Mitochondrial DNA (mtDNA)—Relatedness among Formae Speciales of *Fusarium oxysporum* in the Cucurbitaceae

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ABSTRACT

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Thirty-nine isolates of *Fusarium oxysporum* encompassing five formae speciales causing vascular wilt in cucurbits were examined for genetic similarity by restriction fragment length polymorphisms (RFLP) analysis of mitochondrial DNA (mtDNA). Total DNA was digested with three enzymes (*Pst*I, *Hind*III, and *Eco*RI), Southern blotted, and hybridized with a *F. o. niveum* mtDNA polyprobe (pFON2a–pFON8b). The presence or absence of mtDNA fragments was examined by the unweighted-paired-group method using averages (UPGMA) and parsimony analysis. A total of 14 mtDNA RFLP groups were detected. Within each forma specialis there were unique RFLPs; however, one pattern generally occurred most frequently for each forma specialis. Two RFLP groups (RFLPG-fspI and IX) occurred in four and two formae speciales, respectively. The RFLP haplotype most common in *F. o. niveum* (RFLPG-fspI) also occurred in one or more isolates from every other formae speciales except *F. o. luffae* and was present in isolates from North America, Europe, and Asia. Genetic distances generated by UPGMA suggest that *F. o. niveum* was the least diverse forma specialis, while *F. o. cucumerinum* was the most diverse. However, both cluster analysis and parsimony analysis indicated that all of *F. oxysporum* formae speciales in the cucurbits are closely related and, in some cases, isolates of different formae speciales were genetically more similar than isolates of the same forma specialis.

Formae speciales of *Fusarium oxysporum* Schlechtend.:Fr., causal agents of many vascular wilt diseases, have been defined on the basis of host specificity (35). Over 90 different formae speciales of *F. oxysporum* are recognized, and many are further divided into pathological races based on pathogenicity to a set of differential host cultivars, each with a unique combination of resistance genes (1,35). Five different formae speciales have been identified that cause wilt in cucurbitaceous plants: *F. o.* f. sp. *niveum* (E. F. Sm.) Snyd. & Hans. on watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), *F. o.* f. sp. *melonis* W. C. Snyder & H. N. Hans on muskmelon (*Cucumis melo* L.), *F. o.* f. sp. *cucumerinum* J. H. Owens on cucumber (*Cucumis sativus* L.), *F. o.* f. sp. *lagenaria* Matuo & Yamamota on calabash gourd (*Lagenaria siceraria* (Molina) Standley, and *F. o.* f. sp. *luffae* Kawai, Suzuki, & Kawai on vegetable sponge (*Luffa aegyptiaca* Miller) (1,19,25,29,32). Among these five formae speciales, *F. o. niveum*, *melonis*, and *cucumerinum* are globally distributed and more important pathogens from an economic standpoint. The other two formae speciales, *F. o. lagenaria* and *F. o. luffae*, have only been recognized in a restricted geographic area of Japan (19,29).

Several studies have shown the existence of genetically distinct groups within formae speciales of *F. oxysporum* on cucurbits (16,20,21,24). Groups determined by vegetative compatibility or restriction fragment length polymorphisms (RFLP) of mitochondrial DNA (mtDNA) have also been correlated with their host range (3,16,18,22,24). However, a change from one forma specialis (*F. o. niveum*) to another forma specialis (*F. o. melonis*) has been reported (4), and one isolate of *F. o. cucumerinum* from The Netherlands is pathogenic to cucumber, muskmelon, and watermelon (13). These reports suggest that intra-forma specialis variation is present in each forma specialis and that the formae speciales of *F. oxysporum* that attack related hosts may be closely related. The differences between formae speciales may be relatively simple (one or two genes) from a genetic basis, and the determinants of host specificity may be combined (or perhaps lost) in a single strain. Similar concepts were established in *F. o. conglutinans* (Wollenweb.) W. C. Snyder & H. N. Hans, which contains isolates attacking cruciferous plants (1).

Several studies have been done to determine genetic variation at different taxonomic levels of *Fusarium* and include pathogenicity (1), vegetative compatibility grouping (VCG) (3,7,8,15,17, 18,24,33), RFLP (16,20-23,26), rRNA sequence comparison (14), and chromosomal polymorphisms (31). However, appropriate methods and their useful ranges in fungal systematics have recently been discussed (5), and RFLP of mtDNA has been suggested as perhaps the molecular method best suited at the subspecies level (2). Two laboratories have extensively examined the mtDNA RFLP of two formae speciales within the Cucurbitaceae plant family [*F. o. niveum* (20,21) and *F. o. melonis* (16)], and the restriction map of mtDNA from *F. o. niveum* (21) and *F. o. melonis* (16) was very similar, if not identical (21). In this study, the genetic divergence and the relatedness of five formae speciales

within the Cucurbitaceae were determined by RFLP analysis of mtDNA.

