Genetic Diversity Within and Among Races and Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *lycopersici* as Determined by Isozyme Analysis

Karol S. Elias and R. W. Schneider

Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803-1720. Supported in part by grants from USDA Office of International Cooperation and Development (project 58-319R-5-036) and grant US-1408-87 from BARD, The United States-Israel Binational Agricultural Research & Development Fund. Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript 91-38-5444. We thank all contributors for supplying fungal isolates (Table 1).
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ABSTRACT

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A worldwide collection of 111 isolates of *Fusarium oxysporum* f. sp. *lycopersici* from tomato was examined for isozyme polymorphisms. Of 15 enzymes assayed, eight and seven revealed single and multiple bands, respectively, for a total of 30 putative loci. Twenty-two of the 30 loci exhibited complete homogeneity among all isolates surveyed. Eight loci were variable and revealed two to five putative alleles per locus. Thirty-four electrophoretic phenotypes (EPs) were identified. EPs 1 and 2, with 49 and 13 members, respectively, included 53% of all the isolates. Each of nine EPs contained isolates of different races, vegetative compatibility groups (VCGs), or formae speciales. Thus, a large number of isolates from different races, VCGs, geographic origins, or even formae speciales were electrophoretically identical. Similar patterns were revealed when data were analyzed with simple matching coefficients of similarity, cluster analysis, or principal components analysis. Estimates of genetic similarity among isolates of *F. o. lycopersici* within a VCG were greater than that of isolates between VCGs. Some VCGs were more similar to each other or to members of other formae speciales than to another VCG within *F. o. lycopersici*. The majority of EPs that contained isolates from single-member VCGs (VCG 003-) clustered with the multiple-member VCGs, providing evidence for their recent development from the multiple-member VCGs. In general, the distribution of isolates correlated with VCG rather than race, geographic origin, or formae speciales. These results suggest that parasexual recombination does not occur frequently in nature; however, in combination with strong host selection pressures, parasexual recombination could function to introgress genes for altered host specificity. In addition, we conclude that forma specialis *lycopersici* arose from at least two progenitor populations.

Fusarium wilt of tomato (*Lycopersicon esculentum* Mill.), caused by the vascular wilt pathogen *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W. C. Snyder & H. N. Hans., is a devastating disease that occurs in major tomato-growing regions of the world (42). The three known races of *F. o. lycopersici* are distinguished by their pathogenicity to tomato cultivars possessing specific dominant resistance genes (25,40). Races 1 and 2 are found in virtually all major tomato-growing regions, whereas race 3 is presently limited to Australia (18), Florida (41), and California (9).

Very little is known about the mechanisms involved in the development of races of *F. o. lycopersici* or imperfect fungi in general. New races could develop through spontaneous random mutation or genetic recombination. Because *F. oxysporum* is an imperfect fungus, parasexual recombination is the only mechanism by which reassortment of genetic material can occur. Heterokaryon formation, a prerequisite for parasexual recombination, has been demonstrated in several formae speciales of *F. oxysporum*, including *F. o. lycopersici* (24,31,38). In addition, the genes that control heterokaryon formation (i.e., vegetative incompatibility in (*vic*) genes) have been characterized in closely related ascomycetous fungi (2,29,33,34). Thus, we assume that similar mechanisms control vegetative incompatibility and the potential for parasexual recombination in *F. oxysporum*. This is not to say that these phenomena, which can be demonstrated in the laboratory, occur at a high frequency or affect population structure in nature.

In 1985, Puhalla (32) introduced a technique, refined by Correll et al (7), that allows macroscopic observation of heterokaryosis and assignment of fungal strains to vegetative compatibility groups (VCGs). On the basis of the correlation he observed between VCG and pathotype, Puhalla (32) proposed that all strains within a VCG arose from the same ancestral progenitor, are members of a genetically isolated population, and should be more similar to other members of the same VCG than members of other VCGs. Several investigators utilized VCG analysis to differentiate formae speciales, races, and pathogens from nonpathogens (4,8,13,14,20, 21,30). Elias and Schneider (10,12) used this technique to assign 115 strains of *F. o. lycopersici* to VCGs. Correlation between VCG and race was not observed, VCG 0030 contained all three races of *F. o. lycopersici* and had a worldwide distribution, and VCGs 0031 and 0032 each contained races 1 and 2, but their geographic ranges were more limited. These researchers also discussed the roles of evolution, mutation, and stability of genes for virulence and vegetative incompatibility in accounting for the distribution pattern observed, and they proposed the existence of genetic limits to gene flow that would create genetically diverse subpopulations.

