) were surface-disinfested in 10% household bleach (0.053% NaOCl), rinsed in distilled water, blotted dry on absorbent paper and placed on peptone PCNB agar (PPA) dishes (Leslie 1993). After 5–7 d at room temperature (18–25 C), *Fusarium* colonies were subcultured on carnation-leaf agar (CLA) (Leslie and Summerell 2006). Monosporic cultures established from these cultures were placed on CLA and potato dextrose agar (PDA) under cool-white and black-light fluorescent lights set for a 12 h photoperiod for 7–10 d and then used for identification and, if possible, species determination. Representatives from each site were stored for short term culture on silica gel (Windels et al. 1988). In addition, subsets of cultures were deposited at CBS Fungal Biodiversity Centre (CBS, Utrecht, the Netherlands) and National Center for Agricultural Utilization (NRRL, Peoria, Illinois). We performed all following assays on monosporic cultures subcultured from these stored isolates.

Morphology and growth.—Isolates (n = 192) were examined under an Olympus microscope (Olympus, Center Valley, Pennsylvania) BX41 at 100× and 400× magnification and compared to known species of *Fusarium* (Leslie and Summerell 2006). When isolates could not be assigned to a known species, they were assigned to morphological groups or morphospecies (MS) based on phenotypes, such as macroconidial shape and phialide type. We made digital photographs with a Zeiss (Carl Zeiss, Thornwood, New York) Axioplan 2 imaging microscope with differential interference contrast.

Radial growth rates were determined on PDA plates incubated in the dark at 20, 25, 30 and 35 C for 3 d. One agar plug (4 mm diam) was removed from an actively growing culture on CLA and placed in the center of one PDA plate. There were two replicate plates per isolate/temperature, and we repeated the study once.

DNA isolation and PCR amplification.—Monosporic *Fusarium* cultures used in phylogenetic analysis comprised 20 representative cultures from MS1 and 18 cultures from MS2. Isolates from each MS were chosen to represent the breadth of geographic distribution of the study sites (TABLE II). Representative isolates sequenced in the study (TABLE II) are available at the ARS Culture Collection at NCAUR, Peoria, Illinois. Additional cultures sequenced for this study included *F. equiseti*, *F.* cf. *incarnatum*, *F. langsethiae* and *F. sporotrichioides* and as otherwise indicated (TABLE II). These cultures were included due to their similarities, both morphological and genetic, with the *S. alterniflora* test isolates. Cultures were transferred from PDA Petri plates to

TABLE II. Names, sources, origin, virulence and sequence accession numbers of *Fusarium* isolates from *Spartina alterniflora* and reference strains

Species	Source	Host	Origin	GenBank accession numbers		
				β-tub	*Cal*	*tef1*
Fusarium palustre	CaesSaCT10 (= NRRL 54054)	*Spartina alterniflora*	Madison, Connecticut	GQ856991	GQ857032	GQ856949
F. palustre	CaesSaCT2 (= NRRL 54056 = CBS 126796)	*S. alterniflora*	Madison, Connecticut	GQ856983	GQ857024	GQ856941
F. palustre	CaesSaCT3 (= NRRL 54058	*S. alterniflora*	Madison, Connecticut	GQ856984	GQ857025	GQ856942
F. palustre	CaesSaCT4 (= NRRL 54063)	*S. alterniflora*	Madison, Connecticut	GQ856985	GQ857026	GQ856943
F. palustre	CaesSaCT5 (= NRRL 54064 = CBS 126794)	*S. alterniflora*	Madison, Connecticut	GQ856986	GQ857027	GQ856944
F. palustre	CaesSaCT6 (= NRRL 54053)	*S. alterniflora*	Madison, Connecticut	GQ856987	GQ857028	GQ856945
F. palustre	CaesSaCT7 (= NRRL 54055)	*S. alterniflora*	Madison, Connecticut	GQ856988	GQ857029	GQ856946
F. palustre	CaesSaCT8 (= NRRL 54057)	*S. alterniflora*	Madison, Connecticut	GQ856989	GQ857030	GQ856947
F. palustre	CaesSaCT9 (= NRRL 50462)	*S. alterniflora*	Madison, Connecticut	GQ856990	GQ857031	GQ856948
F. palustre	CaesSaMA1 (= NRRL 54059)	*S. alterniflora*	Cape Cod, Massachusetts	GQ856999	GQ857040	GQ856957
F. palustre	CaesSaMA2	*S. alterniflora*	Cape Cod, Massachusetts	GQ857000	GQ857041	GQ856958
F. palustre	CaesSaMA3 (= NRRL 54060)	*S. alterniflora*	Cape Cod, Massachusetts	GQ857001	GQ857042	GQ856959
F. palustre	CaesSaMA4 (= NRRL 54065 = CBS 126793)	*S. alterniflora*	Cape Cod, Massachusetts	GQ857002	GQ857043	GQ856960
F. palustre	CaesSaMA5 (= NRRL 54066)	*S. alterniflora*	Cape Cod, Massachusetts	GQ857003	GQ857044	GQ856961
F. palustre	CaesSaME1	*S. alterniflora*	Wells, Maine	GQ857007	GQ857048	GQ856965
F. palustre	CaesSaME2	*S. alterniflora*	Wells, Maine	GQ857008	GQ857049	GQ856966
F. palustre	CaesSaME3 (= NRRL 54049)	*S. alterniflora*	Wells, Maine	GQ857009	GQ857050	GQ856967
F. palustre	CaesSaMe4 (= NRRL 54050)	*S. alterniflora*	Wells, Maine	GQ857010	GQ857051	GQ856968
F. palustre	CaesSaME6 (= CBS 126767)	*S. alterniflora*	Wells, Maine	GQ857011	GQ857052	GQ856969
F. palustre	CaesSaNY1 (= NRRL 50452 = CBS 126795)	*S. alterniflora*	Long Island, New York	GQ857012	GQ857053	GQ856970
F. cf. *incarnatum*	CaesSaCT11	*S. alterniflora*	Madison, Connecticut	GQ856992	GQ857033	GQ856950
F. cf. *incarnatum*	CaesSaCT12 (= NRRL 54072)	*S. alterniflora*	Madison, Connecticut	GQ856993	GQ857034	GQ856951
F. cf. *incarnatum*	CaesSaDE4 (= NRRL 54074)	*S. alterniflora*	Milton, Delaware	GQ856994	GQ857035	GQ856952
F. cf. *incarnatum*	CaesSaGA1	*S. alterniflora*	Sapelo Island, Georgia	GQ856995	GQ857036	GQ856953
F. cf. *incarnatum*	CaesSaGA2 (= NRRL 54069)	*S. alterniflora*	Sapelo Island, Georgia	GQ856996	GQ857037	GQ856954
F. cf. *incarnatum*	CaesSaGA3 (= NRRL 54070)	*S. alterniflora*	Sapelo Island, Georgia	GQ856997	GQ857038	GQ856955
F. cf. *incarnatum*	CaesSaGA4	*S. alterniflora*	Sapelo Island, Georgia	GQ856998	GQ857039	GQ856956

