Molecular Relationships of Fungi Within the *Fusarium redolens*–*F. hostae* Clade

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ABSTRACT

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The evolutionary relationships of fungi in the *Fusarium redolens*–*F. hostae* clade were investigated by constructing nuclear and mitochondrial gene genealogies for 37 isolates representing the known genetic and pathogenic diversity of this lineage, together with 15 isolates from putative sister groups that include the *Gibberella fujikuroi* and *F. oxysporum* species complexes and related species. Included in the analyses were 29 isolates of *F. redolens* from *Asparagus*, *Convallaria*, *Dianthus*, *Fritillaria*, *Hebe*, *Helleborus*, *Hordeum*, *Linum*, *Pisum*, *Pseudotsuga*, and *Zea* spp., and from soil. Isolates of *F. hostae* analyzed included two reference isolates from *Hosta* spp. and six isolates from *Hyacinthus* spp. that originally were classified as *F. oxysporum* f. sp. *hyacinthi*. DNA sequences

from a portion of the nuclear translation elongation factor 1 α (EF-1 α) gene and the mitochondrial small subunit (mtSSU) ribosomal RNA (rRNA) were analyzed individually and as a combined data set based on results of the nonparametric Wilcoxon signed ranks Templeton combinability test. Maximum parsimony analysis of the combined data set identified the *F. redolens*–*F. hostae* clade as a sister group to a phylogenetically diverse clade in which the *G. fujikuroi* species complex formed the most basal lineage. Also included in this latter clade were two unnamed *Fusarium* spp. that are morphologically similar to *F. oxysporum* and putative sister taxa comprising the *F. oxysporum* complex and a *F. nissikadoi*–*F. miscanthi* clade. Phylogenetic diversity in *F. redolens* was small; all isolates were represented by only three EF-1 α and two mtSSU rDNA haplotypes. Both the isolates of *F. redolens* f. sp. *asparagi* and those of *F. redolens* f. sp. *dianthi* were nearly evenly distributed in the combined molecular phylogeny between the two major subclades within *F. redolens*.

The soilborne fungus *Fusarium oxysporum* Schlechtend.:Fr. is one of the economically most important plant pathogenic fungi worldwide. More than 150 host-specific formae speciales have been described in *F. oxysporum*, many of them subdivided into distinct pathogenic races. *F. oxysporum* was placed in the *Fusarium* section *Elegans* by Wollenweber (42), together with six other taxa (*F. tracheiphilum*, *F. vasinfectum*, *F. vasinfectum* var. *inodorum*, *F. lycopersici*, *F. niveum*, and *F. redolens*) that have been considered synonymous with *F. oxysporum* since Snyder and Hansen (35). In the past decade, molecular studies have shown that these morphologically highly similar fungi have multiple phylogenetic origins (4,30). The fungi involved are currently referred to as the *F. oxysporum* complex, which consists of three well-supported main clades, each in turn consisting of numerous phylogenetic lineages, most of which do not coincide with known formae speciales (4,30).

The position of *F. redolens* Wollenweb. as a member of the *F. oxysporum* complex has been disputed for many years. Wollenweber's morphological differentiation of *F. redolens* and *F. oxysporum* (42) is problematic due to the presence of intermediate forms (3), such that many authors have treated both species as conspecific (24,35), or at best have recognized *F. redolens* as a variety of *F. oxysporum* (10,16) or even *F. solani* (7). Studies employing the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region and gene sequence analyses have since confirmed

that *F. redolens* is distinct from *F. oxysporum* (29,38,39). Gene genealogies indicate that *F. redolens* is not even the sister group of the *F. oxysporum* complex (4,11,29), challenging the phylogenetic validity of *Fusarium* section *Elegans* (38,39). Instead, a sister group relationship has been reported of the *F. oxysporum* complex and a *F. miscanthi*–*F. nissikadoi* clade, two closely related putatively Asian species with the potential to produce pyriform conidia (11).

Like the *F. oxysporum* complex, *F. redolens* harbors isolates causing a wide range of diseases including wilts, seedling damping-off, and cortical rot; several of these show host specificity (10,14,15). The original description was based on an isolate from *Pisum sativum* that was identified as a vascular parasite and the cause of wilt and foot disease (42), later followed by isolates from rotten pea seeds (43). Booth (10) reported that *F. redolens* occurs in association with *F. oxysporum* f. sp. *pisi* in a wilt of broad bean and of pea, and that each of these pathogens can cause wilting of pea separately, producing symptoms which are indistinguishable from one another. Gerlach and Pag (15) reported on a form from *Dianthus* spp., *F. redolens* f. sp. *dianthi*. This form colonizes xylem vessels and causes wilt symptoms that are indistinguishable from those induced by *F. oxysporum* f. sp. *dianthi* (3). *F. redolens* f. sp. *dianthi* was later shown to harbor four vegetative compatibility groups (VCGs), each of which represents a distinct race (1,6,21). Additional associations between pathogenic forms of *F. redolens* and *F. oxysporum* have been reported. Thus, root and spear rot of asparagus can be caused by *F. redolens* f. sp. *asparagi* and by *F. oxysporum* f. sp. *asparagi*; both taxa cause cortical rot symptoms that are indistinguishable from one another, while the xylem is unaffected (5,8). Oil palm is susceptible to *F. oxysporum* f. sp. *elaeidis* as well as *F. redolens* (17). In addition, wilt and root

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rot of spinach can be caused by *F. redolens* f. sp. *spinaciae* and *F. oxysporum* f. sp. *spinaciae* (20,36).