MATERIALS AND METHODS

Fungal strains. Thirty-nine isolates representing five formae speciales of *F. oxysporum* were analyzed, including a representative isolate from each of six mtDNA RFLP groups detected among 50 isolates of *F. o. niveum* (21). Twenty isolates of *F. o. melonis*, including nine obtained from T. R. Gordon (University of California, Berkeley, CA) and representing at least one isolate of each of the five mtDNA RFLP groups (RFLP A, B, C, D, F) detected in the study by Jacobson and Gordon (16), were examined. Nine isolates of *F. o. cucumerinum* were examined, including three from The Netherlands that were reported to have pathogenicity to several hosts (13). Two isolates each of *F. o. lagenaria* (ATCC 18143 and 38363) and *F. o. luffae* (ATCC 28860 and 42327) were also included. Pathogenicity of each isolate except the three from The Netherlands was verified by root-dip inoculations in previous studies (21,27,28, and Martyn, unpublished). An isolate of *F. oxysporum* isolated from the root of a wilted watermelon plant and shown to be nonpathogenic to watermelon was also included as an outgroup member. Race identity, geographic origin, and mtDNA RFLP groups of each isolate are listed in Table 1.

Pathogenicity tests. Pathogenicity of the three Netherlands isolates to different hosts was confirmed by root-dip inoculation on the appropriate host species. Two watermelon cultivars, Black Diamond and Calhoun Gray; two muskmelon cultivars, Top Mark and Perlita; and one cucumber cultivar, Pionsett 76, were selected as prospective hosts. When the first true leaf was evident, the seedlings were inoculated by dipping the roots into a suspension of microconidia (1×10^6/ml) of each isolate. Inoculated seedlings were transplanted into plastic pots (23 cm diameter), five seedlings per pot, and maintained in a growth room. There were two replicated pots for each inoculum, and plants root-dipped in distilled water served as a control. Observations for symptoms of disease were made daily for 3 wk.

DNA extraction and hybridization. Total DNA from each isolate was extracted using a minipreparation procedure described previously (21). One milligram of total DNA was digested with three restriction endonucleases (*Eco*RI, *Hind*III, and *Pst*I) according to the manufacturer's recommendations (Promega, Madison, WI, or BRL, Gaithersburg, MA). Restriction fragments were separated by 0.6–1.0% agarose gel electrophoresis using TBE (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) buffer and a gradient potential of <5V/cm for 12–16 h and transferred to nylon hybridization membrane for Southern hybridization (GeneScreenPlus, Du Pont, Wilmington, DE). A polyprobe consisting of the mixture of equal molar ratios of nine *Pst*I-cloned fragments of mtDNA from *F. o. niveum* (20,21) was labeled with [α-^{32}P]dATP by the method of Feinberg and Vogelstein (9) and used for hybridization to DNA from each isolate. Hybridizations were conducted at 65 C, and posthybridization washes were conducted at 65 C, according to the manufacturer's recommendation.

RFLP analysis. The restriction pattern for each enzyme digestion was analyzed separately to calculate the phenograms and parsimony trees using a binomial system based on the presence or absence of a given fragment: 1 if a given fragment was present for an isolate and 0 if it was absent. The relationship among isolates was analyzed by two computer-assisted methods: cluster analysis using NTSYS 1.50 (34) and parsimony analysis using PAUP 3.0 (36). In the cluster analysis, the unweighted paired group method using arithmetic means (UPGMA) with Jaccard's similarity coefficients was used for each enzyme digestion to produce a phenogram. In the case of PAUP, most parsimonious trees were searched with an initial MAXTREES setting of 100, and a strict consensus tree was determined from those trees using CONTREE. To determine the significance of the branches in the tree, the bootstrap method was applied with 100 replications (10,11).

TABLE 1. Isolates of *Fusarium oxysporum* formae speciales

Isolate	Pathotype[a]	Origin	RFLPG-fsp
FL-60-3A	FON 0	Florida	I
FL-64-2	FON 1	Florida	III
IS-59(73)	FON 2	Israel	IV
NC-EE2-A(87)	FON 1	N. Carolina	V
TX-CART-CG-1A(87)	FON 2	Texas	VIII
CHN(Cl)-PRCF6(89)	FON 1	China	IX
K-419	FOM 1	Mexico	XI
E-466C	FOM 0	Maryland	VI
E-660A	FOM 1	Maryland	VI
ATCC62938	FOM 0	Texas	VI
CA-FY-1	FOM 2	Canada	I
FR-mm-1(89)	FOM 1,2	France	VI
ATCC16418	FOM 2	Canada	I
ATCC28862	FOM 1	Israel	VI
A47M2	FOM 0	France	VI
R-1	FOM 1	France	VI
P-2	FOM 2	California	I
R-12Y	FOM 1,2y	France	VII
Pt-1	FOM 2	California	I
ATCC28858	FOM 1,2w	France	X
ATCC28861	FOM 2	Japan	I
ATCC28862	FOM 1	Israel	VI
ATCC66052	FOM 0	Israel	X
FOM-1451	FOM 1	Israel	VI
FOM-KNH	FOM 1,2	Israel	X
FOM-RM2	FOM 2	Israel	X
PSU-1265	FOC	Canada	XIII
PSU-1086	FOC	Florida	IX
PSU-1266	FOC	Canada	XIII
PSU-1098	FOC	Florida	IX
PSU-1267	FOC	Canada	XIV
ATCC16416	FOC	Florida	IX
NETH10782	FOC	The Netherlands	I
NETH11179	FOC	The Netherlands	I
NETH20286	FOC	The Netherlands	XII
ATCC18143	FOLa	Japan	I
ATCC38363	FOLa	Japan	I
ATCC28860	FOLu	Japan	II
ATCC42327	FOLu	Japan	II