TABLE II. Continued

Species	Source	Host	Origin	GenBank accession numbers		
				β-tub	*Cal*	*tef1*
F. cf. *incarnatum*	CaesSaMA6 (= NRRL 54073)	*S. alterniflora*	Cape Cod, Massachusetts	GQ857004	GQ857045	GQ856962
F. cf. *incarnatum*	CaesSaMA7 (= NRRL 54075)	*S. alterniflora*	Cape Cod, Massachusetts	GQ857005	GQ857046	GQ856963
F. cf. *incarnatum*	CaesSaMA8	*S. alterniflora*	Cape Cod, Massachusetts	GQ857006	GQ857047	GQ856964
F. cf. *incarnatum*	CaesSaNY2 (= NRRL 54067)	*S. alterniflora*	Long Island, New York	GQ857013	GQ857054	GQ856971
F. cf. *incarnatum*	CaesSaVA1 (= NRRL 54078)	*S. alterniflora*	Nassawadox, Virginia	GQ857014	GQ857055	GQ856972
F. cf. *incarnatum*	CaesSaVA2 (= NRRL 50479)	*S. alterniflora*	Nassawadox, Virginia	GQ857015	GQ857056	GQ856973
F. cf. *incarnatum*	CaesSaVA3	*S. alterniflora*	Nassawadox, Virginia	GQ857016	GQ857057	GQ856974
F. cf. *incarnatum*	CaesSaVA4 (= NRRL 54081)	*S. alterniflora*	Nassawadox, Virginia	GQ857017	GQ857058	GQ856975
F. cf. *incarnatum*	CaesSaVA5 (= NRRL 54082)	*S. alterniflora*	Nassawadox, Virginia	GQ857018	GQ857059	GQ856976
F. cf. *incarnatum*	CaesSaVA6 (= NRRL 54083)	*S. alterniflora*	Nassawadox, Virginia	GQ857019	GQ857060	GQ856977
F. cf. *incarnatum*	CaesSaVA7	*S. alterniflora*	Nassawadox, Virginia	GQ857020	GQ857061	GQ856978
F. equiseti	CaesCvKR3	*Coreopsis verticilliata*	Michigan	N/A	GQ857062	GQ856979
F. incarnatum	CaesAoCTa	*Asparagus officinalis*	Connecticut	GQ857021	GQ857063	GQ856980
F. oxysporum[a]	NRRL 20433	*Vicia faba*	Germany	U34435	AF158366	AF008479
F. langsethiae	NRRL 34176	unknown	unknown	GQ857022	GQ857064	GQ856981
F. sporotrichioides	CaesZmN205	*Zea mays*	Franklin, Connecticut	GQ857023	GQ857065	GQ856982
Gibberella zeae	PH-1 (= NRRL 31084)	*Z. mays*	Michigan	NT086558	NT086521	NT086557

[a] Originally identified as *F. inflexum*.

sterile 2.0 mL tubes containing 1.0 mL sterile potato dextrose broth (PDB), then allowed to grow without shaking 1 wk at room temperature. Mycelium was harvested by centrifugation, rinsed once with sterile distilled water and lyophilized. Total genomic DNA was extracted from lyophilized mycelium by the hexadecyltrimethyl-ammonium bromide (CTAB, Sigma, St Louis, Missouri) protocol described by O'Donnell et al. (1998b) and was used in PCR reactions at final concentrations of approximately 0.1 ng/μL. PCR was done on a Bio-Rad iCycler (Bio-Rad, Hercules, California) in 30 μL volumes with 0.5 units of Invitrogen (Carlsbad, California) High-Fidelity Platinum Taq, 2.5 mM $MgSO_4$, 0.2 mM each of the four nucleotides and 0.25 μM each primer in the accompanying PCR Buffer (60 mM Tris-SO_4 pH 8.9, 180 mM ammonium sulfate). The pairs of primers used in the reactions were T1/T22 (O'Donnell and Cigelnik 1997) for a portion of the beta-tubulin (β-*tub*) gene, EF1/EF2 (O'Donnell et al. 1998a) for a portion of the translational elongation factor 1-alpha (*tef1*) gene and CL1/CL2A (O'Donnell 2000) for a portion of the calmodulin (*cal*) gene. Cycling parameters for all three genes were 3 min at 94 C, followed by 40 cycles of 35 s at 94 C, 55 s at 52 C, and 2 min at 68 C, followed by 10 min at 68 C and a 4 C soak. The annealing temperature was increased to 55 C for amplification of β-*tub* in *F. equiseti*, *F. incarnatum*, *F. langsethiae* and *F. sporotrichioides* to improve sequence quality. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Valencia, California), then electrophoresed and stained them with ethidium-bromide to determine concentration. Amplicons were then sequenced using BigDye Terminator Cycle Sequencing components (Applied Biosystems, Foster City, California) on an Applied Biosystems 3730 DNA Analyzer. Amplicons were then sequenced using as sequencing primers for all genes. Raw sequence data were edited and assembled with ChromasPro 1.49 (Technelysium Pty Ltd., Queensland, Australia). Coding regions were distinguished from noncoding regions using as a guide the corresponding gene data for *Gibberella zeae* strain PH-1 (GenBank accessions numbers NT086558, NT086521 and NT086557 for β-*tub*, *cal* and *tef1*). Annotated sequences were then deposited in GenBank (TABLE II).

β-*tub* amplification from *F. langsethiae* DNA produced two bands, one ~ 1300 bp as expected and the other ~ 100 bp larger. We were unable to identify thermal cycling conditions that favored amplification of one amplicon over the other. Therefore, to sequence the two bands separately, they were electrophoresed ~ 2 cm apart, excised from the gel, cleaned with the Promega (Madison, Wisconsin) Wizard SV Gel and PCR Clean-Up System, then sequenced as described above.

Sequence analysis.—The unannotated sequences were aligned with MEGA 4.1b3 (Tamura et al. 2007). Additional alignments were created using exons only. The alignments included the *G. zeae* PH-1 GenBank sequences referenced above as well as those from *F. oxysporum* NRRL20433 (GenBank accession numbers U34435, AF158366 and AF008479 for β-*tub*, *cal* and *tef1* respectively). Gaps and unalignable nucleotide positions were excluded from the analyses. All characters were unordered and given equal weight in the phylogenetic analyses, which were performed with maximum parsimony (MP) in MEGA 4 and maximum likelihood (ML) in PhyML 3.0 (Guindon and Gascuel 2003). MP analysis was performed as a heuristic search with 10 random addition sequences, using the Close-Neighbor-Interchange algorithm at search level 7. Bootstrap support for internal branches was obtained using 1000 replicates with parsimony settings as described and 10 random addition sequences per replicate. MP analyses first were performed on the individual gene datasets; once topological concordance was established among the three loci they then were analyzed as a combined dataset with relative branch supports obtained with 1000 bootstrap replicates and all other settings as previously described.

Phylogenetic relationships were also inferred using ML analysis on the combined dataset, using a PhyML-generated neighbor-joining tree, the BIONJ option in PhyML, as the starting tree, with tree topologies optimized and estimated with subtree pruning and regrafting (SPR). Additional settings in PhyML included equilibrium frequencies optimized, proportion of invariable sites estimated, substitution rates divided discretely into 10 categories, with the middle of each substitution rate class determined using the median, and the gamma distribution parameter estimated. Branch support was estimated with the approximate likelihood ratio test (aLRT) (Anisimova and Gascuel 2006) as implemented in PhyML 3.0 with the nonparametric Shimodaira-Hasegawa-like (SH-like) option to test significance of the likelihood ratio test statistics. Five substitution models were tested on the combined dataset under these conditions: Jukes-Cantor 1-parameter (JC69), Kimura 2-parameter (K2P), Hasegawa-Kishino-Yano (HKY85), Tamura-Nei 3-parameter (TN93), and general time reversible (GTR). The GTR model yielded the highest log-likelihood statistic and therefore was chosen for subsequent interpretation and comparison with the MP bootstrap consensus tree. All trees were rooted by the outgroup method with *F. oxysporum* NRRL 20433. Alignments and tree files were deposited with TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10911).

Pathogenicity tests.—Stem inoculations were done on healthy *S. alterniflora* plants that had been removed from a healthy marsh with no SVD. After washing the roots, plants were divided and repotted into 1 L plastic pots filled with 1 : 4 (v/v) sand/soil mixture. Pots were placed in a greenhouse bench lined with a commercial swimming pool liner with water added to maintain moisture. Two sets of pathogenicity tests were performed on a subset of isolates (experiment one, n = 51; experiment two, n = 54) that were representative of each morphospecies from each locale. Isolates were cultured on quarter-strength PDA (9.75 g Difco PDA plus 15 g agar/L), from which colonized agar plugs (4 mm diam) were removed and stab-inoculated below the node of a healthy *S. alterniflora* stem. Inoculation sites were wrapped with Parafilm (American National Can, Chicago, Illinois) to prevent dehydration. Stems inoculated with sterile agar plugs served as controls. After 1 mo the length of the lesion was measured to the nearest millimeter and compared to controls that were stab-inoculated with sterile agar plugs. There were two inoculations per isolate on different plants, and we repeated the experiment 3 mo later. Lesion length from each species or morphospecies was subjected to Kruskal-Wallis one-way analysis of variance at $P < 0.05$.