F. hostae, a species described from *Hosta* plants suffering root and crown rot in nurseries in South Carolina, was recently identified as a sister of *F. redolens* by Geiser et al. (13). This species is similar to *F. redolens* morphologically in that it produces similar asexual spores, has similar gross colony characteristics, and often produces a distinctive odor. However, *F. hostae* differs from *F. redolens* in that it occasionally produces simple polyphialides and grows more slowly on potato-dextrose agar (PDA). Also, a teleomorph has been described for *F. hostae* (*Gibberella hostae*), whereas sexual reproduction is unknown for *F. redolens*. With no more than a single host, no data on host specificity in *F. hostae* are available.

Although the phylogenetic relationships within the *F. oxysporum* complex and the *F. nisikadoi*–*F. miscanthi* clade have been studied in detail in the past years, little attention has been paid so far to the phylogenetic relationships and pathogenic diversity in the *F. redolens*–*F. hostae* clade. A major reason for this lack of data is the difficult access to reliable isolates: *F. redolens* is a rare species that is often mistaken for *F. oxysporum*, and *F. hostae* has been described only in 2001 (13). The authors have accumulated as wide a collection as possible of *F. redolens* and *F. hostae*, representing the known genetic and pathogenic diversity in these two species, to fill this gap. The aim of this study was to examine the evolutionary diversity within the *F. redolens*–*F. hostae* clade, and to test whether host specialization in these fungi can be related to evolutionary origin. This was done by constructing nuclear and mitochondrial gene genealogies, using isolates of putative sister

groups (the *F. oxysporum* complex and related species, and the *Gibberella fujikuroi* species complex) as a reference, and *F. beomiforme* as outgroup species.

MATERIALS AND METHODS

Fungal isolates. This study comprises all isolates of *F. redolens* in the collections of the first and second authors, covering nearly all isolates currently available of this rare species. We included isolates of *F. redolens* f. sp. *asparagi* (5), isolates of *F. redolens* f. sp. *dianthi* (6) belonging to VCG 0001, 0002, 0003, and 0004, as well as a putatively nonpathogenic VCG from *Dianthus* sp. (1), and isolates from nine additional host species (Table 1). Pathogenicity of isolates was not tested; therefore, isolates not belonging to *F. redolens* f. sp. *asparagi* and f. sp. *dianthi* may or may not be pathogenic to the host species from which they were isolated, unless otherwise specified. Six isolates of *F. oxysporum* f. sp. *hyacinthi* from hyacinth and suspected to belong to *F. redolens* were also included. The recently published species *F. hostae* was represented by reference isolates of the two genotypes of this species (13). Isolates of the *F. oxysporum* complex and *G. fujikuroi* complex included representatives of the three major clades within each of these large species complexes (4,11,29,30) together with phylogenetically related *Fusarium* spp. (Table 1). Isolates examined in this study have been deposited in the Agricultural Research Service Culture Collection (National Center for Agricultural Utilization Research, Peoria, IL), abbreviated as NRRL after its former name Northern Regional Research Laboratory, where they are stored cryogenically at –175°C.

TABLE 1. Isolates of *Fusarium redolens*, *F. hostae*, and related species included in this study

Host, substrate	Forma specialis (race) ^a	VCG ^b	Origin	NRRL ^c	Original code ^d
<i>Fusarium beomiforme</i>					
Soil	...	...	New Caledonia	25174	BBA 65829
<i>Fusarium hostae</i>					
<i>Hosta</i> sp.	...	...	United States	29889	FRC O-2074
<i>Hosta</i> sp.	...	...	United States	29891	FRC O-2083
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29642	LBO Hy1
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29643	LBO Hy3
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29644	LBO Hy4
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29645	LBO Hy5
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29646	LBO Hy6
<i>Hyacinthus</i> sp.	...	...	The Netherlands	29647	LBO Hy7
<i>Fusarium miscanthi</i>					
<i>Miscanthus sinensis</i>	...	...	Denmark	26231	CBS 578.97
<i>Miscanthus sinensis</i>	...	...	Denmark	26239	S-1296-59
<i>Fusarium nisikadoi</i>					
<i>Phyllostachys nigra</i>	...	...	Japan	25179	MAFF 237509
<i>Phyllostachys nigra</i>	...	...	Japan	25183	MAFF 237510
<i>Fusarium oxysporum</i>					
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	1001	United States	28973 ^e	FGSC 6608
<i>Cucumis melo</i>	f. sp. <i>melonis</i>	0136	United States	26406 ^e	K-419
<i>Dianthus caryophyllus</i>	f. sp. <i>dianthi</i> (1)	0022	Italy	26964 ^f	F100
<i>Linum usitatissimum</i>	f. sp. <i>lini</i>	044–	The Netherlands	28928 ^f	N1
<i>Musa</i> sp.	f. sp. <i>cubense</i>	0120	Australia	25603 ^g	A2

