Gene Genealogies and AFLP Analyses in the *Fusarium oxysporum* Complex Identify Monophyletic and Nonmonophyletic Formae Speciales Causing Wilt and Rot Disease

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ABSTRACT

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The monophyletic origin of host-specific taxa in the plant-pathogenic *Fusarium oxysporum* complex was tested by constructing nuclear and mitochondrial gene genealogies and amplified fragment length polymorphism (AFLP)-based phylogenies for 89 strains representing the known genetic and pathogenic diversity in 8 formae speciales associated with wilt diseases and root and bulb rot. We included strains from clonal lineages of *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, *lilii*, *lini*, *opuntiarum*, *spinaciae*, and *tulipae*. Putatively nonpathogenic strains from carnation and lily were included and a reference strain from each of the three main clades identified previously in the *F. oxysporum* complex; sequences from related species were used as outgroups. DNA sequences from the nuclear translation elongation factor 1α and the mitochondrial small subunit (mtSSU) ribosomal RNA genes were combined for phylogenetic analysis. Strains in vegetative compatibility groups (VCGs) shared identical sequences and AFLP profiles, supporting the monophyly of the two single-VCG formae speciales, *lilii* and *tulipae*. Identical genotypes were also found for the three VCGs in *F. oxysporum* f. sp. *spinaciae*. In contrast, multiple evolutionary origins were apparent for *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, *lini*, and *opuntiarum*, although different VCGs within each of these formae speciales often clustered close together or shared identical EF-1α and mtSSU rDNA haplotypes. Kishino-Hasegawa analyses of constraints forcing the monophyly of these formae speciales supported the exclusive origin of *F. oxysporum* f. sp. *opuntiarum* but not the monophyly of *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, and *lini*. Most of the putatively nonpathogenic strains from carnation and lily, representing unique VCGs, were unrelated to *F. oxysporum* f. spp. *dianthi* and *lilii*, respectively. Putatively nonpathogenic or rot-inducing strains did not form exclusive groups within the molecular phylogeny. Parsimony analyses of AFLP fingerprint data supported the gene genealogy-based phylogram; however, AFLP-based phylogenies were considerably more homoplasious than the gene genealogies. The predictive value of the forma specialis naming system within the *F. oxysporum* complex is questioned.

Fusarium oxysporum Schlechtend.: Fr. is an ubiquitous complex of cosmopolitan soilborne plant pathogens. Although a teleomorph is unknown, DNA sequence-based phylogenetic analyses place this complex unambiguously in the *Gibberella* clade, close to the *G. fujikuroi* species complex (36–39). Related species include *F. nisikadoi* (35), *F. miscanthi* (17), and *F. redolens* (39). Both *F. oxysporum* and *F. redolens* have been classified in *Fusarium* section *Elegans* Wollenw. (53) together with *F. udum* (19). However, the latter species is nested within the *G. fujikuroi* species complex, i.e., *Fusarium* section *Liseola* (39).

More than 150 host-specific formae speciales have been described in the *F. oxysporum* complex (FOC), each of them consisting of one or more vegetative compatibility groups (VCGs) and often distinct pathogenic races. VCGs appear to represent clonal lineages and generally have unique restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) fingerprints (3,6,24). While some formae speciales harbor a single VCG (3,11,23,46), many comprise up to 10 or more VCGs, of which only a few are common and widespread. Katan (22) provides an overview of the current status of VCGs in the FOC. Population-genetic studies on this complex are mostly biased, because isolates from agricultural crops generally are investigated, resulting in an overestimation of clonality through human dissemination of infected propagative materials. Clonality is less common in putatively nonpathogenic populations (2), and some formae speciales harbor large numbers of VCGs, of which none is dominant (14). The same has been observed for *F. proliferatum*, a related species in the section *Liseola* with a rarely encountered teleomorph nested in the *G. fujikuroi* species complex (previously referred to as *G. fujikuroi*) (13,19,29). Although a teleomorph has not yet been found, the sexual cycle may still be active in the FOC, in addition to parasexuality (47).

The genetic basis of host specificity (i.e., formae speciales) and cultivar specificity (i.e., pathogenic races) in the FOC is unknown. Nevertheless, a forma specialis is often assumed to have a single common ancestor from which all VCGs and races in that forma specialis have been derived (monophyly). Alternately, multiple

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VCGs and races within a given forma specialis could have multiple independent origins, with pathogenicity and virulence evolving more than once through mutation or transposition or spreading to distantly related strains through parasexuality or horizontal gene transfer. The monophyly hypothesis was recently tested by O'Donnell et al. (41) for four formae speciales, including *F. oxysporum* f. sp. *cubense*, causal agent of Panama disease of banana, by comparing DNA sequences of nuclear and mitochondrial genes. Concordant evidence from the respective gene genealogies revealed that *F. oxysporum* f. sp. *cubense* harbors at least five lineages with independent evolutionary origins (41). These phylogenetic analyses have been extended to ≈500 strains from a wide range of formae speciales, resulting in two important discoveries: (i) ≈80% of formae speciales with two or more VCGs appear to be para- or polyphyletic; and (ii) the FOC possesses considerable phylogenetic structure, as evidenced by numerous clades in the gene genealogies (38) (K. O'Donnell, *unpublished data*).

The present study focused on the evolutionary relationships between VCGs from eight formae speciales associated with various rot and wilt diseases of flower bulbs, cacti, carnation, asparagus, flax, and spinach. Isolates representing all published VCGs and races were selected for the *F. oxysporum* f. spp. *asparagi* (eight VCGs), *dianthi* (six VCGs), *gladioli* (six VCGs), *lilii* (one VCG), *spinaciae* (three VCGs), and *tulipae* (one VCG) (1,3,6,14, 15,33,44). A wider range of isolates was selected from *F. oxysporum* f. spp. *lini* and *opuntiarum*, from which no VCGs had been identified. We included a reference strain from each of the three main clades, previously published in the FOC (41), and sequences from related species as outgroups. Gene genealogies were constructed from DNA sequences of translation elongation factor EF-1α, and the mitochondrial small subunit (mtSSU) ribosomal RNA genes and compared with independent phylograms from parsimony analyses of amplified fragment length polymorphism (AFLP) fingerprints. AFLP is a powerful tool in molecular fingerprinting and for studying relationships among isolates of fungi at the population, species, and supraspecific level (9,32,42,49). Implications of the results of this study for the forma specialis naming system are discussed.

MATERIALS AND METHODS

Fungal strains. Strains used in the study (Table 1) were stored cryogenically at −175°C in the Agricultural Research Service (NRRL) Culture Collection (National Center for Agricultural Utilization Research, Peoria, IL). We selected strains representing all published VCGs in *F. oxysporum* f. spp. *asparagi* (VCG 1001 to 1008) (14), *dianthi* (VCG 0020 to 0028 plus six additional, putatively nonpathogenic isolates from carnation; [1,6]), *gladioli* (VCG 0340 to 0345; [44] E. Roebroeck, *unpublished data*), *lilii* (VCG 0190 plus seven additional, putatively nonpathogenic isolates from lily [3]), *spinaciae* (current VCG 0330 to 0332; published as VCG 1 to 3 by Fiely et al. [15]), and *tulipae* (VCG 0230 [3]). Also included were 13 strains of *F. oxysporum* f. sp. *lini* (26,28) and 22 strains of *F. oxysporum* f. sp. *opuntiarum* currently assigned to VCGs. Three strains of *F. oxysporum* f. sp. *cubense* and one of *F. oxysporum* f. sp. *lycopersici* were selected as references for FOC clades 1, 2, and 3, respectively (41).