Three experiments were conducted to assess root infection. Isolates of *Fusarium* (n = 27) were cultured on sterile, moistened millet seed 10 d, air-dried and ground in a blender. Millet colonized by the test isolates was mixed into the soil mixture described above at 2 g millet/L soil mix (Elmer 1995, 2003). We placed three healthy *S. alterniflora* plants produced as described above in a 1 L plastic pot and placed them on greenhouse benches in separate trays. Soil was kept saturated by maintaining at least 5–10 cm water. Three healthy plants were placed in soil amended with sterile millet to serve as a control. After 15 wk plants were removed from the pots, washed, photographed and rated for disease on the scale of 0–5 where 0 = roots were white, no discoloration, 1 = less than 10% of root system discolored, 2 = 11–25% of root system discolored, 3 = 26–50% of root system discolored, 4 = 51–75% of root system discolored and 5 = 76–100% of root system discolored or dead. Fresh and dry above-ground weights were recorded. Data were analyzed by to Kruskal-Wallis one-way analysis of variance at $P < 0.05$.

The ability to infect roots was assessed twice with 4 mo old seedlings produced from seed collected from a Madison, Connecticut, marsh with no SVD. Seeds were disinfested in 0.053% NaOCl (10% household bleach) 1 min, rinsed and germinated in sand. Seedlings were fertilized with 20-10-20 soluble fertilizer once a week for 4 mo. In the first set of experiments with seedlings, *Fusarium* cultures (n = 45 plus one nonpathogenic isolate from the *F. solani* species complex) were grown on PDA dishes 10–12 d, macerated in a Waring blender 1 min with 100 mL dH_2O and mixed into 1 L potting mix (Promix BX, Premier Brand, New Rochelle, New York). The infested mix was dispensed into four 8.4 cm diam (250 cc) plastic pots. One *S. alterniflora* seedling was transplanted into each pot and set on greenhouse benches. Plants were rated after 2 mo on a scale of 1–5 where 1 = green and turgid plants with no

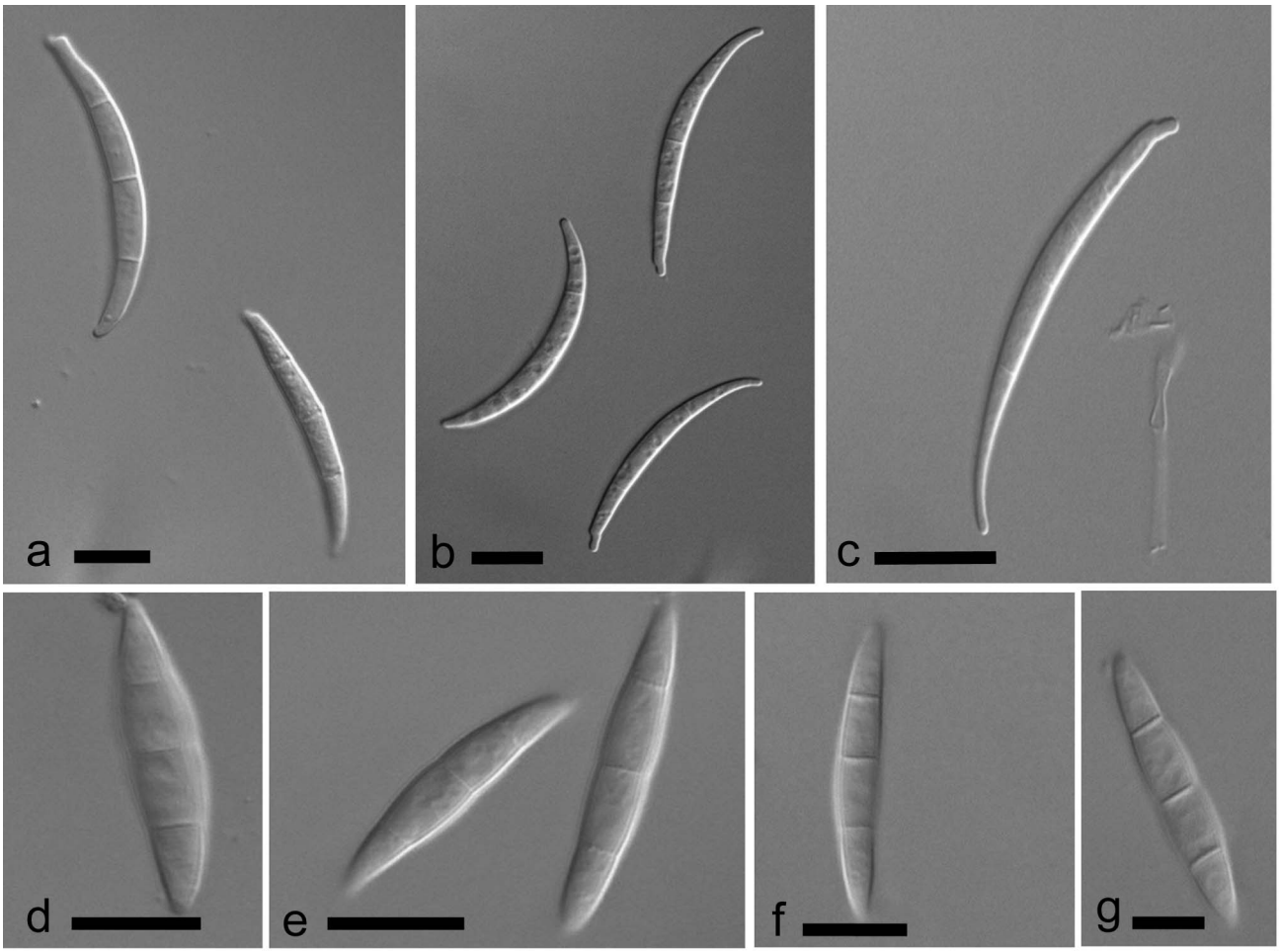

FIG. 1. *Fusarium palustre* (W. Elmer & Marra), isolate CaesSaCT5 (= NRRL 54064). a–c. Macrocondia from sporodochia, bar = 10 μm. e–h. Conidia from aerial mycelium, bar = 10 μm.

disease, 2 = slightly stunted plants, 3 = stunted and chlorotic plants, 4 = stunted with dead stems and 5 = dead. The pathogen was re-isolated from wilted stem tissue. The experiment was repeated on seedlings with a new subset of test isolates (n = 41 plus one nonpathogenic isolate of *F. oxysporum*). In this experiment millet inoculum was prepared as described above and incorporated into autoclaved 1 : 1 peat/sand mix at 3 g per L soil, then dispensed into four 10 cm (370 cc) plastic pots. Inoculum density, assessed by serial dilution onto PPA agar, was 0.5–2 × 10^6 cfu/g soil. Healthy seedlings were transplanted into the pots and placed on greenhouse benches. After 2 mo, the plants were evaluated as described above. Data were analyzed using Kruskal-Wallis one-way analysis of variance at $P < 0.05$.