[a] Abbreviations for forma specialis and race: FON = *Fusarium oxysporum* f. sp. *niveum*; FOM = *F. o.* f. sp. *melonis*; FOC = *F. o.* f. sp. *cucumerinum*; FOLa = *F. o.* f. sp. *lagenaria*; FOLu = *F. o.* f. sp. *luffae*.

RESULTS

Pathogenicity. Pathogenicity data of the three Netherlands isolates on five cultivars of the three major host species (watermelon, cantaloupe, and cucumber) are presented in Table 2. All three isolates showed cross infectivity to the three major host species; however, there was a difference in the aggressiveness of isolates based on the number of dead plants. Both muskmelon cultivars, Top Mark and Perlita, and cucumber cultivar, Pionsett 76, were equally susceptible to each isolate. Watermelon cultivar, Calhoun Gray, which is resistant to races 0 and 1 of *F. o. niveum*, was the least susceptible to two of the isolates (NETH 10782 and NETH 11179). Black Diamond was moderately susceptible

TABLE 2. Pathogenicity of three isolates of *F. o.* f. sp. *cucumerinum* from The Netherlands on different cucurbit hosts

		Isolates			
Host[a]	Cultivars	Neth 10782	Neth 11179	Neth 20286	Control
CU	Pionsett 76	9[b]	9	8	0
MM	Topmark	9	10	10	0
MM	Perlita	10	10	10	0
WM	Black Diamond	3	6	6	0
WM	Calhoun Gray	1	1	10	0
Total		32/50	36/50	44/50	0/50

[a] CU = cucumber; MM = muskmelon; WM = watermelon.
[b] Numbers of dead plants out of a total of 10.

to all three isolates. Isolate NETH 20286 was more aggressive on Calhoun Gray than the other two and the most aggressive across all hosts, killing 44 out of 50 plants compared to 32 and 36, respectively, for NETH 10782 and NETH 11179 (Table 2).

RFLP haplotypes. RFLP patterns detected were assigned numbers (I–XIV) and designated as RFLP group–f. sp. (RFLPG-fsp). Six RFLP haplotypes (RFLPG-fspI, III, IV, V, VIII, and IX) were detected in Southern blots of *F. o. niveum* after digestions with the three enzymes using the polyprobe. These six haplotypes correspond to RFLPG I–VI previously reported (21). A single pattern each was detected from *F. o. lagenaria* and *F. o. luffae* isolates, RFLPG-fsp I and II, respectively. Five haplotypes (RFLPG-fspI, VI, VII, X, and XI) were detected in *F. o. melonis* and corresponded closely to the five RFLP groups (RFLP A, B, C, D, and F) previously described (16). However, among the five groups reported for *F. o. melonis* (16), we detected only four (RFLPs A, C, D, and F) as distinct patterns (RFLPG-fspI, X, VI, and XI, respectively) but could not differentiate RFLP B from RFLP A. These were originally differentiated on the basis of an additional *Hae*III restriction site in the mtDNA (16), an enzyme not used in our study. An additional pattern (RFLPG-fspVII) in *F. o. melonis* was detected in our study by *Hind*III digestion (Fig. 1), which was not observed in the previous study (16). Five RFLP groups (RFLPG-fspI, IX, XII, XIII, and XIV) also were detected from among the *F. o. cucumerinum* isolates. Among The Netherlands isolates, the two less aggressive strains (NETH 10782 and NETH 11179) had identical patterns (RFLPG-fspI), and the most aggressive one (NETH 20286) was the only isolate in RFLPG-fspXII.