Vegetative compatibility. VCG analysis in *F. oxysporum* f. spp. *lini* and *opuntiarum* was carried out as previously described (3,6) by generating *nit*1, *nit*3, and NitM mutants and pairing these on minimal medium. Strains of which the *nit* mutants complemented each other were placed in the same VCG. Novel strains from carnation, lily, and tulip were assigned to existing VCGs by generating *nit* mutants and pairing these with tester mutants of all known VCGs of *F. oxysporum* f. spp. *dianthi*, *lilii*, and *tulipae*, respectively.

Pathogenicity. Pathogenicity of strain NRRL 26442, received as *F. oxysporum* f. sp. *lilii*, was tested on universally susceptible lily cv. Esther with detached bulb scales placed in heavily infested soil, as described previously (3). Control plantlets were placed in uninfested soil. Experiments were terminated 8 weeks after planting, and plantlets were uprooted and evaluated for the presence of rot symptoms and suppression of adventitious root development.

DNA amplification and sequencing. Genomic DNA was prepared, as described previously (37). Polymerase chain reaction (PCR) amplification and sequencing of the mtSSU ribosomal DNA (rDNA) was performed with reagents and primers described by White et al. (52) and O'Donnell and Cigelnik (37). EF-1α was amplified with primers EF-1 and EF-2, which prime within conserved exons (41). In addition to the amplification primers, one forward (EF-11) and two reverse internal sequencing primers (EF-21 and EF-22) (41) were used with the fluorescent-labeled DyeDeoxy protocol on an automated sequencer (models 373 and 377; Perkin-Elmer Applied Biosystems, Foster City, CA). EF-1α and mtSSU rDNA sequences of all exemplars were deposited in GenBank under Accession numbers AF008453, AF008456, AF008459, AF008468, AF246832 to AF246887, AF246889 to AF246892, AF250560 to AF250619, U34509, U34519, and U61608. The aligned sequences are available from K. O'Donnell upon request.

Phylogenetic analysis. Phylogenetic analyses were conducted with PAUP* version 4.0b1 (Sinauer Associates Inc., Sunderland, MA), on the combined data set of mtSSU rDNA and EF-1α gene DNA sequences. Unweighted parsimony analyses were performed with the heuristic search option and 1,000 random addition sequences with the MULPARS function on and with tree bisection-reconnection branch swapping. The outgroup species selected for rooting the gene trees represent a putative sister group to the FOC (37,39). Clade stability was assessed by 1,000 parsimony bootstrap replications and decay indices calculated with TREEROT. The Kishino-Hasegawa (K-H) likelihood test implemented in PAUP* compared various constrained and unconstrained topologies. Alternative topologies were rejected with 95% confidence when they were >1.96 standard deviation (SD) less likely than the most parsimonious tree (MPT).

AFLP analysis. Strains were grown in potato dextrose broth (Difco Laboratories, Detroit) at 24.5°C for 3 days. DNA was isolated from lyophilized mycelium with a Puregene kit (Gentra/Biozym, Landgraaf, the Netherlands) according to the instructions of the manufacturer, except that an additional protein precipitation step was performed. DNA (250 ng) was digested in a 50-µl reaction volume with *Eco*RI (10 units [U]) and *Msp*I (10 U) for 5 h at 37°C in restriction ligation buffer (10 mM Tris/Hac at pH 7.5, 10 mM MgAc, 50 mM Kac, 5 mM dithiothreitol, and bovine serum albumin at 50 ng/µl), and adapters were ligated overnight to the restriction fragments in 40 µl of reaction product (10 µl for gel) at 10 to 12°C. Final concentrations were 2.4 U of T4 DNA ligase (Pharmacia, Uppsala, Sweden), 0.1 µM *Eco*R1 adapter (5′ CTCGTAGACTGCGTACC/CATCTGACGCATGGTTAA 5′), 1.0 µM *Msp*I adapter (5′ GACGATGAGTCCTGAT/CTACTCAGGACTAGC 5′), and 0.2 mM ATP (49). Ligation products were diluted 1:9 with MilliQ water. Nonselective amplification was performed with 5 µl of diluted ligation product added to 20 µl of buffer (10 mM Tris/HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) with 60 µM dNTP, primers *Eco*00 (5′ GACTGCGTACCAATTC) and *Msp*00 (5′ GATGAGTCCTGATCGG) at 5 ng/µl, and 1 U of *Taq* DNA polymerase (Boehringer GmbH, Mannheim, Germany). Reactions were performed in a PTC200 thermocycler (MJ Research, Watertown, MA) programmed as follows: 2 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 56°C, and 90 s at 72°C; and final extension of 10 min at 72°C and cooling to 4°C. Amplicons were checked on 1.0% agarose gels and visualized with ethidium bromide and UV illumination.

Selective PCR was performed on 5 µl of 20× diluted amplicons in a 20-µl final reaction volume of the buffer mentioned above but with 200 µM dNTP and 5 ng of Cy5-labeled fluorescent *Eco*20 primer (5′ GACTGCGTACCAATTCGC), and 30 ng of *Msp*15 (5′

GATGAGTCCTGATCGGCA) or *Msp*16 (5′ GATGAGTCCTGATCGGCC) primer. Reactions were performed under the following conditions: 2 min at 94°C; 13 cycles of 30 s at 94°C, 30 s at 65°C, and 60 s at 72°C (annealing temperature was lowered by 0.7°C during each cycle); followed by 23 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C; and a final extension of 10 min at 72°C and cooling to 4°C. Products were run on an ALFexpress automatic sequencer (Amersham Pharmacia Biotech, Roosendaal, the Netherlands) with a 50-bp ladder (Amersham) as a reference.

AFLP patterns were analyzed qualitatively with Imagemaster 1D software (Amersham) after manual correction for faint bands and obvious mismatches. The highly standardized procedure for generating AFLP patterns, including the ALFexpress and fluorescent instead of radioactively labeled gels, allowed identification of a total of 315 distinct and reproducible bands (185 bands with *Msp*15 and 130 bands with *Msp*16). A binary matrix was constructed for the presence or absence of these 315 bands in all 49 isolates. Phylogenetic analyses were performed on the binary matrix, as described above for the combined data set of DNA sequences. The matrix is available from R. P. Baayen upon request.