RESULTS

Phenotypes.—We obtained 192 *Fusarium* colonies from the *S. alterniflora* samples, of which 75 percent were from above-crown tissue. On examination on CLA and PDA, fewer than 12% of the monosporic cultures could be assigned to known species. The remaining isolates were placed tentatively into one of two morphospecies (MS1 and MS2) based on distinct phenotypic characteristics observed on CLA and PDA. Isolates in MS1 produced beige pigmentation on PDA and produced thick-walled 3–4-septate macroconidia from sporodochia (mean length = 36.5 ± 0.6 μm) and conidia from the aerial mycelium (mean length = 32.5 ± 1.0 μm mean length) (FIG. 1). Macroconidia from sporodochia and conidia in the aerial mycelium were born in monophialides (FIG. 2). Microconidia were absent. Chlamydospores appeared singularly but more frequently in intercalary chains in the aerial mycelium (FIG. 2). Isolates in MS2 were beige with brown pigmentation on PDA. Macroconidia were 4–5-septate with more variable shape and length (mean length = 35.0 ± 2.5 μm) (FIG. 3). Conidia in the aerial mycelium were borne on polyphialides similar to the rabbit ears characteristic of *F. incarnatum* (synonym *F. semitectum*) with single chlamydospores borne terminally and/or multiple intercalary chla-

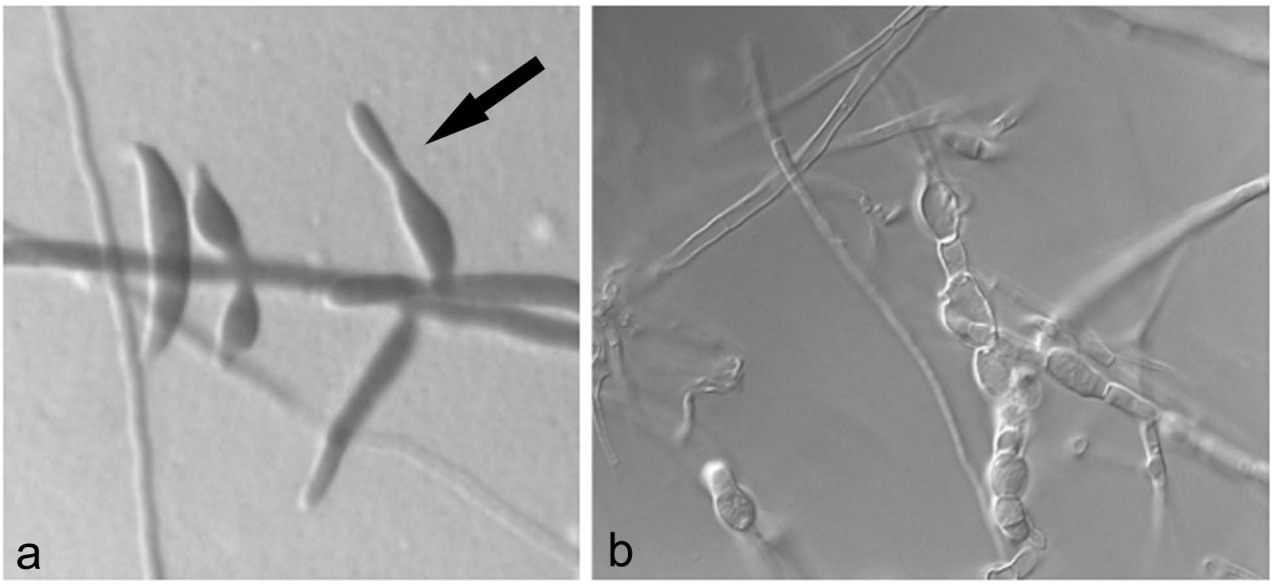

FIG. 2. *Fusarium palustre* (W. Elmer & Marra), isolate CaesSaCT5 (= NRRL 54064). a. Conidia born in monophialides, bar = 10 μm. b. Chlamydospores in chains, bar = 10 μm.

mydospores formed in the aerial mycelium (FIG. 4). All remaining isolates were identified as *F.* cf. *equiseti*, *F. oxysporum*, *F. proliferatum* or *F. solani*.

Radial growth measurements revealed that MS2 isolates grew more slowly than MS1 isolates at all temperatures (FIG. 5). Differences were most pronounced at 20 C and 35 C. All isolates grew fastest at 20 C.

Phylogenetic analyses.—Three alignments were used in the initial single-gene analyses. Each alignment consisted of partial sequences from three genes, β-*tub*, *cal* and *tef1*, from 44 taxa. The ML analyses of individual gene datasets produced trees with nearly identical branching topologies (individual trees not shown), with the GTR model yielding the highest log-likelihood values (−1527.6, −2322.6 and −2753.9 for

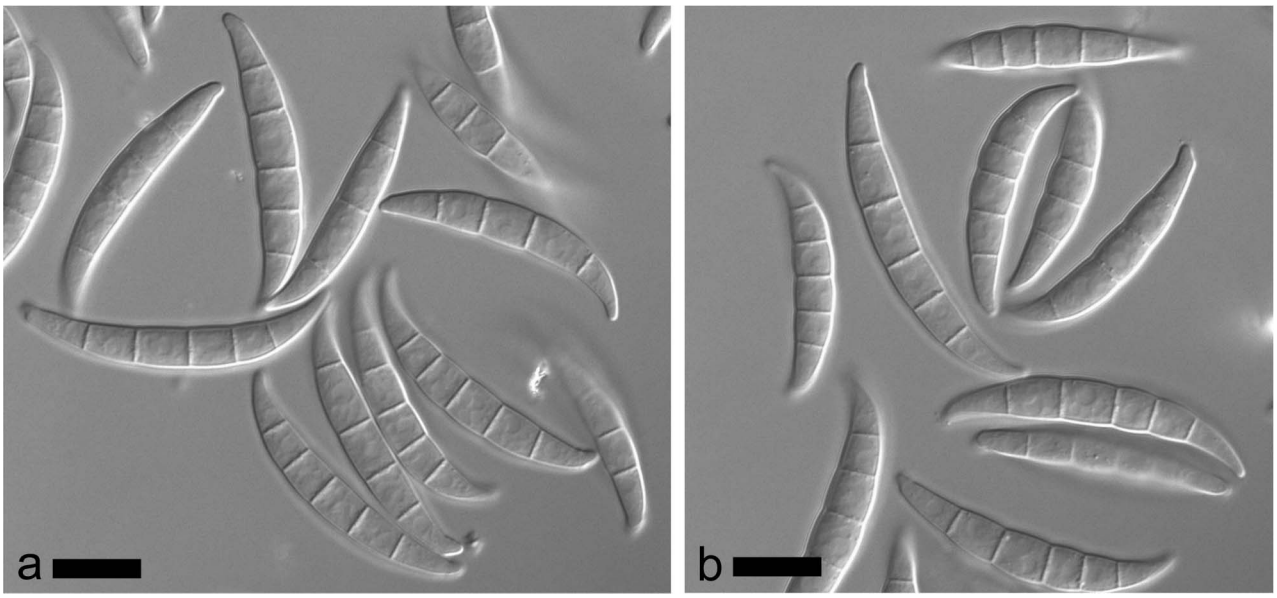

FIG. 3. *Fusarium* cf. *incarnatum*, isolate CaesMa7 (= NRRL 54075). a–b. Macroconidia, bar = 10 μm.

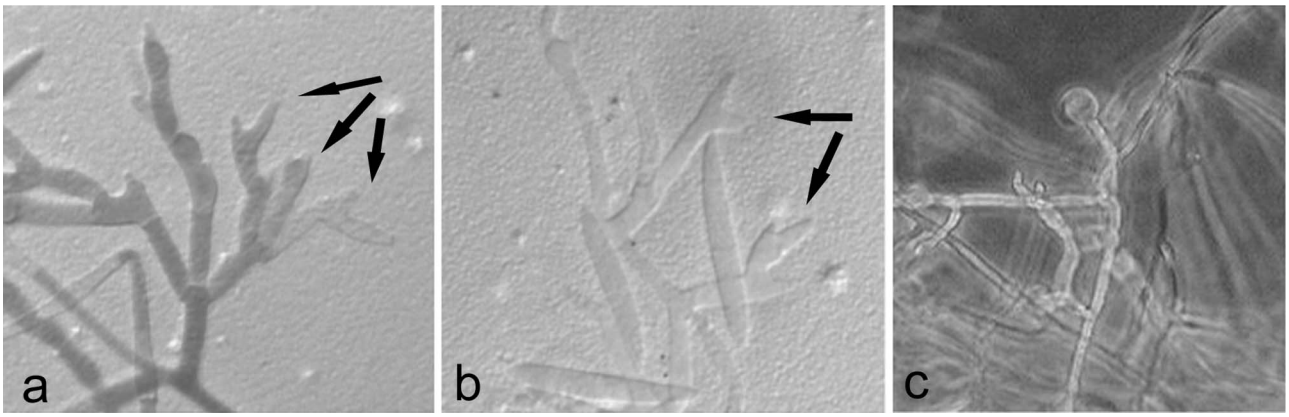

FIG. 4. *Fusarium* cf. *incarnatum*, isolate CaesMa7 (= NRRL 54075). a–b. Polyphialides, bar = 10 μm. c. Chlamydospores borne terminally, bar = 10 μm.