Fourteen RFLP haplotypes were detected among all isolates examined (Table 3), and a schematic diagram of all RFLPG-fsp based on the *Pst*I-polyprobe combination is represented in Figure 2. There were unique RFLPs within each forma specialis; however, one pattern generally was most common for each forma specialis, i.e., RFLPG-fspI in *F. o. niveum*, RFLPG-fspVI in *melonis*, and RFLPG-fspIX in *cucumerinum*. Two patterns (RFLPG-fspI and RFLPG-fspIX) occurred in more than one forma specialis. RFLPG-fspI, which was most common among 50 isolates of *F. o. niveum* (21), occurred in *F. o. melonis*, *cucumerinum*, and *lagenaria* and had a 75% similarity with RFLPG-fspII, which contained *F. o. luffae* based on the *Pst*I

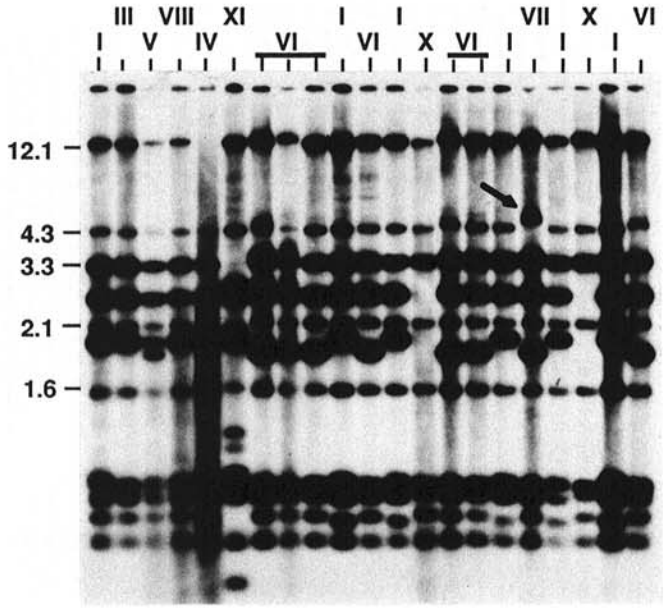

Fig. 1. Hybridization patterns of *Hind*III-digested DNA from *F. oxysporum*. The arrow points to the DNA fragment showing the difference between RFLPG-fspVI and VII. Numbers on the top of the gel refer to the RFLP pattern of the formae speciales of *F. oxysporum*. Numbers on the left refer to the fragments sizes (kb).

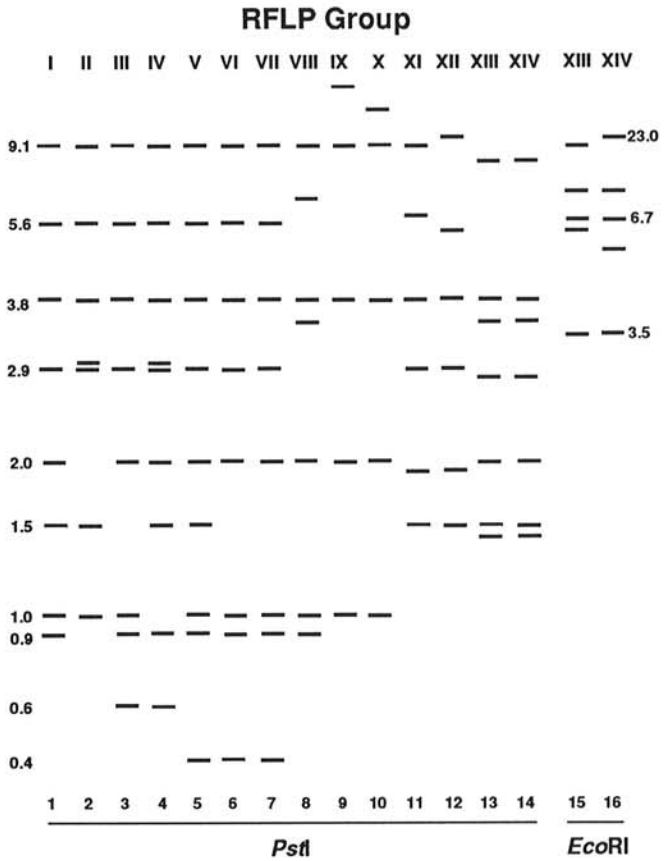

Fig. 2. Schematic diagram of Southern hybridization patterns of the 14 RFLP groups detected with a *Pst*I digestion and the polyprobe. RFLPG-fspVI and RFLPG-fspVII have the same pattern with *Pst*I, but are further differentiated by *Hind*III digestion and hybridized with the polyprobe (see Fig. 1). Similarly, RFLPG-fspXIII and XIV were not differentiated by the *Pst*I digestion, but are by *Eco*RI shown in lanes 15 and 16. Numbers on the left and right refer to the fragments sizes (kb) of *Pst*I and *Eco*RI digestions, respectively. Each RFLP group is designated at the top of diagram. Numbers at the bottom refer to the lane number.