RESULTS

Two independent loci were examined, one from the nuclear (EF-1α) and one from the mitochondrial (mtSSU rDNA) genome. Strains within a particular VCG had identical EF-1α and mtSSU rDNA genotypes. Therefore, only one isolate per VCG is shown in Figure 1, 1 of 100 most parsimonious phylograms based on the combined EF-1α gene and mtSSU rDNA data set rooted with sequences of two outgroup species, *Fusarium* spp. NRRL 25184 and NRRL 28387. The ingroup in both data sets was strongly supported by bootstrapping (100%; Fig. 1). The earliest diverging lineage (82% bootstrap support) comprised two reference strains of *F. oxysporum* f. sp. *cubense* representing clade 1 sensu O'Donnell et al. (41), as well as a putatively nonpathogenic strain from lily. The reference for clade 2, *F. oxysporum* f. sp. *cubense* NRRL 25609, was nested in this clade (87% bootstrap support) with *F. oxysporum* f. spp. *lilii* and *spinaciae*, part of f. spp. *gladioli*, *lini*, and *dianthi*, and two putatively nonpathogenic strains from lily. Clade 3 reference *F. oxysporum* f. sp. *lycopersici* NRRL 26383 fell in a third clade with 87% bootstrap support together with *F. oxysporum* f. spp. *asparagi*, *opuntiarum*, and *tulipae*, part of f. sp. *gladioli*, and putatively nonpathogenic strains from lily and carnation. The remaining strains fell in three small lineages that were unresolved with respect to clades 2 and 3 (Fig. 1) but were nested in clade 2 in a phylogram derived from a subset of strains (bootstrap support 62%; Fig. 2B).

A subset of strains was selected to cover most of the genetic diversity indicated by the sequence data and was analyzed by AFLP. Parsimony analysis of these AFLP data yielded a phylogram that resolved many of the clades from the sequence-based phylogram. Although clade stability was distinctly less, the close concordance of sequence and AFLP-based trees was striking (Fig. 2A and B). With a consistency index of 0.43, the AFLP-based tree (Fig. 2A) was considerably more homoplasious than the sequence-based tree (Fig. 2B), that had a consistency index of 0.97. The number of nodes with high bootstrap values (13 nodes ≥ 90%; 22 nodes ≥ 70%) was higher in the AFLP-based tree than in the sequence-based tree (7 nodes ≥ 90% and 12 nodes ≥ 70%) (Table 2). Moreover, 12 nodes in the AFLP tree with ≥90% bootstrap support corresponded to strains with identical genotypes in the sequence-based tree (Fig. 2).

F. oxysporum f. sp. *lilii* (NRRL 28395 and 26955) VCG 0190 was resolved as a distinct lineage in clade 2 in the AFLP and DNA sequence-based phylograms. Except for NRRL 26442, all putatively nonpathogenic strains tested from lily proved unrelated to VCG 0190 (Fig. 1). Strain NRRL 26442 was received as *F. oxysporum* f. sp. *lilii* but proved nonpathogenic to universally susceptible lily cv. Esther and formed a single-member VCG distinct from VCG 0190 (Fig. 1). The identity of NRRL 26442, thus, remains unresolved. While *F. oxysporum* f. sp. *lilii* belonged to clade 2, two formae speciales (*F. oxysporum* f. spp. *tulipae* and *asparagi*) pathogenic to related liliaceous genera were nested within clade 3. *F. oxysporum* f. sp. *tulipae* NRRL 26954 (VCG 0230) was close to *F. oxysporum* f. sp. *asparagi* NRRL 28372 (VCG 1006) both in the AFLP phylogram and in the gene genealogies (Fig. 2A and B). A close relationship was observed for VCGs 1001, 1003, 1004, 1005, and 1007 of *F. oxysporum* f. sp. *asparagi* (Figs. 1 and 2); the remaining three VCGs of this forma specialis belonged to different lineages in clade 3 sensu O'Donnell et al. (41).

Five distinct lineages were identified in *F. oxysporum* f. sp. *gladioli* among six VCGs from iridaceous genera. Three VCGs (0341, 0342, and 0343) were nested in clade 2 (Fig. 2B) and three in clade 3 (0340, 0344, and 0345). Self-incompatible NRRL 28918, the type strain of *F. oxysporum* f. sp. *gladioli* originally studied by Massey (31), appeared to be more closely related to VCG 0343 NRRL 26993 (Fig. 1) than to the presently common VCG 0340. A schematic representation of phylogenetic lineages, VCGs, and host specificity (races) in *F. oxysporum* f. sp. *gladioli* is given in Table 3.

Both AFLP and sequence data identified a close relationship between *F. oxysporum* f. sp. *gladioli* NRRL 28406, VCG 0341, and *F. oxysporum* f. sp. *dianthi* NRRL 28401, VCG 0021, rather than a relationship of either VCG with other VCGs in the two respective formae speciales (Fig. 2). VCG 0021 harbored the dominant, cosmopolitan race 2 of *F. oxysporum* f. sp. *dianthi*. The remaining five VCGs (0020, 0022, 0025, 0027, and 0028) belonged to clade 2. Resolution in clade 2 was relatively poor using both methods (Fig. 2), leaving phylogenetic relationships within *F. oxysporum* f. sp. *dianthi* partially unresolved. In contrast, putatively nonpathogenic strains from carnation, including members of a large carnation-associated VCG with potential for biological control of Fusarium wilt represented by NRRL 26994 and NRRL 28376 (1,43), were all nested in clade 3, thereby excluding a close relationship to strains of *F. oxysporum* f. sp. *dianthi*. An overview of phylogenetic lineages, VCGs, pathogenicity, and cultivar specificity (races) is given in Table 4.

Attempts to identify VCGs among isolates of *F. oxysporum* f. sp. *lini* were largely unsuccessful. Apart from two compatible strains from Russia (NRRL 29084 and NRRL 29094), all strains of *F. oxysporum* f. sp. *lini* were either self-incompatible or proved to have a *nit*-mutant rather than wild-type phenotype from the start. Despite the availability of mutants from all strains, including several NitM mutants, no complementation reactions were observed. Of the four *F. oxysporum* f. sp. *lini*-containing lineages that received strong bootstrap support (99 to 100%) in the AFLP phylogram, two coincided with strongly supported *F. oxysporum* f. sp. *lini* clades in the sequence-based phylogram (Fig. 2A). Among the f. sp. *lini* lineages, two were found exclusively in Russia (NRRL 29084 and 29094) and Argentina (NRRL 28919 and 28920) (Table 5). The three VCGs in *F. oxysporum* f. sp. *spinaciae* (VCGs 0330 to 0332, corresponding to VCG 1 to 3 of Fiely et al. [15]) formed a monophyletic lineage in clade 2 with VCG 0342 of *F. oxysporum* f. sp. *gladioli*.

VCG diversity was high among isolates from Cactaceae (Table 1). Six isolates were vegetatively incompatible with all others. Only two isolates from different hosts (NRRL 28391 from *Dolichotele* sp. and NRRL 29274 from *Cereus* sp.) belonged to the same VCG (0455); those in VCG 0450 were recovered from two cactus genera, *Rhipsalidopsis* and *Schlumbergera*, from the same commercial greenhouse. Isolates NRRL 28368 from *Disco* sp. and NRRL 28391 from *Dolichotele* sp. were vegetatively incompatible but had virtually identical AFLP profiles (Fig. 2) and formed a well-supported (96% bootstrap interval) lineage in the gene genealogy (Fig. 1).

F. oxysporum f. sp. *opuntiarum*, with the exception of NRRL 28169, appeared to be monophyletic. Results of a K-H analysis of *F. oxysporum* f. sp. *opuntiarum* monophyly constraint strongly support (P = 0.8023; Table 6) the exclusive origin of this forma specialis. Constraints that forced the monophyly of *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, and *lini* were significantly worse than the MPT (Table 6).