β-*tub*, *cal* and *tef1* respectively) among the five models tested. In the absence of phylogenetic conflict among the three gene regions, we performed a ML analysis on the combined gene alignment. The GTR model also yielded the highest log-likelihood value (−6722.9) for the combined gene dataset, with JC69 producing the lowest (−6687.7); therefore GTR was chosen as the best model for this analysis (FIG. 6). The ML tree based on the combined gene dataset is rooted on *F. oxysporum* as outgroup (FIG. 6). Numbers above each branch indicate the ML probability of a branch being correct, given the data, based on the significance of the aLRT statistics (Anisimova and Gascuel 2006) generated in the PhyML analysis.

For the MP analysis of a total of 450 characters in the β-*tub* alignment 65 were parsimony informative and MP analysis resulted in 13385 equally parsimonious trees. Of 614 characters in the *cal* alignment 130 were parsimony informative and MP analysis resulted in 9502 equally parsimonious trees. Of 514 characters in the *tef1* alignment 126 were parsimony informative and MP analysis resulted in 5217 equally parsimonious trees. As with the ML analyses, the branching topologies for the three separate gene trees were nearly identical, with differences only in the order of isolates within each of the terminal clades. Nearly all major nodes had high (> 80%) bootstrap support in the MP trees produced from the individual gene analyses, demonstrating that there was no phylogenetic conflict among these three gene regions (results for individual trees not shown). The MP analysis of the combined three-gene alignment (1128 characters, 256 of which were parsimony informative) resulted in 2598 equally parsimonious trees, with a consistency index (CI) of 0.714 and a retention index (RI) of 0.960.

MP analysis of the combined dataset produced a tree nearly identical in topology to the ML tree (FIG. 6). MP bootstrap support, expressed as the percentage of bootstrap replicate trees in which the associated taxa also clustered, is indicated below the ML statistic at each major branch. Numbers in parentheses alongside the bootstrap values are the percentages of the 2598 equally parsimonious trees in which the associated taxa also clustered.

The taxa used in the ML and MP analyses, excluding *G. zeae* and *F. oxysporum*, can be divided into two subgroups (FIG. 6). The MS1 subgroup (FIG. 6) clusters with *F. langsethiae* and *F. sporotrichioides*, a topology that was equally apparent in the phylogenies of the individual loci (results not shown), strongly suggesting that isolates in this group belong to a single heretofore undescribed species, which we designate *F. palustre* sp. nov. Although isolate CaesSaCT9 clustered with MS1 in all analyses and was morphologically indistinguishable from them, it branches at a node that

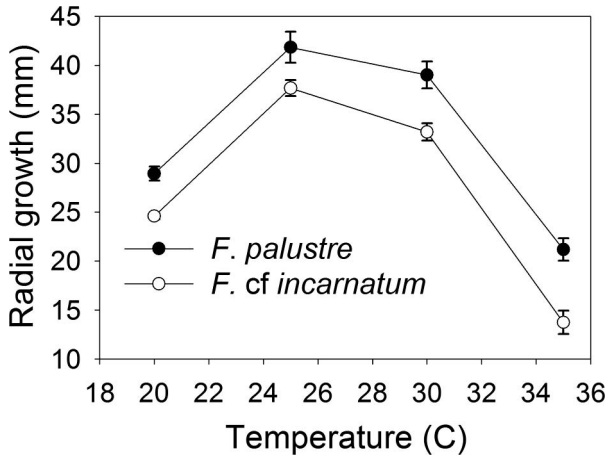

FIG. 5. Mean radial growth rates of isolates of *Fusarium palustre* (isolates CaesSaCT10, CaesSaMA1, CaesSaMA2, CaesSaVA4, CaesSaVA5, CaesSaMA4) and *F.* cf. *incarnatum* (isolates CaesSaCT10, CaesSaMA1, CaesSaMA2, CaesSaVA4, CaesSaVA5, CaesSaMA4) at 20, 25, 30 and 35 C.

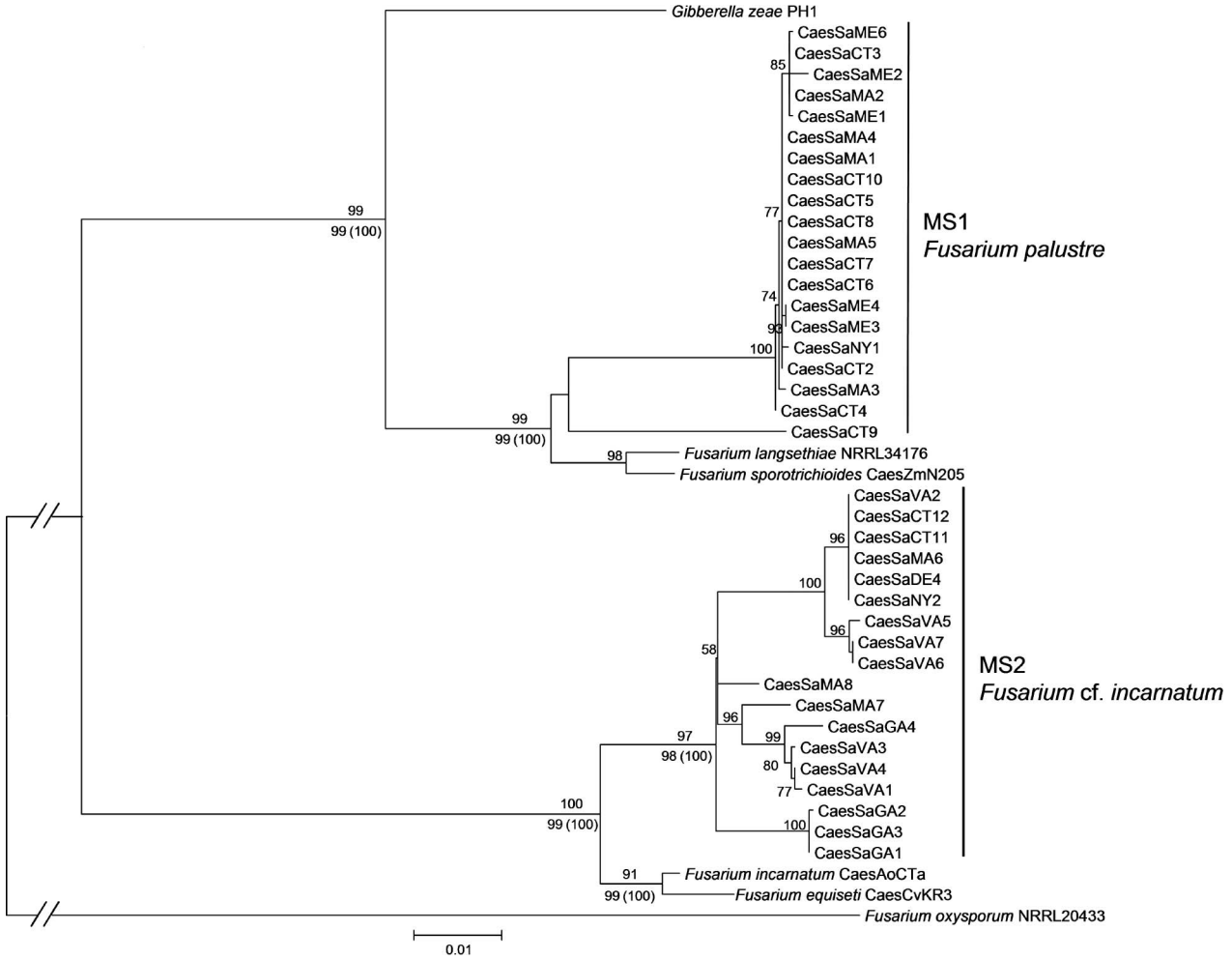

FIG. 6. Phylogenetic relationships among *Fusarium* taxa associated with sudden vegetation dieback in marshes of eastern North America. Other than reference and outgroup strains, the taxa used in the analysis belong to either MS1 or MS2, as indicated in the figure and described in the text. The maximum likelihood (ML) tree is based on a combined analysis of partial gene sequences from *β*-tubulin, calmodulin, and translation-elongation factor 1-α. The tree is rooted with the outgroup method, using *F. oxysporum*. *F. langsethiae* and *F. sporotrichioides* share morphological similarities with MS1, while *F. incarnatum* and *F. equiseti* share such similarities with MS2. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths calculated as the number of substitions per site. Numbers above the branches are the ML branch support values, expressed as the aLRT-based probability that the branch is correct, given the data. MP bootstrap support values are shown below the branches. Numbers in parentheses alongside the MP bootstrap support values represent the percentage of equally parsimonious trees in which the associated taxa also clustered.