The objective of this research was to estimate genetic diversity within and among VCGs and races of *F. o. lycopersici* by examining enzyme polymorphisms. These data were then analyzed and interpreted with regard to the origin of VCGs and races. A preliminary report was published (11).

MATERIALS AND METHODS

Fungal strains. One hundred and eleven isolates of *F. o. lycopersici* were isolated or received from diverse locations (Table 1). Isolates were received as actively growing cultures on agar slants or plates, as lyophilized samples, or on dried filter paper. Single spores of each isolate were transferred to potato-dextrose agar (PDA), and isolates were then stored on silica gel at 4 C (28). The race designation of all strains was verified by pathogenicity tests in the greenhouse by use of the appropriate differential tomato cultivars (12). In addition, seven isolates of *F. oxysporum* from other formae speciales were included for comparison (Table 1).

Tissue preparation. Each isolate was grown in three 250-ml Erlenmeyer flasks containing 100 ml each of potato-dextrose broth (PDB) for 5 days on an orbital shaker (125 rpm) at room

TABLE 1. Isolates of *Fusarium oxysporum* f. sp. *lycopersici* designated by race, source, electrophoretic phenotype, origin, and vegetative compatibility group

Isolate[v]	Race[w]	Source[x]	Electrophoretic phenotype[y]	Origin
VCG 0030[z]				
LSU-3	1	a	2	Louisiana
LSU-5	1	a	2	Louisiana
LSU-6	1	a	1	Louisiana
FRC 0-1078	2	b	1	Florida
FRC 0-1079	2	b	1	Florida
A-22068	1	c	2	Australia
A-18947	2	c	1	Australia
A-19156	2	c	1	Australia
A-21990	3	c	1	Australia
A-21991	3	c	1	Australia
OSU-409	1	d	1	Ohio
OSU-460	2	d	1	Ohio
UM-1 (FRC 0-1118)	1	e	1	California
HMS-2 (66)	1	f	3	California
HMS-3 (74)	1	f	1	California
HMS-6 (80)	2	f	1	California
NK-1	2	g	1	Florida
NK-2	2	g	1	Florida
NK-3	2	g	1	Florida
NK-4	2	g	1	Florida
JBF-4 (548-4-10)	2	h	1	Florida
JBF-5 (8)	2	h	1	Florida
JBF-6 (761-Sp6)	3	h	1	Florida
JBF-7 (761-Sp4)	3	h	1	Florida
BFOL-53	2	i	8	Louisiana
BFOL-65	2	i	8	Louisiana
PS-5	3	j	1	California
IK-1 (FOL-I)	1	k	2	Israel
IK-2 (FOL-I-MX)	1	k	7	Israel
IK-3 (FOL-R)	2	k	2	Israel
IK-4 (FOL-649)	1	k	6	Israel
IK-5 (FOL-650)	1	k	1	Israel
IK-7 (FOL-1295)	1	k	1	Israel
MA-1 (E7C)	1	l	1	Morocco
MA-2 (OE2)	1	l	3	Morocco
MA-3 (MH197)	1	l	4	Morocco
MA-4 (MEXII)	1	l	3	Morocco
MA-5 (KE1)	1	l	5	Morocco
MA-6 (FK3)	1	l	2	Morocco
MA-7 (MB6)	1	l	1	Morocco
MA-8 (M6)	1	l	2	Morocco
MA-9 (F1V1)	1	l	3	Morocco
MA-10 (MVH2)	1	l	5	Morocco
FA-4 (FOL15)	2	l	2	Tunisia
FA-7 (FOL27)	2	l	1	France
FA-8 (FOL28)	2	l	1	France
FA-9 (FOL29)	1	l	1	France
FA-11 (FOL31)	2	l	5	France
FA-13 (FOL33)	2	l	1	France