DISCUSSION

In contrast to what is generally assumed, formae speciales in the FOC are frequently nonmonophyletic. Evidence for a paraphyletic or polyphyletic origin was presented for the first time by O'Donnell et al. (41) for *F. oxysporum* f. sp. *cubense*. Their results were supported by cluster analysis of DNA amplification finger-

TABLE 1. Strains of the *Fusarium oxysporum* complex and outgroups sequenced in this study

Taxon, VCG,[a] and race	Host/substrate	Origin	NRRL[b]	Source[c]	AFLP[d]
F. oxysporum f. sp. *asparagi*					
VCG 1001	*Asparagus officinalis*	United States	28973	FGSC 6608	+
VCG 1002	*Asparagus officinalis*	United States	28362	FGSC 6609	+
VCG 1003	*Asparagus officinalis*	United States	28404	FGSC 6611	+
VCG 1004	*Asparagus officinalis*	United States	28378	FGSC 6613	+
VCG 1005	*Asparagus officinalis*	United States	28398	FGSC 6615	+
VCG 1006	*Asparagus officinalis*	United States	28372	FGSC 6617	+
VCG 1007	*Asparagus officinalis*	United States	28384	FGSC 6619	+
VCG 1008	*Asparagus officinalis*	United States	28379	FGSC 6621	+
F. oxysporum f. sp. *cubense*					
VCG 0120	*Musa* sp.	Australia	25603[clade 1]	Kistler A2	–
VCG 01210	*Musa* sp.	United States	26029[clade 1]	Kistler A15	–
VCG 01214	*Musa* sp.	Malawi	25609[clade 2]	Kistler MW2	+
F. oxysporum f. sp. *dianthi*					
VCG 0020, race 4	*Dianthus caryophyllus*	United States	26147	IMI 141130	+
VCG 0020, race 4	*Dianthus caryophyllus*	Italy	26965	Garibaldi F140	+
VCG 0020, race 4	*Dianthus caryophyllus*	Italy	28902	Garibaldi F310	+
VCG 0020, race 4	*Dianthus caryophyllus*	Italy	28903	Garibaldi F79	+
VCG 0020, race 4	*Dianthus caryophyllus*	Italy	28904	Garibaldi F261	+
VCG 0020, race 4	*Dianthus caryophyllus*	Italy	28905	Garibaldi F828	+
VCG 0021, race 2	*Dianthus caryophyllus*	Israel	26222	CBS 416.90	–
VCG 0021, race 2	*Dianthus caryophyllus*	The Netherlands	28401	IPO WCS816	+
VCG 0021, race 2	*Dianthus caryophyllus*	Italy	28403	Garibaldi F107	–
VCG 0022, race 1	*Dianthus caryophyllus*	Italy	26964	Garibaldi F100	+
VCG 0022, race 1	*Dianthus caryophyllus*	Italy	28389	Garibaldi F521	+
VCG 0022, race 8	*Dianthus caryophyllus*	Italy	28356	Garibaldi F639	+
VCG 0022, race 8	*Dianthus caryophyllus*	Italy	28399	Garibaldi F773	+
VCG 0025, race 11	*Dianthus caryophyllus*	The Netherlands	26960	PD 90/291	+
VCG 0027, race 10	*Dianthus caryophyllus*	The Netherlands	26962	IPO WCS842	+
VCG 0027, race 10	*Dianthus caryophyllus*	The Netherlands	28365	IPO NAKS3	–
VCG 0028, race 9	*Dianthus caryophyllus*	Australia	26961	Kalc Wright 70	+
VCG 0028, race 9	*Dianthus caryophyllus*	Australia	28377	Kalc Wright 68	–
VCG 0028, race 9	*Dianthus caryophyllus*	Australia	28906	Kalc Wright B6D214/2	–
VCG 0028, race 9	*Dianthus caryophyllus*	Australia	28907	Kalc Wright B9D221/2	–
F. oxysporum f. sp. *gladioli*					
VCG 0340, race 1	*Gladiolus* × *grandiflorus*	France	26992	LBO G2	+
VCG 0340, race 1	*Gladiolus italicus*	The Netherlands	28394	LBO G15	–
VCG 0340, race 2n	*Gladiolus* × *nanus*	The Netherlands	28911	LBO G6	–
VCG 0340, race 2n	*Ixia* sp.	The Netherlands	28912	LBO X1	–
VCG 0340, race 2i	*Iris* × *hollandica*	The Netherlands	28385	LBO Ir2	–
VCG 0340, race 2i	*Iris* × *hollandica*	The Netherlands	28913	LBO Ir1	–
VCG 0340, race 3	*Crocus* sp.	The Netherlands	28914	LBO Cr7	–
VCG 0340, race 3	*Crocus* sp.	The Netherlands	28915	LBO Cr12	–
VCG 0340, race 4	*Crocus* sp.	The Netherlands	28916	LBO Cr4	–
VCG 0340, race 4	*Crocus* sp.	The Netherlands	28917	LBO Cr8	–
VCG 0341, race 5	*Freesia* sp.	The Netherlands	26988	LBO Fr10	–
VCG 0341, race 5	*Gladiolus* × *colvillei*	The Netherlands	28406	LBO G23	+
VCG 0342, race 2c	*Crocus* sp.	The Netherlands	26991	LBO Cr1	+
VCG 0342, race 2c	*Crocus* sp.	Unknown	28388	LBO Cr2	–
VCG 0343, race 1it	*Gladiolus* × *grandiflorus*	Italy	26993	LBO G82	+
VCG 0343, race 1it	*Gladiolus* × *grandiflorus*	Italy	28360	LBO G76	–
VCG 0344, new race	*Freesia* sp.	The Netherlands	26989	LBO Fr11	+
VCG 0345, new race	*Freesia* sp.	The Netherlands	26990	LBO Fr12	+
VCG 034–si	*Gladiolus* sp.	United States	28918	CBS 151.27	–
F. oxysporum f. sp. *lilii*					
VCG 0190	*Lilium* sp.	The Netherlands	26955	CPRO Fol4	+

(*continued on next page*)

[a] VCG = vegetative compatibility group; * = vegetatively incompatible with any other VCG from carnation; ** = vegetatively incompatible with any other VCG from lily; *** = vegetative compatibility tested but not sequenced.

[b] Reference strains for clades 1, 2, and 3 of O'Donnell et al. (41) in superscript.

[c] Strain source: FGSC = Fungal Genetics Stock Center, Kansas; Kistler = H. C. Kistler, Cereal Disease Laboratory, USDA/ARS, St. Paul, MN; IMI = International Mycological Institute (CABI Bioscience), Egham, U.K.; Garibaldi = A. Garibaldi, Turin University, Turin, Italy;. CBS = Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; IPO = Research Institute for Plant Protection, Wageningen, the Netherlands; Kalc Wright = G. F. Kalc Wright, Parkville, Australia; LBO = Bulb Research Centre, Lisse, the Netherlands; CPRO = Centre for Plant Breeding and Reproduction Research, Wageningen, the Netherlands; PD = Plantenziektenkundige Dienst, Wageningen, the Netherlands; DAOM = Department of Agriculture (Mycology) and Agri-Food, Ottawa, Canada; BBA = Biologische Bundesanstalt für Landund Forstwirtschaft, Berlin; Fiely = M. B. Fiely, University of Arkansas, Fayetteville; BCRI = British Columbia Research Inc., Vancouver, Canada.