(continued on next page)

^a ... Indicates none described.

^b Vegetative compatibility groups (VCGs) 0011, 0012, and 0013 correspond with VCGs 1, 12, and 9 from Blok and Bollen (8). The remaining isolates that did not react with reference tester strains from these VCGs, nor with each other, were placed in VCG 001–.

^c Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, formerly Northern Regional Research Laboratory.

^d Isolate source: BBA = Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin; BCRI = British Columbia Research Inc., Vancouver, Canada; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CWB = W. J. Blok, Wageningen University, Wageningen, The Netherlands; DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; FGSC = Fungal Genetics Stock Center, Kansas; FRC = Fusarium Research Center, State College, PA; IMI = CABI Bioscience (formerly International Mycological Institute), Egham, U.K.; IPO = Plant Research International (formerly Instituut voor Plantenziektenkundig Onderzoek), Wageningen, The Netherlands; LBO = Bulb Research Centre, Lisse, The Netherlands; MAFF = Ministry of Agriculture, Forestry, and Fisheries, NIAR, Tsukuba, Japan; PD = Plantenziektenkundige Dienst, Wageningen, The Netherlands.

^e Member of *F. oxysporum* complex clade 3.

^f Member of *F. oxysporum* complex clade 2.

^g Member of *F. oxysporum* complex clade 1.

^h According to Baayen et al. (6).

ⁱ According to Aloï and Baayen (1) these two isolates belong the same VCG.

DNA amplification and sequencing. Genomic DNA was prepared as described previously (28). Polymerase chain reaction amplification and sequencing of a portion of the mitochondrial small subunit (mtSSU) rDNA was performed by using reagents and primers described in White et al. (41) and O'Donnell and Cigelnik (28). A partial fragment of the elongation factor 1 α (EF-1 α) gene was amplified with primers EF-1 and EF-2, which prime within conserved exons (30). One forward (EF-11) and two reverse internal sequencing primers (EF-21 and EF-22) (30) were used with the fluorescent-labeled dyedexy chemistry on an Applied Biosystems 377 automated sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). EF-1 α and mtSSU rDNA sequences have been deposited in GenBank under accession numbers AF324256–324333, or under numbers cited in O'Donnell et al. (30), Baayen et al. (4), and Geiser et al. (13). The aligned sequences are available from the second author upon request.

Phylogenetic analysis. Phylogenetic analyses of the individual and combined data sets of partial mtSSU rDNA and EF-1 α gene sequences were conducted using PAUP (version 4.0b3a; Sinauer Associates Inc., Sunderland, MA). Unweighted parsimony analyses were performed using the heuristic search option with 1,000 random addition sequences with mulpars on and with tree bisection-reconnection branch swapping. Sequences of *F. beomiforme* were selected for rooting the gene trees by the outgroup method based on more inclusive phylogenetic analyses (11,28,29). Clade stability was assessed by 1,000 parsimony bootstrap replications. The nonparametric Wilcoxon signed-ranks (WS-R) Templeton test implemented in PAUP 4.0b3a was used to assess whether the two individual partitions could be combined. The consistency index

(CI), a measure of the amount of homoplasy in a character relative to a given cladogram, was calculated as the ratio of m , the minimum number of steps a character can exhibit on any cladogram, to s , the minimum number of steps the same character can exhibit on the cladogram in question. The retention index (RI), a measure of the amount of similarity in a character that can be interpreted as synapomorphy on a given cladogram, was calculated as the ratio of $(g - s)$ to $(g - m)$, where g is the greatest number of steps a character can exhibit on any cladogram.