TABLE 3. Mitochondrial DNA (mtDNA) RFLP groups of *Fusarium oxysporum* causing vascular wilt in cucurbits

Formae speciales[x]	No. of isolates	No. of RFLP groups	RFLP groups													
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
FON	6[y]	6	X		X	X	X			X	X					
FOM	20[z]	5	X					X	X			X	X			
FOC	9	5	X								X			X	X	X
FOLa	2	1	X													
FOLu	2	1		X												
Total	39	18														

[x] FON = *F. o. niveum*; FOM = *F. o. cucumerinum*; FOLa = *F. o. lagenariae*; FOLu = *F. o. luffae*.
[y] These isolates represent each of the six mtDNA haplotypes described from 50 FON isolates in a previous study (21).
[z] Five of these isolates were selected as representatives of the mtDNA haplotypes described from a larger collection (16).

digestion. In addition, RFLPG-fspIX is the same pattern as RFLPG VI reported previously in *F. o. niveum* (21) and also occurred in *F. o. cucumerinum*. Considering the small number of *F. o. cucumerinum* isolates used and the number of RFLP patterns detected, it appears that it is the most heterogeneous forma specialis.

Cluster analysis of mtDNA haplotypes. A cluster analysis of the mtDNA haplotypes was derived separately for each of the three restriction enzymes (*Eco*RI, *Hind*III, and *Pst*I) using Jaccard's similarity coefficients. The cophenetic correlation coefficients showing the goodness of fit of the data matrix to the tree were very high, 0.99, 0.99, and 0.94, respectively, for each enzyme. *Eco*RI digests hybridized to the polyprobe detected only five of the 14 total RFLP patterns among the isolates (Fig. 3A). One branch had two RFLP groups from *F. o. cucumerinum* (RFLPG-fspXIII and XIV) and was the most distant from all the other groups with only a 2% similarity. The second most distant cluster consisted of RFLPG-fspXII from *F. o. cucumerinum* and RFLPG-fspXI from *F. o. melonis*, with a similarity of 25% to the remaining branches. Eight of the RFLP groups from five formae speciales were not distinguishable by this probe-enzyme combination and were lumped together on a single branch. This was closest to the cluster of RFLPG-fspIX and X with 64% similarity. *Hind*III digestion detected seven RFLP groups (Fig. 3B) and, again, the branch containing the two clusters of RFLPG-fspXIII, XIV and RFLPG-fspXI, XII was the most distant from the remaining groups with a 32% similarity. The remaining RFLP groups were grouped together with a 73% or better similarity (Fig. 3B). *Pst*I digestion detected the largest number of RFLP groups (RFLPG), 12 out of a total of 14 (Fig. 3C). The most distant branch contained two RFLP groups of *F. o. cucumerinum* (RFLPG-fspXIII and XIV) and had a similarity of only 15%. The second most distant cluster consisted of one group from *F. o. cucumerinum* (RFLPG-fspXII) and one from *F. o. melonis* (RFLPG-fspXI) with a similarity of 33% to the remaining groups.

In all three restriction enzyme analyses, one cluster containing two RFLP groups from *F. o. cucumerinum* was always the most distant, while the second most distant cluster always consisted of one RFLPG from *F. o. cucumerinum* (RFLPG-fspXII) and one from *F. o. melonis* (RFLPG-fspXI). The remaining 10 RFLPG detected among the five different formae speciales were consistently clustered to form complex branches (Fig. 3). RFLPG-fspI was the most common group and contained isolates from four formae speciales. RFLPG-fspII from *F. o. luffae* was most similar to RFLPG-fsp I. The five RFLP groups detected in *F. o. cucumerinum* were the most divergent for isolates within the same forma specialis with a maximum range of 32% similarity, while the six RFLP groups in *F. o. niveum* were the most similar for a given forma specialis with at least 42% similarity for all isolates. Two RFLP groups (RFLPG-fspI and RFLPG-fspXII), which included the cross-infective Netherlands isolates, were only distantly similar (33%) to the other groups.

Parsimony analysis of *F. oxysporum*. Strict consensus trees for each enzyme in the unrooted parsimony analysis using a nonpathogenic isolate of *F. oxysporum* recovered from a wilted watermelon plant as an outgroup are shown in Figure 4, along with confidence intervals generated by bootstrap. The branching patterns of the consensus trees were identical to that of UPGMA except for RFLPG-fspXI and XII in the *Pst*I digestion. A branch and bound search for most parsimonious trees of the *Eco*RI digestion resulted in a single tree (Fig. 4A). The clusters containing RFLPG-fspXIII and RFLPG-fspXIV, and RFLPG-fspXI and RFLPG-fspXII were significantly supported at the level of 93% or better (Fig. 4A). *Hind*III digestion recognized 55 parsimonious trees in a heuristic search, and three branches were significantly supported at the level of 93% or better (Fig. 4B). *Pst*I resulted in seven parsimonious trees by a branch and bound search, and the branch containing RFLPG-fspXIII and RFLPG-fspXIV was the only one that was significantly supported (99%) (Fig. 4C). This indicates that several arrangements of these similar haplotypes can give equally parsimonious solutions, and the disagree-