IA-7 (FOL77)	1	l	5	Italy
IA-11 (FOLV)	1	l	1	Italy
VCG 0031				
BFOL-51	1	i	13	Louisiana
OSU-451	2	d	10	Ohio
HMS-4 (65)	2	f	9	California
HMS-5 (67)	2	f	9	California
PS-2	2	j	10	California
PS-3	2	j	12	California
UCD-2 (1776)	2	m	11	California
CIC-2	2	n	13	California
VCG 0032				
LSU-2	2	a	3	Louisiana
LSU-4	1	a	2	Louisiana
LSU-7	2	a	1	Louisiana
BFOL-70	2	i	8	Louisiana
VCG 003-				
PS-1	1	j	1	California
PS-4	2	j	1	California
CIC-1	1	n	1	California
JBF-1 (626-6B)	1	h	1	Florida
JBF-2 (626K-1)	1	h	1	Florida
JBF-3 (626K-2)	1	h	1	Florida
FDA-1 (FTCC854)	1	o	14	Florida
FDA-3 (FTCC979)	3	o	15	Florida
FRC 0-1081	1	b	1	Florida
FRC 0-1082	1	b	1	Florida
UCD-1 (1775)	1	m	31	California
OSU-415	1	d	1	Ohio
OSU-416	2	d	1	Ohio
FOL-84-1590	1	p	1	Michigan
FO-14	1	q	33	Michigan
T-1	1	r	1	Taiwan
FOL-R5-6	1	p	1	Wisconsin
HMS-1 (60)	1	f	5	California
F1E	1	s	29	Maryland
F22 (701-2)	2	s	3	Florida
F23	2	s	10	Maryland
F34	1	s	10	Maryland
F37	2	s	30	Maryland
BFOL-54	1	i	27	Louisiana
BFOL-56	1	i	8	Arkansas
BFOL-57	2	i	5	Arkansas
BFOL-63	1	i	28	Louisiana
BFOL-67	1	i	23	Louisiana
BFOL-69	1	i	28	Louisiana
BFOL-75	2	i	8	Louisiana
PHW-554	1	t	2	Wisconsin
PHW-555	2	t	29	Wisconsin
FA-3 (FOL8)	1	l	20	France
FA-5 (FOL24)	1	l	19	France
FA-6 (FOL26)	1	l	19	France
FA-10 (FOL30)	1	l	21	France
FA-12 (FOL32)	1	l	22	France
FA-14 (FOL62)	1	l	1	Senegal
IA-1 (FOLAL)	1	l	16	Italy
IA-2 (FOL82)	1	l	17	Italy
IA-3 (FOLBari)	1	l	18	Italy
IA-4 (FOL81)	1	l	1	Italy
IA-5 (FOL74)	1	l	14	Italy
IA-6 (FOL84)	1	l	2	Italy
IA-8 (FOLD77)	1	l	25	Italy
IA-9 (FOL1)	2	l	2	Italy
IA-10 (FOL2)	2	l	26	Italy
IA-12 (FOLVA)	1	l	4	Italy
Other f. spp.				
FA-1 (FORL-19) (*F. o. radicis-lycopersici*)	ND	l	1	France
FA-2 (FORL-22) (*F. o. radicis-lycopersici*)	ND	l	1	France
TAM-2 (*F. o. cucumerinum*)	ND	u	34	Texas
TAM-3 (*F. o. vasinfectum*)	ND	u	32	Texas
TAM-4 (*F. o. niveum*)	ND	u	1	Texas
CSU-1 (*F. o. dianthi*)	ND	v	2	Colorado
FOA-2 (*F. o. asparagi*)	ND	q	24	Michigan

[v] Original strain number in parentheses.

[w] Race designation was determined with the differential tomato cultivars Walter (resistant to races 1 and 2), Supersonic (resistant to race 1), and Fantastic (susceptible to all three races). ND = not done.

[x] a = Authors; b = Fusarium Research Center, Pennsylvania State University, University Park; c = R. G. O'Brien; d = R. C. Rowe; e = C. E. Windels; f = K. A. Kimble; g = R. B. Volin; h = J. P. Jones; i = L. L. Black; j = J. C. Watterson; k = T. Katan; l = C. Alabouvette; m = R. G. Grogan; n = H. A. Bolkan; o = C. L. Schoulties; p = T. S. Isakeit; q = W. H. Elmer; r = S. K. Sun; s = T. H. Barksdale; t = P. W. Bosland; u = R. Martyn; v = R. Baker.