RESULTS

The EF-1 α data set consisted of 662 nucleotide characters, of which 82 were autapomorphic and 118 were cladistically informative. Maximum parsimony analysis, including autapomorphic characters, yielded 96 equally most-parsimonious trees (MPTs) (Fig. 1) of 315 steps (CI = 0.778, RI = 0.926). All of the ingroup isolates (i.e., the group under investigation in a cladistic analysis in order to resolve the relationships of its members) fell within one of two strongly supported sister clades. Of these, the basal-most clade (91% bootstrap) was the most phylogenetically diverse and included a *F. nisikadoi*–*F. miscanthi* clade (99% bootstrap), two unnamed *Fusarium* spp. morphologically similar to *F. oxysporum* (one represented by isolate NRRL 25184, the other by isolates NRRL 28058 and 28387; K. O'Donnell, K. Skovgaard, and H. I. Nirenberg, unpublished data), and the *G. fujikuroi* complex (isolates NRRL 22016, 22057, and 22172) and *F. oxysporum* complex (isolates NRRL 25603, 26406, 26964, 28928, and 28973). All of the remaining ingroup isolates were represented by

TABLE 1. (Continued from preceding page)

Host, substrate	Forma specialis (race) ^a	VCG ^b	Origin	NRRL ^c	Original code ^d
<i>Fusarium proliferatum</i>	...	...	...	22057	JFL 4853
<i>Fusarium redolens</i>					
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	0011	The Netherlands	28409	CWB 1
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	0012	The Netherlands	28390	CWB 33
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	0013	The Netherlands	28412	CWB 42
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	001–	The Netherlands	28975	IPO 97-13
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	001–	The Netherlands	28909	IPO 97-143
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	001–	The Netherlands	28381	IPO 97-158
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	001–	Germany	28444	DSM 62380
<i>Asparagus officinalis</i>	f. sp. <i>asparagi</i>	001–	Poland	28417	KFL63
<i>Convallaria majalis</i>	...	...	Germany	28420	DSM 62385
<i>Dianthus barbatus</i>	f. sp. <i>dianthi</i> (1)	0001 ^h	Germany	28421	DSM 62393
<i>Dianthus barbatus</i>	f. sp. <i>dianthi</i> (1)	0001	The Netherlands	26967	PD 89/1825
<i>Dianthus barbatus</i>	f. sp. <i>dianthi</i> (2)	0002 ^h	The Netherlands	26966	PD 90/1882
<i>Dianthus caryophyllus</i>	f. sp. <i>dianthi</i> (3)	0003 ^h	Italy	26969	621
<i>Dianthus caryophyllus</i>	f. sp. <i>dianthi</i> (3)	0003	Italy	28425	622
<i>Dianthus caryophyllus</i>	f. sp. <i>dianthi</i> (4)	0004 ^h	Germany	25600	DSM 62390
<i>Dianthus caryophyllus</i>	f. sp. <i>dianthi</i> (4)	0004	Germany	28419	DSM 62392
<i>Dianthus caryophyllus</i>	...	=28355 ⁱ	Germany	28411	DSM 62378
<i>Dianthus caryophyllus</i>	...	=28411 ⁱ	Germany	28355	DSM 62379
<i>Fritillaria</i> sp.	...	...	Germany	28423	DSM 62386
<i>Hebe buxifolia</i>	...	...	United States	26440	IMI 141126
<i>Helleborus</i> sp.	...	...	Germany	28422	DSM 62383
<i>Hordeum vulgare</i>	...	...	Finland	28246	BBA 70530
<i>Linum usitatissimum</i>	...	...	France	28927	F60
<i>Linum usitatissimum</i>	...	...	France	26425	CBS 530.96
<i>Linum usitatissimum</i>	...	...	Italy	26762	FO25
<i>Pisum sativum</i>	...	...	Germany	28424	DSM 64613
<i>Pseudotsuga menziesii</i>	...	...	Canada	22901	BCRI F30
<i>Zea mays</i>	...	...	Austria	28181	MA 1209
Soil	...	...	France	26623	92302
<i>Fusarium subglutinans</i>					
<i>Zea mays</i>	...	...	United States	22016	JFL 2192
<i>Fusarium</i> sp.					
<i>Dianthus caryophyllus</i>	...	...	The Netherlands	28387	PD 90/1377
Peat soil	...	...	Germany	25184	BBA 65467
River sediment	...	...	Japan	28058	FRC 01173
<i>Fusarium verticillioides</i>					
<i>Zea mays</i>	...	...	Germany	22172	BBA 11778

a strongly supported *F. redolens*–*F. hostae* clade (100% bootstrap). Parsimony analysis of the 706-bp mtSSU rDNA partition (15 autapomorphies, 49 synapomorphies) yielded >10,000 equally MPTs 115 steps in length (CI = 0.774, RI = 0.908) (Fig. 2). The *G. fujikuroi* complex (88% bootstrap) formed a basal sister group to the remaining ingroup taxa. The remaining ingroup isolates

formed a clade (87% bootstrap) composed of the following sister clades: (i) *Fusarium* spp., *F. nisikadoi*–*F. miscanthi*, and the *F. oxysporum* complex (92% bootstrap); and (ii) *F. hostae*–*F. redolens* (73% bootstrap). A strongly supported *F. nisikadoi*–*F. miscanthi* clade (100% bootstrap) was resolved as the putative sister group of the *F. oxysporum* complex (82% bootstrap). Re-