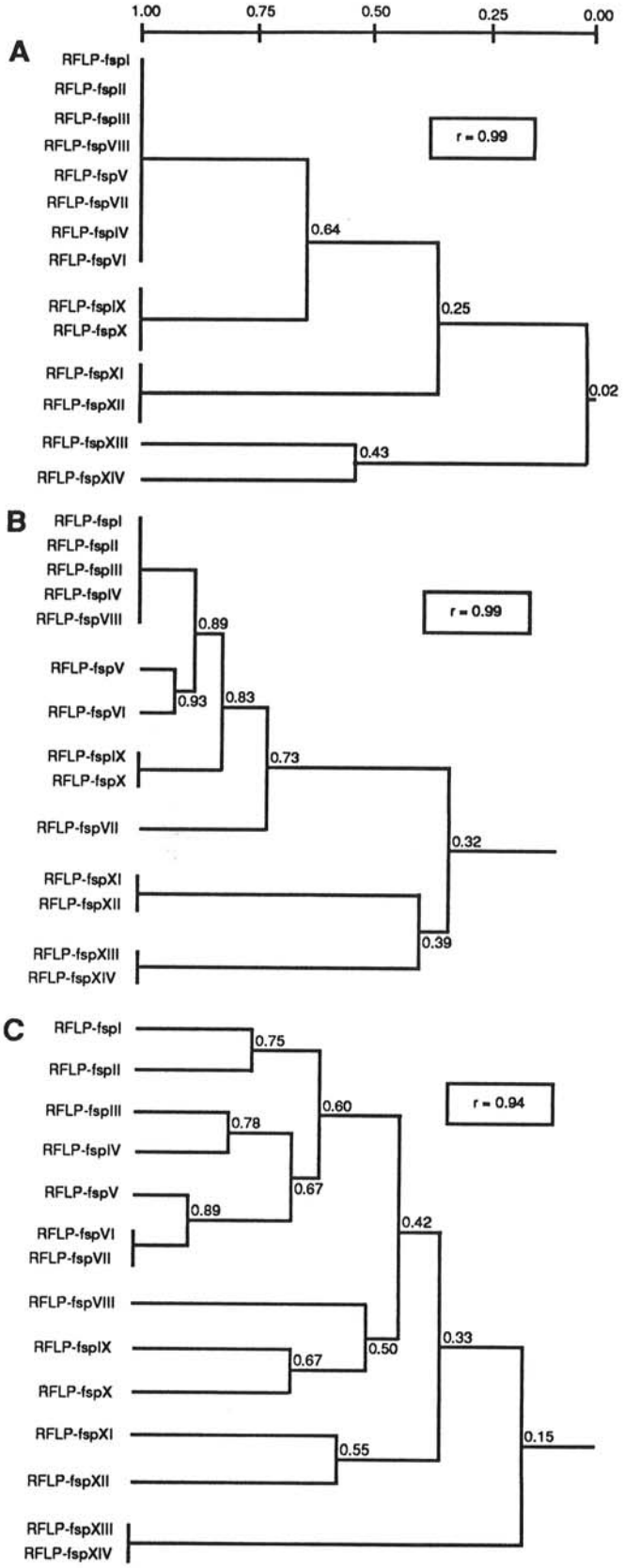

Fig. 3. Phenograms of mtDNA RFLP groups from different formae speciales of *F. oxysporum* based on UPGMA. **A,** Phenogram generated by the *Eco*RI-polyprobe combination. Only five RFLP groups were differentiated. **B,** Phenogram generated by the *Hind*III-polyprobe combination. Seven RFLP groups were differentiated. **C,** Phenogram generated by *Pst*I-polyprobe combination. Twelve RFLP groups were detected. RFLP groups with identical hybridization pattern in the specific probe-enzyme combination are at the same termini of the branch. Numbers refer to similarity between the cluster based on Jaccard's similarity coefficient. Cophenetic correlation coefficients are shown in the upper box.