[d] AFLP = amplified fragment length polymorphism. Strains subjected (+) and not subjected (–) to AFLP analysis.

prints (7). The present study provides evidence for multiple evolutionary origins of *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, and *lini*. A phylogram inferred from the AFLP data set was strikingly similar to one inferred from combined EF-1α and mtSSU rDNA sequences. Both approaches complement each other: AFLP analysis, in theory, samples loci throughout the entire genome, while gene genealogies examine the evolution of nuclear and mitochondrial gene sequences. Clades that are supported by AFLP and gene genealogies are likely to reflect independent measures of the evolutionary history of these fungi. The con-

TABLE 1. (*continued from preceding page*)

Taxon, VCG[a], and race	Host/substrate	Origin	NRRL[b]	Source[c]	AFLP[d]
VCG 0190	*Lilium* sp.	Italy	28395	CPRO Fol28	+
VCG 0190	*Lilium* sp.	Poland	28908	CPRO Fol80	–
F. oxysporum f. sp. *lini*					
VCG 0440	*Linum usitatissimum*	Russia	29084	CPRO U2	+
VCG 0440	*Linum usitatissimum*	Russia	29094	CPRO U1	+
VCG 044–	*Linum usitatissimum*	Argentina	28919	CPRO A1	+
VCG 044–	*Linum usitatissimum*	Argentina	28920	CPRO A2	+
VCG 044–	*Linum usitatissimum*	Belgium	28921	CPRO B1	+
VCG 044–	*Linum usitatissimum*	Belgium	28922	CPRO B2	+
VCG 044–	*Linum usitatissimum*	Canada	28923	CPRO C1	+
VCG 044–	*Linum usitatissimum*	Canada	28924	CPRO C2	+
VCG 044–	*Linum usitatissimum*	France	28925	CPRO F1	+
VCG 044–	*Linum usitatissimum*	France	28926	CPRO F26	+
VCG 044–	*Linum usitatissimum*	The Netherlands	28928	CPRO N1	+
VCG 044–	*Linum usitatissimum*	The Netherlands	28929	CPRO N3	+
VCG 044–	*Linum usitatissimum*	The Netherlands	28930	CPRO N10	+
F. oxysporum f. sp. *lycopersici*					
VCG unknown	*Lycopersicon esculentum*		26383[clade 3]		+
F. oxysporum f. sp. *opuntiarum*					
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28363	IPO 96-11	+
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28367	IPO 96-15	+
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28370	IPO 96-18	–
VCG 0450	*Schlumbergera* sp.	The Netherlands	28402	IPO 96-32	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28405	IPO 96-21	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28933	IPO 96-13	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28934	IPO 96-14	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28937	IPO 96-23	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	28938	IPO 96-24	–
VCG 0450	*Schlumbergera* sp.	The Netherlands	28939	IPO 96-33	–
VCG 0450	*Schlumbergera* sp.	The Netherlands	28940	IPO 96-34	–
VCG 0450	*Schlumbergera* sp.	The Netherlands	28941	IPO 96-36	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	29126	IPO 96-16NitM	–
VCG 0450	*Rhipsalidopsis* sp.	The Netherlands	29127	IPO 96-17*nit*3	–
VCG 0451	*Disco placentiformis*	The Netherlands	28368	PD 94/123	+
VCG 0452	Cactus	Canada	28169	DAOM 215628	–
VCG 0453	*Zygocactus* sp.	Germany	28243	BBA 64709	–
VCG 0454	*Ferocactus* sp.	Germany	28279	BBA 62346	+
VCG 0455	*Dolichotele* sp.	The Netherlands	28391	PD 95/1408	+
VCG 0455	*Cereus* sp.	The Netherlands	29274***	PD 99/3057	–
VCG 0456	*Opuntia microdasys*	The Netherlands	29272***	PD 99/6057	–
VCG 045–	*Zygocactus* sp.	Germany	22548	BBA 62349	–
F. oxysporum f. sp. *spinaciae*					
VCG 0330	*Spinacea oleracea*	United States	26874	Fiely MF 15	–
VCG 0331	*Spinacea oleracea*	United States	26875	Fiely MF 34	–
VCG 0332	*Spinacea oleracea*	United States	26876	Fiely MF 42	–
F. oxysporum f. sp. *tulipae*					
VCG 0230	*Tulipa* × *gesneriana*	Germany	22556	CBS 242.59; BBA 8248	–
VCG 0230	*Tulipa* × *gesneriana*	The Netherlands	26954	LBO Tu10	+
VCG 0230	*Tulipa* × *gesneriana*	The Netherlands	28974	LBO Tu4	+
F. oxysporum, putatively nonpathogenic					
VCG 1	*Dianthus caryophyllus*	The Netherlands	26994	PD 89/1523	+
VCG 1	*Dianthus caryophyllus*	Israel	28376	Manicom X40	–
Unique VCG*	*Dianthus caryophyllus*	The Netherlands	28369	PD 90/1580.2	–
Unique VCG*	*Dianthus caryophyllus*	The Netherlands	28392	PD 90/1580.1	–
Unique VCG*	*Dianthus caryophyllus*	The Netherlands	28380	PD 90/440	–
Unique VCG*	*Dianthus caryophyllus*	Israel	28396	Manicom X48	–
Unique VCG**	*Lilium* sp.	United States	26442	IMI 141108	–
Unique VCG**	*Lilium* sp.	The Netherlands	28358	CPRO Fol17	–
Unique VCG**	*Lilium* sp.	The Netherlands	28359	CPRO Fol14	–
Unique VCG**	*Lilium* sp.	Italy	28361	CPRO Fol77	+
Unique VCG**	*Lilium* sp.	United States	28366	CPRO Fol67	+
Unique VCG**	*Lilium* sp.	United States	28371	CPRO Fol65	+
Unique VCG**	*Lilium* sp.	The Netherlands	28397	CPRO Fol6	+
Fusarium					
VCG not tested	*Pseudotsuga menziesii*	United States	22903	BCRI 3139	–
VCG not tested	*Dianthus caryophyllus*	The Netherlands	28387	PD 90/1377	+
Fusarium					
VCG not tested	Peat soil	Germany	25184	BBA 65467	–

sistency index was considerably lower with the AFLP-based phylogeny. This is in accord with a recent phylogenetic analysis of RAPD data for seven FOC isolates from maize and three outgroup strains, which revealed the same underlying phylogeny as EF-1α and mtSSU rDNA gene genealogies but with a much higher level of homoplasy than sequence data (40). Recent phylogenetic analysis of exclusively RAPD data from strains from cucurbitaceous hosts also supports the nonmonophyletic nature