[y] Electrophoretic phenotypes represent a composite of all enzymes scored.

[z] Vegetative compatibility groups numbered according to Puhalla (32). VCG 003- is an artificial group containing isolates that are single members of a VCG.

temperature. Mycelium was collected on Whatman No. 1 filter paper by vacuum filtration, resuspended in 300 ml of sterile distilled water, and collected by vacuum filtration again. The mycelium was transferred to a petri dish, frozen overnight (−20 C), lyophilized, and stored at −70 C until needed for protein extraction.

Protein extraction. Lyophilized fungal mycelium was ground to a fine powder with a chilled mortar and pestle. A 300-mg aliquot of the powdered mycelium was mixed with 2.25 ml of 50 mM Tris-HCl (pH 7.1) in a small centrifuge tube and incubated for 15 min on ice. The sample then was centrifuged at 18,000 rpm (Beckman SW55TI rotor, Beckman Instruments, Fullerton, CA) for 27 min at 4 C. The supernatant was collected from the tube without disturbing the lipid upper layer or the pellet, dispensed into 10 microfuge tubes in 100-μl aliquots, and stored at −70 C until needed for electrophoresis.

Electrophoresis. Protein extracts were allowed to thaw on ice, then 10-μl aliquots were applied to filter paper wicks (Whatman No. 3). The wicks were loaded onto a 15- by 20-cm horizontal potato starch gel (12% w/v) and subjected to electrophoresis at 50 mA for 4–6 h depending on the buffer system used. Gels were cooled with ice packs for the duration of the run. After completion of electrophoresis, each gel was sliced horizontally into six 1.5-mm-thick slices and treated with specific enzyme visualization reagents (19,26,35,37). Four electrophoretic buffer systems (23) and 34 enzyme systems were tested initially for activity, resolution, and consistent appearance of bands. Of these, 15 enzymes that produced resolvable banding patterns were selected for further use (Table 2). As many as 30 samples, including one standard isolate (LSU-2) with known banding patterns, were analyzed in each gel slice. All isolates were analyzed at least twice in separate electrophoretic runs.

Data analysis. Electrophoretic mobility, or distance of migration (cm) from the origin, was recorded for all enzyme bands (Table 3). The relative intensity of bands was ignored. Enzyme bands were numbered in the order of fastest to slowest migration. Although there is no genetic data for the inheritance of the enzyme polymorphism, we refer to the enzyme bands as putative loci and alleles as described by Bosland and Williams (4). A composite of all locus and allele phenotype numbers was tabulated for each isolate, compared to other isolates, and electrophoretic phenotypes (EPs) were designated. These data were used to calculate simple matching coefficients (3) between all pairs of isolates according to the equation:

$$Sm = \frac{(++) + (--)}{(++) + (--) + (+-) + (-+)}$$

TABLE 2. Enzymes, abbreviations, Enzyme Commission numbers, and buffer systems used in this study

Enzyme	Abbreviation	EC number	Buffer[z]
Aconitase	ACON	4.2.1.3	C
Aspartate amino transferase	AAT	2.6.1.1	A
Esterase	EST	3.1.1.1	C
Fumarase	FUM	4.2.1.2	C
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	C
Glucose phosphate isomerase	GPI	5.3.1.9	A
Hexokinase	HEX	2.7.1.1	C
Isocitrate dehydrogenase	IDH	1.1.1.42	C
Lactate dehydrogenase	LDH	1.1.1.27	C
Malate dehydrogenase	MDH	1.1.1.37	A
Malic enzyme	ME	1.1.1.40	A
Mannose phosphate isomerase	MPI	5.3.1.8	C
Phosphoglucomutase	PGM	2.7.5.1	A
6-Phosphoglucose dehydrogenase	6PGD	1.1.1.42	C
Superoxide dismutase	SOD	1.15.1.1	A

[z] Buffer systems used are from Leung and Williams (23). A = amine-citrate, pH 6.1; B = Tris-borate-EDTA, pH 8.0; C = Tris-citrate/lithium-borate, pH 8.5; and D = Tris-citrate, pH 6.3.

in which Sm = simple matching coefficient; ++ = positive matches (an enzyme band present in both isolates being compared); −− = negative matches (an enzyme band present in another isolate but absent in both isolates being compared); and (+−) and (−+) = mismatches. The coefficients were then used to construct a similarity matrix (3) among the 34 EPs that represented all 118 isolates of *F. oxysporum*. We subjected the matrix to cluster analysis by using the unweighted paired-group method with arithmetic averaging (UPGMA) (39) to construct a dendrogram. Principal components analysis (17) also was employed in an attempt to resolve phylogenetic groups, and product-moment correlation coefficients among variables were calculated. Eigenvectors were extracted from the correlation matrix, and the data were projected onto the eigenvectors. The computer program NTSYS-pc version 1.60 (Exeter Publishing Inc., Setauket, NY) was used for these analyses.