Fig. 1. One of 96 most-parsimonious phylograms inferred from the 662 aligned elongation factor 1 α (EF-1 α) nucleotide characters showing phylogenetic relationships of the *Fusarium redolens*–*F. hostae* clade (100% bootstrap interval) and related species. Three closely related EF-1 α haplotypes were resolved within *F. redolens*. A sequence of *F. beomiforme* was used to root the tree by the outgroup method. Bootstrap values >50% from 1,000 maximum parsimony replications implemented in PAUP (version 4.0b3a) are indicated above nodes. CI = consistency index and RI = retention index.

sults of the WS-R Templeton test implemented in PAUP indicated that the EF-1 α and mtSSU rDNA data sets could be combined ($P = 0.0956$ constraining mtSSU rDNA data to EF-1 α 70% bootstrap consensus, and $P = 0.007$ constraining EF-1 α data to mtSSU rDNA bootstrap consensus). Maximum parsimony searches on the combined data set (Fig. 3) yielded >5,000 MPTs 441 steps in

length (CI = 0.575, RI = 0.912). A strongly supported *F. hostae*–*F. redolens* clade (100% bootstrap) was resolved as sister to the remaining ingroup taxa, which formed a moderately well supported clade (79% bootstrap). A *F. nisikadoi*–*F. miscanthi* clade (100% bootstrap) formed a putative sister group to the *F. oxysporum* complex (<50% bootstrap support).

Fig. 2. One of >10,000 most-probable trees based on maximum parsimony analysis of the aligned mitochondrial small subunit (mtSSU) ribosomal (rDNA) sequences rooted with a sequence of *Fusarium beomiforme*. Numbers at the nodes represent bootstrap support >50% from 1,000 replications. Every isolate of *F. redolens* except one shared an identical mtSSU rDNA haplotype. Note that phylogenetic relationships of the *Gibberella fujikuroi* species complex were unresolved. CI = consistency index and RI = retention index.

Genetic diversity within *F. redolens* was small as reflected in the discovery of only three EF-1 α (Fig. 1) and two mtSSU rDNA (Fig. 2) haplotypes. Isolates belonging to the same VCG always shared the same multilocus haplotype (Fig. 3). Of the 14 isolates sharing identical EF-1 α and mtSSU rDNA haplotypes at the root of the *F. redolens* clade (Fig. 3), four were *F. redolens* f. sp.

asparagi, six were *F. redolens* f. sp. *dianthi* (all members of VCGs 0001, 0003, and 0004), two represented a putatively non-pathogenic VCG from carnation, and the remaining two were from *Fritillaria* and *Hebe* spp. (the latter isolate was received as *F. oxysporum* f. sp. *hebae*). A pathogenic isolate from *Linum usitatissimum* (NRRL 28927) (19) differed from the basal haplo-

Fig. 3. One of >5,000 most-parsimonious phylograms inferred from the combined elongation factor 1 α (EF-1 α) and mitochondrial small subunit (mtSSU) ribosomal DNA (rDNA) data set. Bootstrap intervals from 1,000 replicates are indicated above nodes. Note that the *Fusarium redolens*–*F. hostae* clade is strongly supported as monophyletic (100% bootstrap). Except for two isolates that possess unique haplotypes, *F. redolens* isolates are nearly evenly divided among two closely related multilocus haplotypes. Note that isolates pathogenic to *Asparagus* (f. sp. *asparagi*), *Dianthus* (f. sp. *dianthi*), and *Linum* spp. are nested within each of the two major subclades within *F. redolens*. CI = consistency index and RI = retention index.

type by a single substitution. Thirteen *F. redolens* isolates, representing a third EF-1 α and mtSSU rDNA haplotype, formed a clade (Fig. 3; 94% bootstrap) composed of the following: two isolates from *L. usitatissimum* (NRRL 26425 and 26762), one isolate of VCG 0002 of *F. redolens* f. sp. *dianthi* (NRRL 26966), four isolates of *F. redolens* f. sp. *asparagi* together with isolates from *Convallaria*, *Helleborus*, *Pisum*, *Hordeum*, and *Zea* spp., and one isolate from soil. A fourth haplotype which differed by seven substitutions from the basal haplotype was represented by a single isolate (NRRL 22901) from *Pseudotsuga menziesii*.

Results of the molecular phylogeny indicate that the six isolates from hyacinth collected in The Netherlands as *F. oxysporum* f. sp. *hyacinthi*, and suspected by us to belong to *F. redolens*, are *F. hostae*. The six *F. hostae* isolates from hyacinth represent a random subset of a larger collection isolated from various cultivars of this host in The Netherlands. In a preliminary study, restriction digest patterns of the ITS region of the rDNA had classified these isolates as distinct from *F. oxysporum*; the ITS-restriction fragment length polymorphisms were identical to that of *F. redolens* and a number of species in the *G. fujikuroi* complex. Morphological reexamination of these isolates, however, revealed that they produce thick macroconidia as well as polyphialides, confirming their identification as *F. hostae* rather than *F. oxysporum* or *F. redolens*.