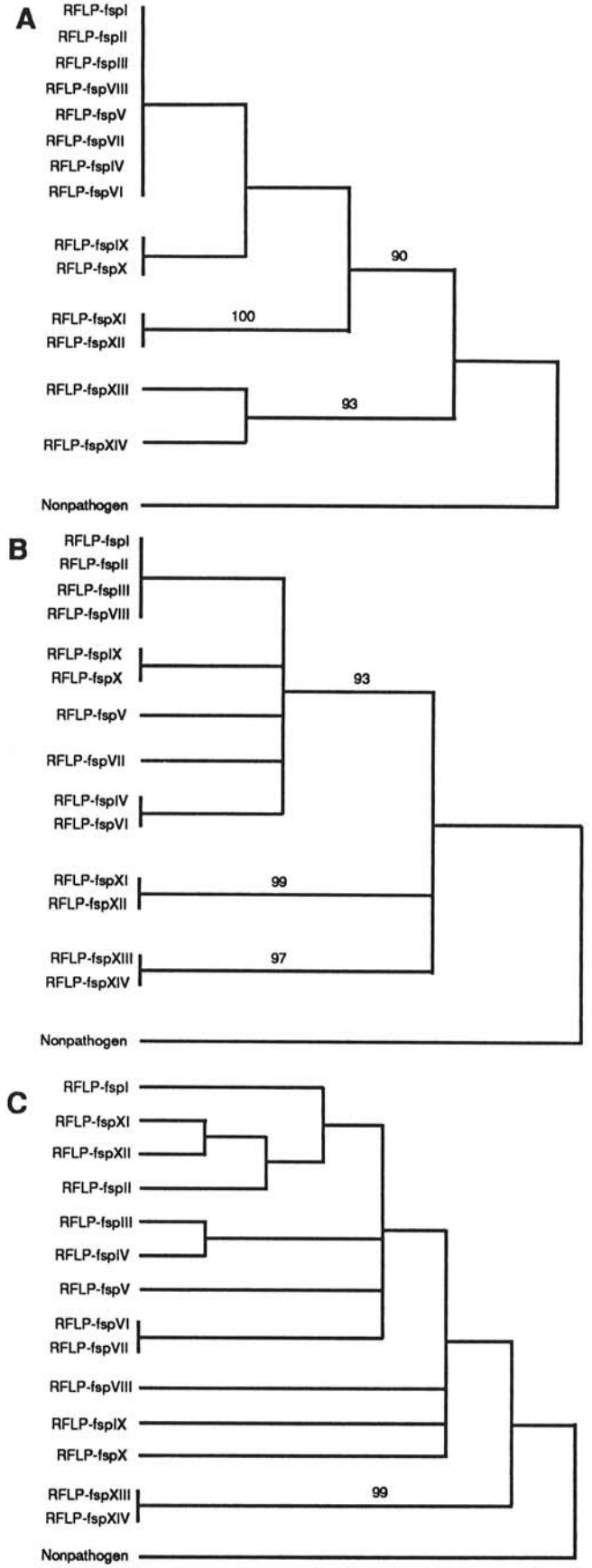

Fig. 4. Phylogenetic relationship of mtDNA RFLP groups from different formae speciales of *F. oxysporum*. The most parsimonious trees were produced by using the PAUP microcomputer program (PAUP 3.0). **A,** Strict consensus tree based on the *Eco*RI-polyprobe combination. **B,** Strict consensus tree based on the *Hind*III-polyprobe combination. **C,** Strict consensus tree based on the *Pst*I-polyprobe combination. Numbers refer to the confidence interval determined by bootstrapping with 100 replications.

ment of branching pattern of RFLPG-fspXII and XI was not significantly supported.

No single forma specialis clustered exclusively under a significantly supported branch, i.e., isolates from a single forma specialis were grouped along multiple branches with isolates from the different formae speciales, and more than one forma specialis occurred within a single branch. However, two RFLP groups of *F. o. cucumerinum* (RFLPG-fspXIV and XIII) consistently separated out first and with a high level of significance in all trials. Although the three cross-infective Netherlands isolates divided into two RFLP groups (RFLPG-fspI and XII), their separation was not always significantly supported, but they were still related to each other. All remaining isolates and formae speciales still appear to be very similar. All five formae speciales were observed within the multichotonous branches, which suggests a close relationship with one another.

DISCUSSION

The reported pathogenicity of the three Netherlands isolates of *F. o. cucumerinum* to cucumber, watermelon, and muskmelon was confirmed. These strains were originally isolated from cucumber (13). Similarly, a *F. oxysporum* isolate from wilted cucumber from the Bahamas also was reported to cause wilt in watermelon (30); however, that isolate is not available for independent confirmation. The fact that some isolates exist within this forma specialis which are cross-infective on different hosts suggests that they are closely related and share characteristics necessary for pathogenicity.

The formae speciales concept proposed by Snyder and Hansen (35) for *F. oxysporum* in 1940 was based on a strict host specificity of these strains. However, exceptions to this rule occur. Within the cucurbitaceous group three notable cases have been described. In the early 1970s, Bouhot (4) showed that one forma specialis could be mutated into another and the pathogenicity factors in these forms could coexist in one isolate. Secondly, in 1986, McMillan (30) reported that isolates of *F. oxysporum* obtained from wilted cucumber plants in the Bahamas were pathogenic to cucumber, melon, and watermelon, while Florida isolates were host specific. Thirdly, isolates recovered from wilted cucumber plants in The Netherlands also are pathogenic to watermelon and muskmelon (13). In addition, Martyn and McLaughlin (28) reported that an isolate of *F. o. niveum* also caused wilt in some *Cucurbita pepo* cultivars. These reports led Gerlagh and Blok (13) to suggest that we do away with the individual forma specialis in the Cucurbitaceae family and create a new forma specialis that would encompass all the cucurbit forms. This was proposed to be *F. o.* f. sp. *cucurbitacearum* n. f.