RESULTS

Electrophoresis. In an initial survey, the activity and resolution of 34 enzyme systems in four gel buffer systems were tested. No activity or poor resolution was observed for the enzymes acid phosphatase, adenoside deaminase, alcohol dehydrogenase, adenylate kinase, aldolase, alkaline phosphatase, catalase, diaphorase, glucose dehydrogenase, glutamate dehydrogenase, glycerate dehydrogenase, α-glycerophosphate dehydrogenase, guanine deaminase, β-hydroxybutyrate dehydrogenase, leucine aminopeptidase, nitrate reductase, nucleoside phosphorylase, sorbitol dehydrogenase, and xanthine dehydrogenase regardless of the gel buffer system used. In contrast, aconitase, aspartate amino transferase, esterase, fumarase, glucose-6-phosphate dehydrogenase, glucose phosphate isomerase, hexokinase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic enzyme, mannose phosphate isomerase, phosphoglucomutase, 6-phosphoglucose dehydrogenase, and superoxide

TABLE 3. Allele distribution for 30 putative enzyme loci in 118 isolates of *Fusarium oxysporum*

Locus[y]	Allele number 1	2	3	4	5
Acon	1.1 (118)[z]				
Aat-1	2.1 (118)				
Aat-2	1.5 (4)				
Aat-3	0.1 (28)				
Est-1	2.0 (22)	1.9 (3)	1.8 (3)	1.6 (89)	
Est-2	1.3 (88)	0.9 (1)	0.8 (22)		
Est-3	0.1 (118)				
Est-4	2.8 (22)				
Fum	1.1 (1)	0.8 (2)	0.7 (115)		
G6pdh	0.9 (118)				
Gpi	2.6 (118)				
Hex	1.7 (112)	1.4 (2)	1.3 (4)		
Idh	0.8 (118)				
Ldh-1	1.9 (1)	1.5 (1)	1.0 (2)	0.8 (113)	0.6 (1)
Ldh-2	1.1 (1)	0.7 (1)	0.6 (33)	0.4 (1)	
Mdh-1	1.2 (118)				
Mdh-2	0.8 (118)				
Mdh-3	0.5 (118)				
Mdh-4	3.3 (1)				
Mdh-5	0.1 (1)				
Me-1	3.3 (1)	1.0 (117)			
Me-2	0.7 (118)				
Me-3	0.4 (118)				
Me-4	0.1 (1)				
Mpi	2.5 (118)				
Pgm-1	2.4 (85)	2.1 (4)	2.0 (27)	1.9 (1)	1.8 (1)
Pgm-2	0.8 (117)				
6Pgd	1.3 (118)				
Sod-1	2.0 (118)				
Sod-2	1.5 (118)				

[y] Abbreviations for putative enzyme loci according to Richardson et al. (35).

[z] Migration from origin in centimeters (number of isolates).

dismutase showed strong activity and acceptable resolution in combination with the appropriate gel buffer system (Table 2). Once the optimal gel buffer system had been determined for each enzyme system, electrophoresis of protein extracts and collection of isozyme polymorphism data were straightforward.

Eight and seven of the enzyme systems assayed revealed single and multiple bands, respectively, for a total of 30 putative loci detected in the 15 enzyme systems (Table 3). Twenty-two of the 30 loci exhibited complete homogeneity among all isolates surveyed. Eight of the 30 loci were variable and revealed two to five putative alleles per locus (Table 3).