consistently separates it with strong statistical support from the remainder of the isolates in this clade. The MS2 subgroup (FIG. 6) clusters with *F. equiseti* and *F. incarnatum*. Isolates in this group are designated *F.* cf. *incarnatum*. The arrangement of taxa within each of the terminal or near-terminal groupings (e.g. CaesSaVA1, CaesSaVA3, CaesSaVA4 and CaesSaGA4) is consistent with that observed in the individual gene analyses (results not shown) with strong branch support for each clade.

We encountered abnormalities in the β-*tub* sequences from our isolates of *F. langsethiae* and *F. equiseti*. Regardless of thermal-cycling conditions, amplification from *F. langsethiae* DNA with the β-*tub* primers produced two fragments differing by approximately 100 bp. The sequence of the smaller band grouped tightly with the MS1 group, corroborating the topologies demonstrated in the *cal*, *tef1* and combined-gene phylogenies. By contrast, the β-*tub* sequences from the larger *F. langsethiae* β-*tub* band and from *F. equiseti* could not be aligned with the β-*tub* dataset and will be addressed in another report. Omission of the *F. equiseti* β-*tub* sequence from the combined dataset did not affect its topology; *F.*

TABLE III. Reaction of *Spartina alterniflora* to stem and root inoculation with isolates of *F. palustre* and *F.* cf. *incarnatum* collected from sudden vegetation dieback sites in mid-Atlantic and New England marshes

Stem inoculations	Mean lesion length (mm)[a]	SEM	*n*
Experiment 1			
Control	0.94	0.32	4
F. palustre	19.4	1.85	34
F. cf. *incarnatum*	5.65	1.35	17
	$P < 0.001$[b]		
Experiment 2			
Control	0.51	0.11	2
F. palustre	15.79	2.14	28
F. cf. *incarnatum*	6.25	1.11	26
	$P < 0.001$[b]		
Root inoculations[c]	**Mean disease rating[d]**	**SEM**	***n***
Experiment 1			
Control	2.46	0.27	2
F. palustre	3.31	0.12	30
F. cf. *incarnatum*	3.07	0.15	15
F. solani	1.25	0.25	1
	$P = 0.015$[b]		
Experiment 2			
Control	1.95	0.26	5
F. palustre	3.04	0.14	23
F. cf. *incarnatum*	2.54	0.17	18
F. oxysporum	2	0.72	1
	$P < 0.001$[b]		

[a] Lesion length was based on the length of internal stem discoloration.

[b] Based on Kruskal-Wallis one-way analysis of variance.

[c] Seedling were grown in soil infested with autoclave ground millet colonized by the test fungus.

[d] Disease ratings based on a scale of 0–5.

equiseti clustered tightly with *F. incarnatum* as it did in the individual *cal* and *tef1* analyses.

Pathogenicity tests.—A subset of the MS1 isolates (experiment one = 34 isolates, experiment two = 28 isolates) and the MS2 isolates (experiment one = 17 isolates, experiment two = 26 isolates) were selected for pathogenicity tests. We chose to label an isolate as pathogenic if it produced stem rot lesions longer than 5 mm. Of the 105 isolates tested, 68 were designated pathogens; 51 (82%) of these were in MS1, while only 17 (39%) were in MS2 (TABLE III). MS1 isolates produced a mean lesion 17.3 mm long, which was significantly greater than the control (0.94 mm) ($P < 0.05$). MS2 isolates produced a mean lesion 6.1 mm long, significantly shorter than the MS1 mean and not significantly different from the control.

Twenty-seven of the most virulent isolates in MS1 (n = 15) and MS2 (n = 12) were used to infest soil into which healthy *S. alterniflora* plants had been transplanted. After 15 wk there were no significant differences in the amount of root rot among any isolates or when compared to the control (data not shown), nor were there any differences in the fresh or dry weight of the plants observed (data not shown). Two additional greenhouse experiments were conducted with seedlings. In both experiments the mean disease severity rating of MS1 isolates was significantly higher than that of the controls and the nonpathogenic *F. oxysporum* or *F. solani* isolates. The MS2 isolates were intermediate in pathogenicity between the controls and the MS1 isolates.

TAXONOMY

Fusarium palustre W. Elmer & Marra, sp. nov.

FIGS. 1, 2

MycoBank MB518237

Etymology. *palustre*, from Latin *palus*, referring to the marsh habitat in which this fungus is found.

Coloniae, in Solani tuberosi glucosi agaro 20 C, crecentes 9.2 mm/d (± 0.3 mm/d). Sporodochia sporulentes et pseudecapitula facientes. Conidiophorae erectae vel prostratae. Cellulae conidiogenae monophialidae, doliiformes. Mesoconidia parietibus crassis, 3–4 septata, 31.8–33.2 μm. Macroconidia parietibus crassis, latere dorsali curviore, 36.2–37.4 μm. Cellulae apicales obtusae, cellula basali angustiore. Microconidia carentes. Chlamydosporae intercalares solitariae vel interdum catenatae.

Colonies on potato dextrose agar showing an average growth (mm/d) of 9.2 (± 0.3) at 20 C, 13.9 (± 0.4) at 25 C, 13.0 (± 0.5) at 30 C and 7.1 (± 0.4) at 35 C); colony margin entire, aerial mycelium white orange, lanose. Pigmentation in reverse pale orange to orange-gray center. Sporulation starting early in aerial mycelium in false heads. Sporodochia produced after 2 wk. Conidiophores in aerial mycelium erect or prostrate, conidiophore identical with phialides. Phialides of aerial mycelium monophialidic, doliform. Conidia from aerial mycelium, thick-walled, 3–4-septate, 32.0 μm (± 0.8 μm). Macroconidia from sporodochia thick-walled dorsal side more curved, 36.2–37.4 μm (± 1.0 μm). Apical cells blunt, foot cell foot-shaped. Microconidia absent. Chlamydospores appearing single but more frequently in intercalary chains in aerial mycelium.

Notes. Macroconidia most closely resemble *F. sporotrichioides*, but *F. palustre* can be differentiated by the lack of microconidia.

Holotype. UNITED STATES OF AMERICA, CONNECTICUT: Madison, Hammonasset Beach State Park, 41.337°N, 72.63°W at sea level, from stems of *Spartina alterniflora*, Jun 2006, coll. *Wade H. Elmer*, Isolate *CaesSaCT2*. *Ex-type*: CBS 126796 = NRRL 54056.

DISCUSSION

Since 2000 the number of reports of SVD along the Atlantic Coast of the USA has increased steadily. In this study *Fusarium* spp. were readily isolated from plants taken from SVD sites. However, in contrast to Useman and Schneider (2005), of the 192 isolates collected for this report, more than 88% could not be assigned to any known species based on morphology or genetic analysis. We were able to differentiate these isolates into two groups based on morphology, growth rate, pathogenicity on *Spartina alterniflora* and by multiple gene sequence phylogenies. Isolates in the first group, assigned to MS1, constitute a new species, which we designate *Fusarium palustre* sp. nov. Isolates in the second group, MS2, are referred to as *F.* cf. *incarnatum*, although these also might represent a distinct species in the *F.-incarnatum-equiseti* species complex (FIESC) (O'Donnell et al. 2009).