DISCUSSION

In the present study, partial EF-1 α gene and mtSSU rDNA nucleotide sequences were used to infer phylogenetic relationships within and among a newly discovered lineage, the *F. redolens*-*F. hostae* clade (13), and other *Gibberella* fusaria. The merits and power of multiple gene phylogenies for resolving species phylogenies have been shown in a recent review on phylogenetic species recognition and species concepts in fungi (37). In various lineages of the genus *Fusarium*, the consistent concordance of several nuclear and mitochondrial genealogies indeed offers compelling evidence that the gene trees reflect the underlying phylogenies (4,27–32). Each of the *G. fujikuroi* and *Nectria haematococca* species complexes, once considered by Snyder and Hansen (35) to be represented by *F. moniliforme* and *F. solani*, respectively, are now known to harbor over 40 phylogenetically distinct species with a hypothesized Gondwanic biogeographical history (27,29,32). All of the mating populations (biological species) in each of these two complexes were resolved as exclusive groups using molecular phylogenetics. Moreover, the multigene phylogeny approach facilitated the discovery of species-defining morphological characters within the *G. fujikuroi* complex, thus characterizing hitherto unknown morphological species (25,26). Recently, *F. graminearum* (teleomorph: *G. zeae*), the causal agent of wheat scab, was found to consist of seven biogeographically structured lineages that appear to represent phylogenetically distinct species, among which gene flow has been very limited during their more recent evolutionary history (31). The multigene approach used in the latter study also allowed for the identification of a hybrid isolate between two of the lineages. In all, 15 heterothallic, 7 homothallic, and 4 putatively mitosporic species were identified in the *N. haematococca* species complex (27). In the *F. oxysporum* complex, amplified fragment length polymorphism-based genealogies have been shown to support the multigene phylogenies, resolving numerous evolutionarily distinct clonal lineages (4,30).

As first reported by Gams et al. (11), the *F. oxysporum* complex and the *F. miscanthi*-*F. nisikadoi* clade, together with two unnamed *F. oxysporum*-like species (one represented in the present study by isolate NRRL 25184, the other by isolates NRRL 28058 and 28387) and the *G. fujikuroi* complex, form a monophyletic group independent from *F. redolens*. Our results confirm those of Geiser et al. (13) that *F. redolens* and *F. hostae* form a separate monophyletic lineage. As estimated from EF-1 α and mtSSU

rDNA partial sequences, genetic diversity in the *F. redolens*-*F. hostae* clade was low compared to any of the three major clades in the *F. oxysporum* complex (4,30). Within *F. redolens*, no more than three EF-1 α and two mtSSU rDNA haplotypes were found, and three EF-1 α and a single mtSSU rDNA haplotype in *F. hostae*. Remarkably, all isolates of the latter species from hyacinths shared a unique EF-1 α haplotype different from those described from isolates from *Hosta* spp. (13).

Pathogenic diversity in *F. redolens* resembles that in *F. oxysporum* in that isolates of *F. redolens* have been associated with a wide range of diseases, including wilts, seedling damping-off, and cortical rot (10,14,15). Although isolates within the *F. oxysporum* complex sharing host specificity have been grouped traditionally in formae speciales, only three formae speciales have been described within *F. redolens*: *F. redolens* f. sp. *dianthi* Gerlach (15), *F. redolens* f. sp. *spinaciae* (Sherb.) Subramanian (36), and *F. redolens* f. sp. *asparagi* Baayen (5). It is reasonable to assume, however, that tests for host specificity may reveal that more pathogenic forms exist within this species. For example, isolate NRRL 28927 from *Linum* spp. causes typical root rot symptoms similar to those induced by *F. oxysporum* f. sp. *lini* (this is isolate F60 in Kroes et al. [19]), and it is noteworthy that the original description of *F. redolens* was based on isolates pathogenic to *Pisum sativum* causing disease symptoms similar to those induced by *F. oxysporum* f. sp. *pisi*. Further, because isolate NRRL 26440 from *Hebe buxifolia* was received as *F. oxysporum* f. sp. *hebae* Raabe (12,33,34), additional isolates from *Hebe* spp. should be investigated to determine whether other (or all) members of this forma specialis belong to *F. redolens*.