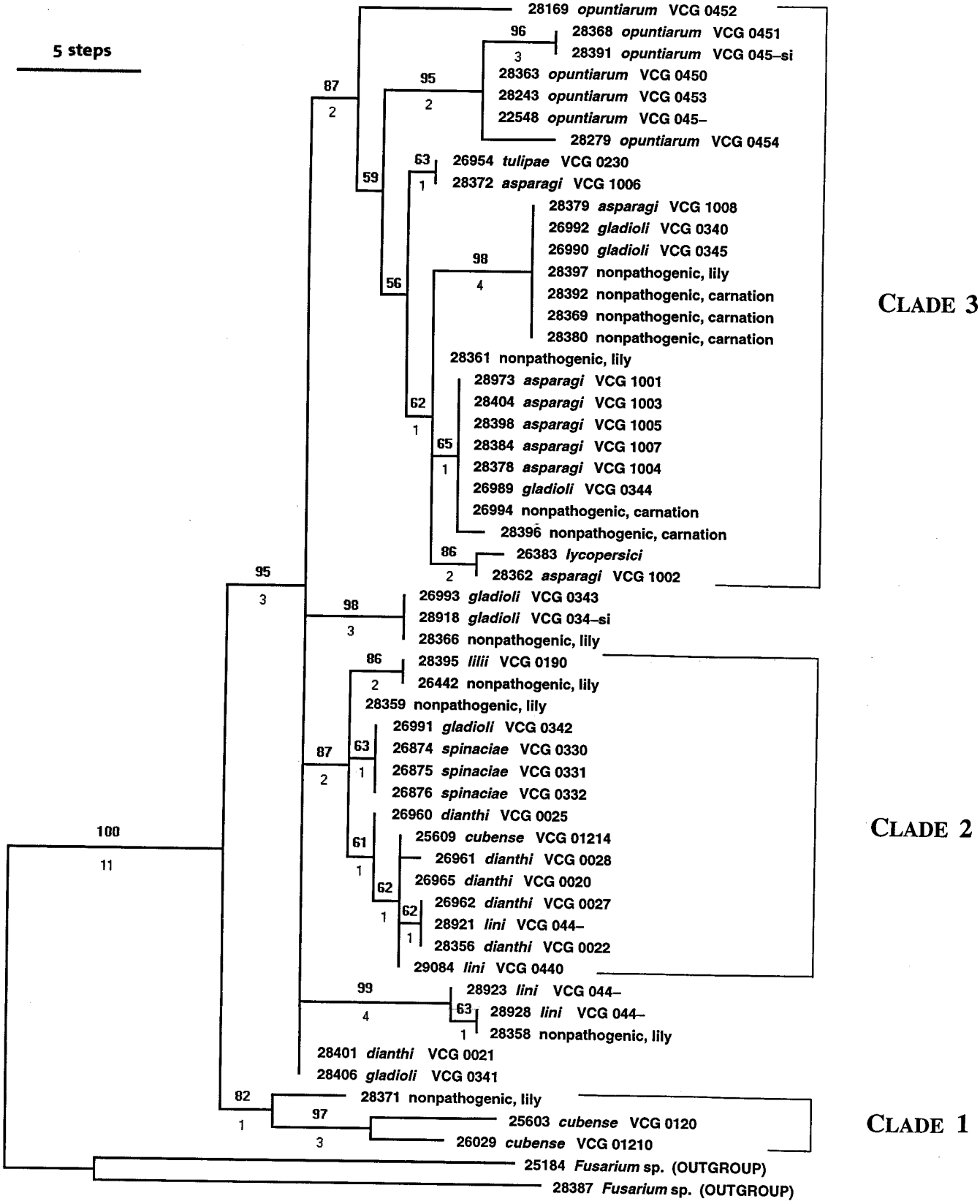

Fig. 1. One of one-hundred most parsimonious phylograms (116 steps; consistency index [CI] = 0.94; retention index [RI] = 0.97; rescaled consistency index [RC] = 0.92) by maximum parsimony implemented in PAUP* based on the combined EF-1α gene and mitochondrial small subunit ribosomal DNA data set for the *Fusarium oxysporum* complex rooted with sequences from *Fusarium* sp. strains NRRL 25184 and 28387. Bootstrap values (1,000 replications) are indicated above nodes. Decay indices calculated with TREEROT are indicated below nodes. Only one strain is shown per vegetative compatibility group (VCG) and genotype.

of some formae speciales (*cucumerinum* and *niveum*), while others appear to be monophyletic (*radicis-cucumerinum* and *melonis*) (48). In the latter study, cross-compatibility between *nit* mutants from strains of *F. oxysporum* f. sp. *radicis-cucumerinum* VCGs 0260 and 0261, which have the same RAPD pattern, indicates that this forma specialis may represent a single recently diverged clonal lineage. Similar results have been reported for VCGs 0030 and 0032 of *F. oxysporum* f. sp. *lycopersici* (34).