In recent years, vegetative compatibility groups (VCGs) have been established for numerous formae speciales of *F. oxysporum*. In many cases, a good correlation among strains has been demonstrated between VCG and virulence (3,16,18) and/or race (6,17,24). However, there is sufficient variability within populations of a given forma specialis and numerous nonconforming isolates (15,33), as well as cases where virtually no correlation exist among strains (7,8), to suggest that VCG alone cannot be used to distinguish strains of *F. oxysporum*. Similarly, relationships between VCG and RFLPs of both genomic and mtDNA have been demonstrated (16,26); however, the degree of correlation among strains in a given forma specialis varies. Very little data are available on the relationship among different formae speciales. Other than the present study, the only other group of related formae speciales examined are those causing vascular wilt in the Cruciferae. Bosland and Williams (3) reported a good correlation between VCG and other characteristics among *F. o. conglutinans*, *F. o. raphani* J. B. Kendrick & W. C. Snyder, and *F. o. matthioli* K. Baker. Kistler et al (22) reported a similar correlation between mtDNA RFLPs and these formae speciales, but no differences in rDNA RFLPs among them.

In the present study, 14 RFLP groups were detected among isolates representing five formae speciales, each causing vascular wilt in a member of the Cucurbitaceae. Eleven of these isolates

(five *F. o. melonis* and six *F. o. niveum*) were selected as being representative of each mtDNA haplotype described previously from larger collections of these two formae speciales (16,21). However, RFLP group B of *F. o. melonis* reported by Jacobson and Gordon (16) was not differentiated from RFLP A (RFLPG-fspI in this study). This is due to the fact that *Hind*III was used in our study rather than *Hae*III used in their study (16). As a result, however, a new group, RFLPG-fspVII, was detected in our study.

According to both cluster analysis and parsimony analysis, a similar relationship among the formae speciales was shown as indicated by the tight grouping of many haplotypes from different formae speciales, including some of the *F. o. cucumerinum*. In addition, one haplotype (RFLPG-fspI) occurred in all formae speciales except one, *F. o. luffae*, and that may be due to the lack of adequate sample size in that one forma specialis. Our mtDNA data on these fungi clearly demonstrate that a close relationship among the different formae speciales exists with a lot of genetic similarity. In some cases, isolates of different formae speciales appear to be more genetically alike than are certain isolates of the same forma specialis. Thus, our data could be used to support the idea of a unified forma specialis in the cucurbitaceous group such as *F. o. cucurbitacearum* (13). However, the fact that the majority of isolates within these formae speciales are still highly host specific continues to validate the formae speciales concept (35). A similar study conducted with strains of *F. oxysporum* representing three formae speciales causing wilt in crucifers showed that there was no variation in mtDNA haplotypes within each forma specialis (22). Thus, strains of *F. oxysporum* on cucurbits probably still have genetic exchange among the formae speciales, while those on crucifers may have stabilized and reached a pathogenic specialization.

Inference of evolutionary relationship based on fragment differences could be misleading due to erroneously high levels of confidence from bootstrapping because of a reported high frequency of length mutations in mtDNA, poor correspondence between the number of fragment differences and that of mutation, and no independence of mutation in small mtDNA (5). Therefore, while it is very tempting to speculate and propose an evolutionary lineage for each RFLP group of this fungi at this time, it is not possible. However, the fact that isolates from a single forma specialis were grouped along multiple branches with isolates from different formae speciales and that more than one forma specialis occurred within a single branch suggest a close evolutionary relationship among the different formae speciales and a monophyletic origin of isolates from cucurbits. In addition, while length mutations in mtDNA are common in some fungi, site mutations were more common in *F. o. niveum* (21). It is also possible that genetic exchange occurs between formae speciales, which is supported by a case of heteroplasmy of mtDNA between *F. o. niveum* and *F. o. melonis* (21). Similarly, using repetitive DNA sequence probes, Kistler et al (23) suggested a phylogenetic relationship among the Cruciferae formae speciales and that, within races of *F. o. conglutinans*, there was a common ancestry but a distinct ancestral difference between *F. o. raphani* and *F. o. matthioli*. With the assumption that the diversity of clonally propagated molecules should increase over time (12), *F. o. cucumerinum* may be the oldest forma specialis. Based on the frequency and wide geographic distribution, it is suggested that RFLPG-fspI may be the most coevolved with its host plant or, alternatively, it could indicate that this group is the oldest and has stabilized (21).

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