Our objectives in conducting phylogenetic analyses of the *Fusarium* spp. isolates associated with *S. alterniflora* in SVD sites were to determine (i) the relationships among these isolates, as well as the relationships between these isolates and described *Fusarium* species, (ii) whether the separation of these isolates into two groups based on morphology and pathogenicity is supported by phylogenetic analyses and (iii) whether phylogenetic analyses support the hypothesis that either or both groups represent newly described *Fusarium* species. We achieved each of these goals with sequences from three gene regions, specifically β-*tub*, *cal* and *tef1*, that have proven useful in other phylogenetic studies of clades within the *Fusarium* genus. For example, O'Donnell et al. (2000) used sequences from these three genes to identify 10 new phylogenetically distinct *Fusarium* species among isolates in the *Gibberella fujikuroi* species complex (2000). Similarly, Schroers et al. (2009) used sequences from four genes, the internal transcribed spacer region, large subunit ribosomal DNA, β-*tub* and *tef1* to resolve phylogenetic relationships among taxa within the *F. dimerum* species group, with the latter two genes providing the most resolution. We obtained a tree topology that was nearly identical whether from individual gene sequences or the combined sequence dataset with either maximum likelihood or maximum parsimony analyses. In all cases the two morphospecies separated into two monophyletic clades with high branch support. The MS1 isolates clustered with *F. sporotrichioides* and *F. langsethiae*, while the MS2 isolates clustered with *F. incarnatum* and *F. equiseti*. The low level of branching observed among the MS1 isolates leads us to hypothesize that these isolates belong to a single species. In contrast, although the MS2 isolates consistently clustered together, we observed more variability among them. Therefore, we designate the isolates in this clade as *F.* cf. *incarnatum*, recognizing that future studies might reveal them to be one or more additional new species belonging to the FIESC (O'Donnell et al. 2009). When *tef1* sequences of these isolates were BLAST queried against the GenBank database, we found only one significant (99%) match between the clade consisting of CaesSaCT11, CaesSaCT12, CaesSaDE4, CaesSaMa6, CaesSaNY2, CaesSaVa2 and *F.* cf. *incarnatum* isolate NRRL 34005, which was isolated from human intravitreal fluid (O'Donnell et al. 2009), underscoring the diversity that probably exists for isolates in FIESC.

The species chosen as outgroups, *F. oxysporum* and *G. zeae*, as expected were distinct genetically from the *Fusarium* species found on *S. alterniflora*. However, we used only *F. oxysporum* to root the trees because *G. zeae* consistently clustered with the *F. palustre/F. langsethiae/F. sporotrichioides* clade. This may not be surprising when one considers the phylogenetic associations among trichothecene-producing *Fusarium* species, which include *F. langsethiae* and *F. sporotrichioides*. In an analysis of *tef1* sequences of trichothecene-producing *Fusarium* species, Kristensen et al. (2005) showed that trichothecene production is a synapomorphy for the *Fusarium* clade that produces this toxin.

Our preliminary investigation into the unusual β-*tub* amplification products from *F. langsethiae* and *F. equiseti* suggests that these might be paralogous and fully functional β-*tub* genes; conserved β-*tub* coding regions, containing no stop codons, were identified in both. Queries of GenBank with BLASTN produced no alignable matches. However a TBLASTX query produced alignable matches with *F. cerealis*, *F. larvarum* and *G. zeae*. We will further investigate the nature of these genes, which will be the subject of a future report. It is worth noting that O'Donnell et al. (2009) identified highly divergent paralogs and xenologs among a collection of strains in the FIESC.

The majority of the mid- and north-Atlantic Coast *Fusarium* isolates described here differ from the species found by Useman and Schneider (2005), all of which belong to the *Gibberella fujikuroi* species complex. Although *F. proliferatum* is a member of the *G. fujikuroi* species complex, it constituted only 3% of the fusaria isolated in the present study. Of the

remaining isolates, 88% belonged to one of the two morphospecies, MS1 and MS2. This result was unexpected, given that in studies of agronomic hosts, more than 10 *Fusarium* species typically were recovered from a single plant species (Burgess et al. 1981, Elmer 1996, Elmer et al. 1997).

The relatively low *Fusarium* species diversity observed in this study might be due to the remarkably low plant diversity of the intertidal marsh, where *S. alterniflora* dominates in an extensive monoculture. In addition, the harshness of the intertidal environment—anaerobic conditions, fluctuating salinity, wind energy and hydrological erosion—may present a further limit on the *Fusarium* species that are able to survive. Last, the salt exudation from the foliage of *Spartina* spp. is likely refractory to many fungi, suggesting that these *Fusarium* spp. might possess an unusual degree of salt tolerance relative to other terrestrial *Fusarium* species.

The symptoms observed in the current study are essentially the same as the stem rot symptoms described by Useman and Schneider (2005), suggesting that these studies describe the same disease, albeit associated with different *Fusarium* species. The phenomenon of different *Fusarium* species causing the same disease is not without precedent. For example, *Fusarium* crown and root rot on asparagus (Elmer et al. 1997), root rot of wheat and barley and of stalk rot of corn (Burgess et al. 1981, Francis and Burgess 1975) are diseases that can be caused by different species of *Fusarium*, depending on geography and climate. It would be of interest to know whether the *Fusarium* species described here are colonists on other *Spartina* species, such as *S. anglica* C.E. Hubb and *S. foliosa* Trin, in Europe and Asia. Other salt marsh plants found in the high marsh, such as *Distichlis spicata* (L.) Greene, Juncus *gerardii* Loisel. and *Spartina patens* (Aiton) Muhl., also might be symptomless hosts for these species.

Plant pathogenic fungi were implicated in the denuding of more than 200 ha of *Spartina townsendii* Groves in the United Kingdom in the 1950s (Goodman 1959). Because the attempt by Goodman et al. (1959) to transmit the dieback from unhealthy plants to healthy plants failed, they concluded a pathogen was not involved. This conclusion was supported further by the observation that of more than 20 fungal species isolated from dead rhizomes on nonselective media, none was demonstrated to be pathogenic; *Fusarium* spp. were not reported. However, our current understanding suggests that any potential *Fusarium* spp. might have been missed easily in their study because dead, not declining plants, were sampled and because selective agars were not used. In addition, their greenhouse studies never considered the role of prestressing agents. We know of only three other reports where *Fusarium* spp. were associated with salt marsh plants. The first report was a brief description of a *Fusarium* sp., called *F. spartinae* Ellis & Everh., observed on leaves of *Spartina stricta* Roth (Ellis and Everhart 1902). However, the species description, made in vivo, was far too generic for any meaningful comparisons with current isolates and no isolates were saved. A survey in the 1970s of fungi in a Rhode Island salt marsh listed a *Fusarium* sp. (Gessner 1977), but the isolates were not identified and no longer are available. Although *Fusarium heterosporum* Nees & T. Nees: Fr. was found in association with the ergots of *Claviceps purpurea* (Fr.) Tul. on *Spartina anglica* in England (Preece et al. 1994), a direct association with *S. anglica* could not be demonstrated, and the effect on *C. purpurea* was minimal (Raybould et al. 1998).

Outbreaks of plant pathogens causing significant disease in salt marshes have been described. For example, relatively high incidences of ergot caused by *C. purpurea* on *Spartina* spp. have been observed in salt marshes of California (Fisher et al. 2007) and Europe (Eleuterius and Meyers 1974). In the case of SVD, a predisposing stressor must be in place for the *Fusarium* pathogens described herein to cause any appreciable damage. Indeed, many organisms can coexist harmlessly with a host until stressors, such as moisture deficit and/or temperature extremes, permit the transition from endophyte to pathogen, for example *F. verticillioides* on corn (Schneider and Pendery 1983) and *F. proliferatum* on asparagus (Elmer and LaMondia 1999). *Fusarium palustre* also may exist as an endophyte because low incidences of *F. palustre* were found in surveys of marshes where no SVD was observed (Elmer unpubl). Although mortality was never observed after inoculation of healthy *S. alterniflora* with *F. palustre*, preliminary data have shown that marked mortality occurs when pots planted with *S. alterniflora* are inoculated with *Fusarium* spp. and stressed by drought (Elmer unpubl). Drought has been associated with SVD in many southern states but does not appear to be implicated in the northern marshes that are inundated by daily tides (Alber et al. 2008).