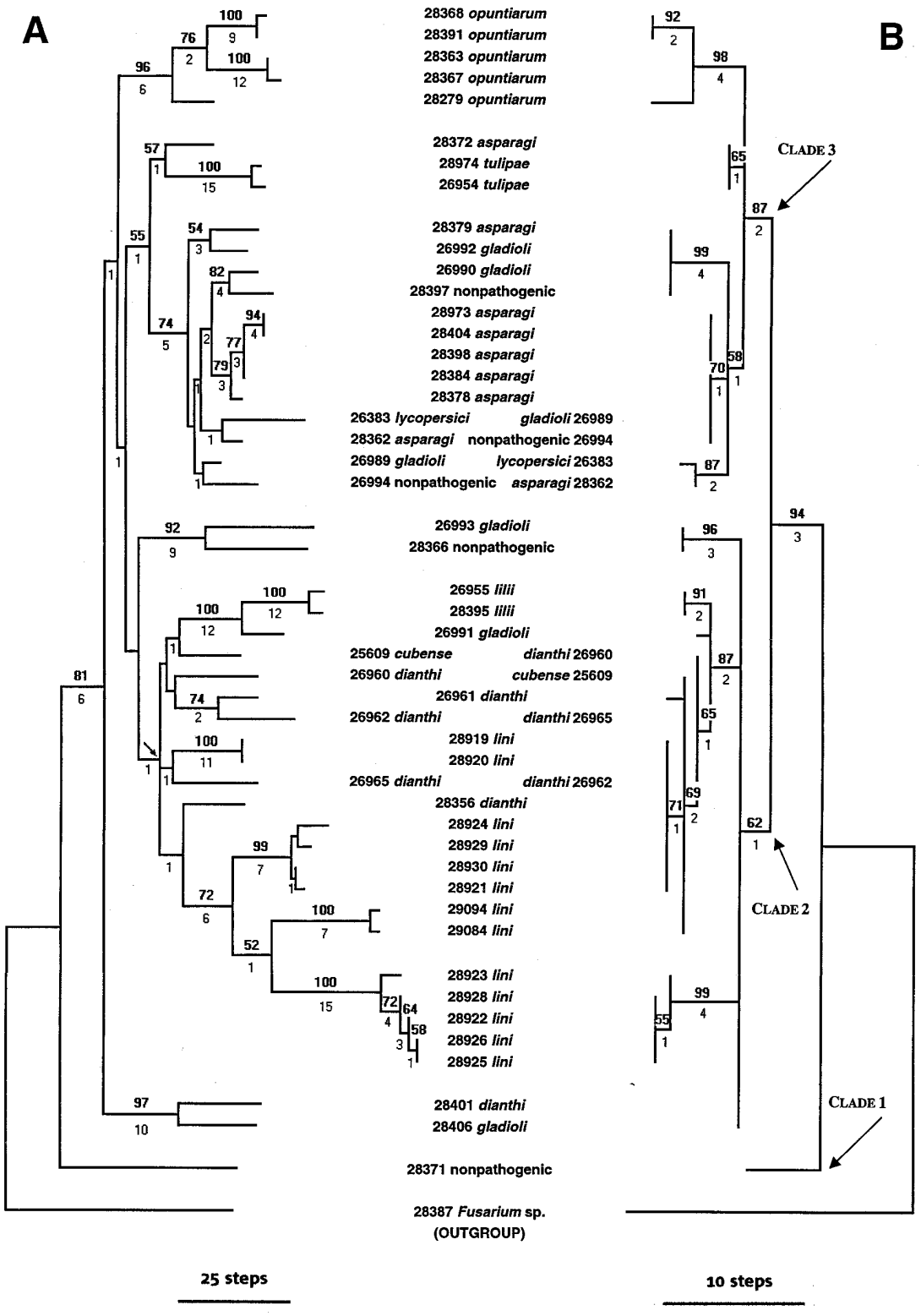

Fig. 2. Concordance of phylograms inferred from **A,** amplified fragment length polymorphism (AFLP) and **B,** combined EF-1α and mitochondrial small subunit (mtSSU) rDNA data sets for the *Fusarium oxysporum* complex rooted by the outgroup method with *Fusarium* sp. strain NRRL 28387. Bootstrap replication frequencies ≥50% are indicated above nodes; decay indices are indicated below nodes. Isolate codes positioned between phylograms belong with the respective AFLP and sequence phylogram branches at that position. When exact matching of the dendrograms through branch rotation was not possible, the proper isolate codes are given next to each other. **A,** Results of 1 of 10 most parsimonious phylograms (710 steps; consistency index [CI] = 0.43; retention index [RI] = 0.69; rescaled consistency index [RC] = 0.30) from the binary AFLP data set. A poorly supported branch (decay index = 1) has been collapsed to a trichotomy (arrow). **B,** Results of 1 of 80 most parsimonious phylograms (78 steps; CI = 0.97; RI = 0.99; RC = 0.97) from the combined EF-1α and mtSSU rDNA data set. Only one strain is generally shown per vegetative compatibility group (VCG), because AFLP fingerprints and sequence genotypes were virtually identical within VCGs.

TABLE 2. Comparison of amplified fragment length polymorphism (AFLP) and gene sequence tree statistics[a]

Analysis	Characters	Parsimony tree length	Trees	Consistency index	Autapomorphies/ synapomorphies	Nodes ≥70% bootstrap	Nodes ≥90% bootstrap
AFLP	315	710	10	0.43	100/205	22	13
mtSSU[b] rDNA + EF-1α	1,396	78	80	0.97	39/40	12	7

[a] Shown in Figure 2.
[b] mtSSU = mitochondrial small subunit.

TABLE 3. Relation of EF-1α and mitochondrial small subunit (mtSSU) ribosomal DNA genotypes in *Fusarium oxysporum* f. sp. *gladioli* with the known vegetative compatibility group (VCG), amplified fragment length polymorphism (AFLP)/restriction fragment length polymorphism (RFLP) and race structure[a]

Genotype	Representative strain	VCG	AFLP/RFLP group	Race
1	NRRL 28406	0341	II	5
2	NRRL 26991	0342	III	2c
3	NRRL 26993	0343	V	1it
	NRRL 28918	034-	IV	Unknown
4	NRRL 26992	0340	I	1
	NRRL 28911	0340	I	2n
	NRRL 28385	0340	Ib	2i
	NRRL 28914	0340	Ic	3
	NRRL 28916	0340	Id	4
	NRRL 26990	0345	XXI	Unknown
5	NRRL 26989	0344	XX	Unknown

[a] Mes et al. (33,44); E. J. A. Roebroeck, *unpublished data*.

TABLE 4. Relation of EF-1α and mitochondrial small subunit (mtSSU) rDNA genotypes in *Fusarium oxysporum* f. sp. *dianthi* with known vegetative compatibility group (VCG), restriction fragment length polymorphism (RFLP), esterase, and race structure[a]

Genotype	Representative strain	VCG	RFLP/esterase group	Race
1	NRRL 28401	0021	II	2
2	NRRL 26964	0022	III	1
	NRRL 28356	0022	III	8
	NRRL 26962	0027	V	10
3	NRRL 26965	0020	I	4
4	NRRL 26961	0028	VI	9
5	NRRL 26960	0025	IV	11

[a] Aloi and Baayen (1) and Baayen et al. (6).

Formae speciales harboring a single VCG, such as *F. oxysporum* f. spp. *lilii* and *tulipae*, are generally assumed to be clonal (3). However, VCGs within given formae speciales may or may not form exclusive groups in the molecular phylogeny. For example, the five VCGs in *F. oxysporum* f. sp. *asparagi* (VCGs 1001, 1003, 1004, 1005, and 1007) form a monophyletic group, as do VCGs 0340 and 0345 of *F. oxysporum* f. sp. *gladioli*. In addition, all three VCGs of *F. oxysporum* f. sp. *spinaciae* (15) form an exclusive group in the gene tree, indicating that this forma specialis is monophyletic. Although *F. oxysporum* f. sp. *opuntiarum* appears to represent a paraphyletic grade, results of a K-H analysis support a possible monophyletic origin. All of the other formae speciales studied have paraphyletic or polyphyletic origins, with *F. oxysporum* f. sp. *gladioli* being the most diverse in evolution.

Phylograms based on EF-1α and mtSSU rDNA sequences resolved five distinct lineages in *F. oxysporum* f. sp. *gladioli*. Races within VCG 0340, left unresolved in the gene genealogies, have recently been resolved with high-resolution AFLP analyses (E. Roebroeck, *unpublished data*). In general, AFLP analyses have a higher resolution at a refined level, while analysis of the EF-1α and mtSSU rDNA gene sequence resolves higher order phylogenies. Phylogenetic analysis of the rDNA internal transcribed spacer (ITS) region, while useful in many organisms, is cladistically uninformative in the *G. fujikuroi* and *F. oxysporum* species complexes and misleading due to the presence of two nonorthologous intragenomic rDNA ITS2 types within all known species (37,39,50,51).

TABLE 5. Relation of EF-1α and mitochondrial small subunit (mtSSU) rDNA genotypes in *Fusarium oxysporum* f. sp. *lini* with vegetative compatibility group (VCG) and amplified fragment length polymorphism (AFLP) structure[a]

Genotype	Representative strain	VCG	AFLP group	Geographic origin
1a	NRRL 28928	044-	1a	The Netherlands, Belgium, France
1b	NRRL 28923	044-	1b	Canada
2	NRRL 29084	0440	2	Russia
3	NRRL 28921	044-	3	The Netherlands, Belgium, Canada
	NRRL 28919	044-	4	Argentina

[a] Kroes (26) and Kroes et al. (28).