Host preference in *F. redolens* did not correlate with the two major subclades resolved within this species by the molecular phylogeny. Three VCGs of *F. redolens* f. sp. *dianthi* and one putatively nonpathogenic VCG from a *Dianthus* sp. had the same genotype, but a fourth VCG, 0002, was phylogenetically distinct. The same was the case for *F. redolens* f. sp. *asparagi*, in which both genotypes were nearly evenly divided among each of the two major subclades within *F. redolens*. Isolates from flax had distinct genotypes as well. Collectively, these results suggest that members of these *F. redolens* formae speciales may have independent evolutionary origins. Within the *F. oxysporum* complex, the majority of formae speciales with two or more VCGs appear to be either para- or polyphyletic as well (4,30). In contrast, formae speciales in the *G. fujikuroi* complex and *N. haematococca* species complex all appear to be monophyletic, except for two polyphyletic cucurbit pathogens within the *N. haematococca* species complex (27), but this is not surprising because so-called formae speciales within these two latter species complexes actually represent phylogenetically and biologically distinct species (22,25,27,29,32).

Although isolates of *F. redolens* f. sp. *spinaciae* (Sherb.) Subramanian were not available for study, Sherbakoff's original description and illustrations of the type isolate of *F. spinaciae* (18) beyond any doubt identify this fungus as *F. redolens* as well. Consequently, Snyder and Hansen (35) were mistaken in placing this fungus in *F. oxysporum*, as *F. oxysporum* Schlechtend.:Fr. f. sp. *spinaciae* (Sherb.) W. C. Snyder & H. N. Hans. To further complicate the matter, molecular studies have shown that *F. oxysporum* also harbors three VCGs (VCG 0330–0332), of which the isolates are pathogenic to spinach (4). These isolates are in lack of a valid name, because the type used by Snyder and Hansen (35) to define f. sp. *spinaciae* belonged to an entirely different species. We therefore propose a new combination, *F. oxysporum* Schlechtend.:Fr. f. sp. *spinaciae* Baayen, for isolates of *F. oxysporum* with pathogenicity to spinach such as those in VCG 0330–0332.

Results of the present study demonstrate that six isolates previously identified as *F. oxysporum* f. sp. *hyacinthi* (9) belong to *F. hostae*. Prior to the present study, the latter species has only been

reported from container-grown *Hosta* spp. in South Carolina (13,40). However, preliminary results suggest that this species is widespread on hyacinths in The Netherlands, where it is associated with various disease symptoms, including bulb rot, root rot, cracking of the basal plate, and skin damage without any root development (2,9,23). Presently it is unknown whether any pathogenic isolates from hyacinth belong to *F. oxysporum*: all 14 isolates at the Bulb Research Center, Lisse, The Netherlands, proved to belong to *F. hostae* (M. de Boer and S. Breeuwsma, unpublished data). Pathogenicity tests are being conducted to determine whether isolates of *F. hostae* from *Hosta* and *Hyacinthus* spp. are pathogenic to both hosts.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. We thank S. Prather for preparing the publication figures.