Although *F. palustre* was found in salt marshes affected by SVD all along the Atlantic and most of the isolates were capable of inciting disease in pathogenicity trials, it is doubtful that *F. palustre* is causing SVD. However, *F. palustre* is strongly associated with *S. alterniflora* in a manner analogous to the parasite/host relationship observed with many *Fusarium* species and hosts (e.g. *F. verticillioides* on maize or *F. thapsini* on sorghum). Given the strong genetic similarities of *F. palustre* with the toxigenic species *F.*

langsethiae and *F. sporotrichioides*, further characterization and study of this new species for mycotoxin production is warranted.

ACKNOWLEDGMENTS

The authors thank Susan Adamowicz, Merryl Alber, Robert Christian, Lisa Fox, Ron Rozsa and Stephen Smith for providing plant material from SVD sites, Peter Thiel, Joan Bravo, Christina Connelly and Caitlin Steckler for technical assistance, Kerry O'Donnell for guidance on phylogenetic analyses, and Amy Rossman for editorial comments and Latin translations.

LITERATURE CITED

Alber M, Swenson EM, Adamowicz SC, Mendelssohn IA. 2008. Salt marsh dieback: an overview of recent events in the US. Estuar Coast Shelf Sci 80:1–11, doi:10.1016/j.ecss.2008.08.009

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate and powerful alternative. Syst Biol 55:539–552, doi:10.1080/10635150600755453

Bertness MD. 2007. Atlantic shorelines: natural history and ecology. Princeton, New Jersey: Princeton Univ Press. 448 p.

Burgess LW, Dodman RL, Pont W, Mayer P. 1981. Fusarium diseases of wheat, maize and grain sorghum in eastern Australia. In: Nelson PE, Tousson TA, Cook RJ, eds. Fusarium: diseases, biology and taxonomy. University Park: Pennyslvania State Univ. Press. p 64–76.

Eleuterius LN, Meyers SP. 1974. *Claviceps purpurea* on *Spartina* in coastal marshes. Mycologia 66:978–986, doi:10.2307/3758316

Ellis JB, Everhart BM. 1902. New species of fungi from various locales. J Mycol 8:10–19.

Elmer WH. 1995. Association between Mn-reducing root bacteria and NaCl applications in suppression of Fusarium crown and root rot of asparagus. Phytopathology 85:1461–1467, doi:10.1094/Phyto-85-1461

———. 1996. Fusarium fruit rot of pumpkin in Connecticut. Plant Dis 80:131–135, doi:10.1094/PD-80-0131

———. 2003. Local and systemic effects of NaCl on root composition, rhizobacteria and fusarium crown and root rot of asparagus. Phytopathology 93:186–192, doi:10.1094/PHYTO.2003.93.2.186

———, LaMondia JA. 1999. Studies on the suppression of Fusarium crown and root rot of asparagus with NaCl. In: Benson B, ed. First International Asparagus Symposium: Acta Horticulturae. p 211–217.

———, Summerell BA, Burgess LW, Backhouse D, Abubaker AA. 1997. Fusarium species associated with asparagus crowns and soil in Australia and New Zealand. Australas Plant Pathol 26:255–261, doi:10.1071/AP97041

Fisher AJ, DiTomaso JM, Gordon TR, Aegerter BJ, Ayres DR. 2007. Salt marsh *Claviceps purpurea* in native and invaded *Spartina* marshes in northern California. Plant Dis 91:380–386, doi:10.1094/PDIS-91-4-0380

Francis RG, Burgess LW. 1975. Surveys of Fusaria and other fungi associated with stalk rot of maize in eastern Australia. Aust J Agric Res 26:801–807, doi:10.1071/AR9750801

Gessner RV. 1977. Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. Mycologia 69:477–491, doi:10.2307/3758551

Goodman PJ. 1959. The possible role of pathogenic fungi in dieback of *Spartina townsendii*. Trans Br Mycol Soc 42:409–415, doi:10.1016/S0007-1536(59)80041-3

———, Braybrooks EM, Lambert JM. 1959. Investigations into dieback in *Spartina townsendii* Groves 1. The present status of *Spartina townsendii* in Britain. J Ecol 47:651–677, doi:10.2307/2257297

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704, doi:10.1080/10635150390235520

Holdredge C, Bertness MD, Altieri AH. 2009. Role of crab herbivory in dieoff of New England salt marshes. Conserv Biol 23:672–679, doi:10.1111/j.1523-1739.2008.01137.x

Kristensen R, Torp M, Kosiak B, Holst-Jensen A. 2005. Phylogeny and toxigenic potential is correlated in *Fusarium* species as revealed by partial translation elongation factor 1-alpha gene sequences. Mycol Res 109:173–186, doi:10.1017/S0953756204002114

Leslie JF. 1993. Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150, doi:10.1146/annurev.py.31.090193.001015

———, Summerell BA. 2006. The Fusarium laboratory manual. Ames, Iowa: Blackwell Publishing. 385 p.

McKee KL, Mendelssohn IA, Materne MD. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? Glob Ecol Biogeogr 13:65–73, doi:10.1111/j.1466-882X.2004.00075.x

Nirenberg HI, O'Donnell K. 1998. New Fusarium species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90:434–458, doi:10.2307/3761403

O'Donnell K. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience 41:61–78, doi:10.1007/BF02464387

———, 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, doi:10.1006/mpev.1996.0376

———, ———, Casper HH. 1998a. Molecular phylogenetic, morphological and mycotoxin data support reidentification of the Quorn mycoprotein fungus as *Fusarium venenatum*. Fungal Genet Biol 23:57–67, doi:10.1006/fgbi.1997.1018

———, ———, Nirenberg HI. 1998b. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465–493, doi:10.2307/3761407

———, Sutton DA, Rinaldi MG, Gueidan C, Crous PW, Geisder DM. 2009. Novel multilocus sequence typing

scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatuirm-F. equseti* and *F. chlamydosporum* species compelexes within the United States. J Clin Microbiol 47:3851–3861, doi:10.1128/JCM.01616-09

Preece TF, Pettit TR, Biggs DT. 1994. *Fusarium heterosporum* growing on ergots (*Claviceps purpurea*) in spikelets of common cord-grass (*Spartina anglica*) in the Isle of Wight. Mycologist 8:9–11, doi:10.1016/S0269-915X(09)80673-3

Raybould AF, Gray AJ, Clarke RT. 1998. The long-term epidemic of *Claviceps purpurea* on *Spartina anglica* in Poole Harbour: pattern of infection, effects on seed production and the role of *Fusarium heterosporum*. New Phytol 138:497–505, doi:10.1046/j.1469-8137.1998.00123.x

Schneider RW, Pendery WE. 1983. Stalk rot of corn—mechanism of predisposition by an early season water stress. Phytopathology 73:863–871, doi:10.1094/Phyto-73-863

Schroers HJ, O'Donnell K, Lamprecht SC, Kammeyer PL, Johnson S, Sutton DA, Rinaldi MG, Geiser DM, Summerbell RC. 2009. Taxonomy and phylogeny of the *Fusarium dimerum* species group. Mycologia 101:44–70, doi:10.3852/08-002

Silliman BR, van de Koppel J, Bertness MD, Stanton LE, Mendelssohn IA. 2005. Drought, snails and large-scale dieoff of southern US salt marshes. Science 310:1803–1806, doi:10.1126/science.1118229

Smith SM. 2009. Multi-decadal changes in salt marshes of Cape Cod, MA: photographic analyses of vegetation loss, species shifts and geomorphic change. Northeast Nat 16:183–208, doi:10.1656/045.016.0203

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA 4: molecular evolutionary genetics analysis (MEGA) software. Mol Biol Evol 24:1596–1599.

Useman S, Schneider RW. 2005. The possible role of plant pathogens in Louisana's brown marsh syndrome. Proceedings of the 14th Biennial Coastal Zone Conference, New Orleans.

Windels CE, Burnes PM, Kommedahl T. 1988. Five-year preservation of *Fusarium* species on silica gel and soil. Phytopathology 78:107–109, doi:10.1094/Phyto-78-107