Rot-associated formae speciales have often been considered less specialized and phylogenetically distinct from xylem vessel-colonizing pathogens. However, our results show that rot- and wilt-associated VCGs are equally dispersed throughout the molecular phylogeny. At present, clearcut distinctions in pathology between both groups are unavailable. *F. oxysporum* f. spp. *lilii* and *tulipae* are cortex colonizers (5). *F. oxysporum* f. sp. *gladioli* colonizes both the cortex and xylem (10). *F. oxysporum* f. sp. *opuntiarum* causes root and stem rot of Cactaceae rather than invading the xylem (18). *F. oxysporum* f. spp. *lini* and *basilici* induce both a typical root-rot syndrome as well as a wilt disease in flax and basil, respectively (16,26). No specific association was found between formae speciales and VCGs associated with bulb and root rot of monocots in Iridaceae and Liliaceae. *F. oxysporum* f. sp. *gladioli* VCG 0340 does not appear to be closely related to *F. oxysporum* f. sp. *lilii* (VCG 0190), even though VCG 0340 is pathogenic to lily (3). As expected, putatively nonpathogenic isolates from lily that do not belong to VCG 0190 proved unrelated to this pathogenic VCG. The status of NRRL 26442 remains unresolved. Although NRRL 26442 was received as *F. oxysporum* f. sp. *lilii*, it is nonpathogenic to susceptible lily cv. Esther and is not a member of VCG 0190.

Six strains isolated from and nonpathogenic to carnation from the study of Aloi and Baayen (1) all belonged to clade 3, which does not contain *F. oxysporum* f. sp. *dianthi*. Two of these strains isolated from the same carnation plant (NRRL 28369 and 28392), though vegetatively incompatible, proved to be phylogenetically related to each other. Two others (NRRL 26994 and 28376) belong to a putatively nonpathogenic VCG that is commonly found as an endophyte in the xylem of healthy carnations. This fungus is presently commercialized as a biocontrol agent against Fusarium wilt (1,43). Apart from a minimal risk of parasexual or vegetative (horizontal) gene exchange with pathogenic strains encountered inside the xylem of carnation, these biocontrol strains are unlikely to become pathogenic.

The single cosmopolitan VCG 0021 of *F. oxysporum* f. sp. *dianthi* proved phylogenetically distinct from the remaining five

TABLE 6. Kishino-Hasegawa likelihood analysis of constrained and unconstrained of trees from combined mitochondrial small subunit (mtSSU) rDNA and EF-1α data set

Tree[a]	Tree length[b]	–ln *L*	Difference –ln *L*	SD[c]	*P*[d]
MPT	116	–2,713.11859			
Fusarium oxysporum f. sp. *asparagi*	123 (+7)	–2,766.47588	–53.35729	17.52455	0.0024
Fusarium oxysporum f. sp. *dianthi*	121 (+5)	–2,755.24375	–42.12516	16.64345	0.0115
Fusarium oxysporum f. sp. *gladioli*	133 (+17)	–2,840.23080	–127.11221	26.60986	<0.0001
Fusarium oxysporum f. sp. *lini*	126 (+10)	–2,789.46793	–77.64955	21.44588	0.0003
Fusarium oxysporum f. sp. *opuntiarum*	116 (+0)	–2,714.74013	–1.62154	6.47521	0.8023

[a] Monophyly constraints enforced with PAUP* were compared with the best most parsimonious tree (MPT).
[b] Includes autapomorphic characters. Numbers in parentheses represent the difference in length between MPT and constrained trees.
[c] Standard deviation of log likelihood.
[d] Probability of a more extreme T value under the null hypothesis of no difference between the two trees by a two-tailed test. All values are significant at $P < 0.05$ except for the *F. oxysporum* f. sp. *opuntiarum* monophyly constraint.

VCGs that have restricted geographic distributions. The latter VCGs do not appear to have evolved from VCG 0021, even though this is the only sympatric VCG for VCGs 0025 and 0027 (restricted to the Netherlands) and VCG 0028 (strictly Australian) (6,21).

Attempts to elucidate the VCG structure in *F. oxysporum* f. sp. *lini* were largely unsuccessful. The self-incompatibility of several of the isolates from flax may have been due to prolonged subculturing of the isolates. Nevertheless, four distinct AFLP groups and three gene genealogy lineages were resolved in this forma specialis. Two of these exhibited restricted geographic distributions. Isolates of *F. oxysporum* f. sp. *lini* have been associated with wilt disease according to some authors, and with root rot according to others (26,27). The possibility that only certain lineages may be able to incite wilt disease, in addition to cortical rot, deserves exploration.

Considerable phylogenetic diversity was found in *F. oxysporum* f. sp. *opuntiarum*, and most isolates formed single-member VCGs. The only large VCG was composed of isolates exclusively from a single nursery. This parallels VCG diversity in *F. oxysporum* f. sp. *asparagi*, where numerous distinct VCGs occur, but none of these appears to have a selective advantage over the others (8,14), a condition normally occurring in freely outcrossing populations. Indeed, Taylor et al. (47) recently presented evidence for the occurrence of recombination among clade 1 members of *F. oxysporum* f. sp. *cubense* by combining and reanalyzing data published by O'Donnell et al. (41), Koenig et al. (25), and Bentley et al. (7).

Although multiple mutation or transposition events may have led to the independent acquisition of pathogenicity to the same host in strains with a different phylogeny, other mechanisms may be involved as well. Parasexuality can occur in *F. oxysporum* (30) and may have contributed to lateral transfer of pathogenicity genes to distantly related strains. The same holds for sexuality, because vegetative incompatibility does not necessarily block the sexual cycle. A single cross between two isolates with different VCG genotypes could result in more than 1,000 progeny VCG genotypes (30), and pathogenicity genes could be partitioned in numerous VCGs. Many fungi may still have an infrequent sexual cycle, and there is no reason to assume that members of the FOC are an exception (47). Mating-type gene sequences recently have been found in all investigated strains of the currently examined formae speciales (C. Waalwijk, *unpublished data*). Horizontal transfer of pathogenicity genes on conditionally dispensable (supernumerary) chromosomes to related strains may also be involved (12). The apparent clustering of most of the VCGs of *F. oxysporum* f. spp. *dianthi* and *lini* in one subclade and those of *F. oxysporum* f. sp. *opuntiarum* in clade 1 is consistent with this hypothesis. In addition, horizontal gene transfer across species boundaries may explain why xylem-colonizing VCGs that cause wilt disease of carnation are found in *F. oxysporum* f. sp. *dianthi* as well as *F. redolens* f. sp. *dianthi* (4,6), why asparagus root- and crown rot-associated VCGs exist in *F. oxysporum* f. sp. *asparagi*, *F. redolens* f. sp. *asparagi*, and *F. proliferatum* f. sp. *asparagi* (13,14,20), and perhaps even why wilt disease-inducing formae speciales exist in *F. udum* (19), a member of the *G. fujikuroi* species complex (37).

The origin of pathogenic diversity and the potential of horizontal spread of pathogenicity genes and gene clusters among members of the FOC deserves further investigation. Horizontal (vegetative) spread of dispensable chromosomes carrying pathogenicity genes or gene clusters across evolutionary distinct lineages (12) might explain why basic pathogenicity to the same host occurs in different clades of the FOC. Such a mechanism, as yet purely speculative, could provide support for the existing concept of formae speciales as introduced by Snyder and Hansen (45), even if the overall evolutionary background of VCGs in a given forma specialis is heterogeneous. More probable, host specificity and pathogenicity genes in different VCGs of a forma specialis are dissimilar generally, rendering the forma specialis concept artificial and of little predictive value. The practical diagnostic value of such an artificial classification system will undoubtedly eventually prove inferior to a novel, VCG-based, and more natural system with greater predictive value than host specificity. Whether a correlation exists between lineages in gene and AFLP trees and the morphologically defined subgroups in section *Elegans*, as circumscribed by Wollenweber and Reinking (53), deserves further study.

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