LITERATURE CITED

- Aloi, C., and Baayen, R. P. 1993. Examination of the relationships between vegetative compatibility groups and races in *Fusarium oxysporum* f. sp. *dianthi*. Plant Pathol. 42:839-850.
- Anonymous. 1983. Pages 44-46 in: Ziekten in bolgewassen 1 (ed. 2). Laboratorium voor Bloembollenonderzoek, Lisse, The Netherlands.
- Baayen, R. P., and Gams, W. 1988. The *Elegans* fusaria causing wilt disease of carnation. I. Taxonomy. Neth. J. Plant Pathol. 94:273-288.
- Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., Roebroek, E. J. A., and Waalwijk, C. 2000. Gene genealogies and AFLP analyses within the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology 90:891-900.
- Baayen, R. P., Van den Boogert, P. H. J. F., Bonants, P. J. M., Poll, J. T. K., Blok, W. J., and Waalwijk, C. 2000. *Fusarium redolens* f. sp. *asparagi*, causal agent of asparagus root rot, crown rot and spear rot. Eur. J. Plant Pathol. 106:907-912.
- Baayen, R. P., Van Dreven, F., Krijger, M. C., and Waalwijk, C. 1997. Genetic diversity in *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi*. Eur. J. Plant Pathol. 103:395-408.
- Bilal, V. I. 1955. Fusarii. Izd Akad Nauk Ukrain SSR, Kiev, USSR.
- Blok, W. J., and Bollen, G. J. 1997. Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum* f. sp. *asparagi*. Can. J. Bot. 75:383-393.
- Boerema, G. H., and Hamers, M. E. C. 1988. Check-list for scientific names of common parasitic fungi. Series 3a: Fungi on bulbs: Liliaceae. Neth. J. Plant Pathol. 94(Suppl.)1:1-32.
- Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, U.K.
- Gams, W., Klammer, M., and O'Donnell, K. 1999. *Fusarium miscanthi* sp. nov. from *Miscanthus* litter. Mycologia 91:263-268.
- Garibaldi, A., Minuto, A., Gilardi, G., and Gullino, M. L. 2000. First report of *Fusarium* wilt on *Hebe* sp. in Italy and Europe. Plant Dis. 84:705.
- Geiser, D. M., Juba, J. H., Wang, B., and Jeffers, S. N. *Fusarium hostae* sp. nov., a relative of *F. redolens* with a *Gibberella* teleomorph. Mycologia. (In press.)
- Gerlach, W. 1961. *Fusarium redolens* Wr., seine Morphologie und systematische Stellung. Ein Beitrag zur Kenntnis der *Elegans*-Fusarien. Phytopathol. Z. 42:150-160.
- Gerlach, W., and Pag, H. 1961. *Fusarium redolens* Wr., seine phytopathologische Bedeutung und eine an *Dianthus*-Arten gefäßparasitäre Form (*F. redolens* Wr. f. *dianthi* Gerlach). Phytopathol. Z. 42:349-361.
- Gordon, W. L. 1952. The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. Can. J. Bot. 30:236-238.
- Ho, Y. W., Varghese, G., and Taylor, G. S. 1985. *Fusarium oxysporum* var. *redolens* from Africa as a cause of vascular wilt disease of oil palm. Phytopathol. Z. 113:373-376.
- Hungerford, C. W. 1923. *Fusarium* wilt of spinach. Phytopathology 13:205-209.
- Kroes, G. M. L. W., Löffler, H. J. M., Parlevliet, J. E., Keizer, L. C. P., and Lange, W. 1999. Interactions of *Fusarium oxysporum* f. sp. *lini*, the flax wilt pathogen, with flax and linseed. Plant Pathol. 48:491-498.
- Larsson, M., and Olofsson, J. 1994. Prevalence and pathogenicity of spinach root pathogens of the genera *Aphanomyces*, *Phytophthora*, *Fusarium*, *Cylindrocarpon*, and *Rhizoctonia* in Sweden. Plant Pathol. 43:251-260.
- Manicom, B. Q., and Baayen, R. P. 1993. Restriction fragment length polymorphisms in *Fusarium oxysporum* f. sp. *dianthi* and other fusaria from *Dianthus* species. Plant Pathol. 42:851-857.
- Matuo, T., and Snyder, W. C. 1973. Use of morphology and mating populations in the identification of formae speciales in *Fusarium solani*. Phytopathology 63:562-565.
- Muller, P. J. 1978. Krasbodems, vethuidigheid en bolrot bij hyacinten. Weekblad voor Bloembollencultuur 89(16):389-391.
- Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species. An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
- Nirenberg, H. I., and O'Donnell, K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90:434-458.
- Nirenberg, H. I., O'Donnell, K., Kroschel, J., Andrianavo, A. P., Frank, J. M., and Mubatanhema, W. 1998. Two new species of *Fusarium*: *Fusarium brevicatenulatum* from the noxious weed *Striga asiatica* in Madagascar and *Fusarium pseudoanthophilum* from *Zea mays* in Zimbabwe. Mycologia 90:459-464.
- O'Donnell, K. 2000. Molecular phylogeny of the *Nectria haematococca*-*Fusarium solani* species complex. Mycologia 92:919-938.
- O'Donnell, K., and 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.
- O'Donnell, K., Cigelnik, E., and Nirenberg, H. I. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465-493.
- O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA 95:2044-2049.
- O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. USA 97:7905-7910.
- O'Donnell, K., Nirenberg, H. I., Aoki, T., and Cigelnik, E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. Mycoscience 41:61-78.
- Raabe, R. D. 1957. *Fusarium* wilt of *Hebe buxifolia*. (Abstr.) Phytopathology 47:532.
- Raabe, R. D. 1985. *Fusarium* wilt of *Hebe* species. Plant Dis. 69:450-451.
- Snyder, W. C., and Hansen, H. N. 1940. The species concept in *Fusarium*. Am. J. Bot. 27:64-67.
- Subramanian, C. V. 1971. Hyphomycetes. An Account of Indian Species, Except *Cercosporae*. Indian Council for Agricultural Research, New Delhi, India.
- Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., and Fisher, M. C. 2001. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 21:21-32.
- Waalwijk, C., Baayen, R. P., De Koning, J. R. A., and Gams, W. 1996. Ribosomal DNA analyses challenge the status of *Fusarium* sections *Liseola* and *Elegans*. Sydowia Ann. Mycol. 48:90-104.
- Waalwijk, C., De Koning, J. R. A., Baayen, R. P., and Gams, W. 1996. Discordant groupings of *Fusarium* spp. from sections *Elegans*, *Liseola* and *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. Mycologia 88:361-368.
- Wang, B., and Jeffers, S. N. 2000. *Fusarium* root and crown rot: A disease of container-grown hostas. Plant Dis. 84:980-988.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innes, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.
- Wollenweber, H. W. 1913. Studies on the *Fusarium* problem. Phytopathology 3:24-50.
- Wollenweber, H. W. 1916-1935. *Fusaria* Autographice Delineata. Berlin.