An EP is defined as one or more fungal isolates with a distinctive combination of isozyme phenotypes. Thirty-four EPs were identified among the 118 isolates surveyed (Table 4). Twenty-one EPs

TABLE 4. Numerical designation of electrophoretic phenotypes observed for 118 isolates of *Fusarium oxysporum*

	Electrophoretic phenotype																	
Locus[y]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Acon	1[z]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Aat-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Aat-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Aat-3	0	0	1	1	1	1	1	0	0	0	1	1	1	0	1	1	1	0
Est-1	4	4	4	1	4	4	4	4	1	1	4	1	1	4	1	4	4	4
Est-2	1	1	1	3	1	1	1	1	3	3	1	3	3	1	3	1	1	1
Est-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Est-4	0	0	0	1	0	0	0	0	1	1	0	1	1	0	1	0	0	0
Fum	3	3	3	3	3	2	2	3	3	3	3	3	3	3	3	3	3	3
G6pdh	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Gpi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hex	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
Idh	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ldh-1	4	4	4	4	4	4	4	4	3	4	4	1	4	4	4	4	5	4
Ldh-2	0	3	3	3	0	3	0	0	0	0	0	2	0	0	3	3	0	4
Mdh-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mdh-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Me-1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Me-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Me-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Me-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mpi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pgm-1	1	1	1	1	1	1	1	3	3	3	3	3	3	2	4	3	1	1
Pgm-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
6Pgd	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sod-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sod-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

	Electrophoretic phenotype																
Locus[y]	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Acon	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Aat-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Aat-2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Aat-3	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Est-1	1	1	1	1	2	2	1	4	3	3	1	1	1	0	2	4
Est-2	3	3	3	3	0	0	3	1	0	0	3	3	3	0	0	2
Est-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Est-4	1	1	1	1	0	0	1	0	0	0	1	1	1	0	0	0
Fum	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3
G6pdh	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Gpi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hex	3	2	3	3	1	1	1	1	1	1	1	1	1	1	1	1
Idh	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ldh-1	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4
Ldh-2	3	0	0	3	0	1	3	3	0	0	3	3	3	0	0	0
Mdh-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Mdh-5	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Me-1	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2
Me-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Me-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Me-4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Mpi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pgm-1	3	3	3	3	5	3	2	1	2	3	3	3	1	1	1	1
Pgm-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6Pgd	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sod-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sod-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[y] Abbreviations for putative enzyme loci according to Richardson et al (35).
[z] Numerical designation of putative alleles detected. 0 = No bands detected.

contained a single isolate, nine EPs contained two to five isolates, and four EPs (EPs 1, 2, 3, and 5) contained six to 49 isolates (Table 5). EP 1, with 49 members, accounted for 42% of all isolates assayed. EPs 1–5, 8, 10, 13, and 29 contained isolates of a different race, VCG, or forma specialis. Thus, a large number of isolates from one or more races, VCGs, or even formae speciales were electrophoretically identical according to the 15 enzyme systems included in this study.

Data analysis. Because many EPs contained more than one race of *F. o. lycopersici* from various geographic origins as well as other formae speciales, no clustering of isolates by formae speciales, geographic origin, or races 1 and 2 was observed (Fig. 1). However, five of the six race 3 isolates were in EP 1. The only other race 3 isolate, FDA-3 from Florida, was in EP 15. There was pronounced clustering of isolates by VCG with minor exceptions (EPs 4 and 11) (Fig. 1). In the dendrogram (Fig. 1), VCGs 0030 and 0032 clustered together on a major branch of the tree, whereas VCG 0031 was on a different branch. EPs 15, 19, 23, and 24 were genetically distant from the other EPs. These EPs, for the most part, contain only one member each (Table 1).

Principal components analysis provided more information regarding the relative position of each isolate with respect to all isolates examined. The first six principal components accounted for 29.6, 23.2, 12.1, 9.6, 6.6, and 5.2% of the variability, respectively. No other factors accounted for greater than 5.0% of the variability. A plot of the first and second principal components is presented in Figure 2. The following general observations can be made. The majority of isolates in VCGs 0030 and 0032 clustered together; several symbols on the plot were representative of more than one isolate. Isolates in VCG 0031 clustered together but at a relatively large distance away from VCGs 0030 and 0032. Other formae speciales clustered with VCGs 0030 and 0032. There was no clustering by race or geographic origin. And, single-member VCGs were clustered closely with the multiple-member groups.

DISCUSSION

In the 1960s and 1970s, analytical techniques were developed for utilizing isozyme data for numerical taxonomy (27,39). Because the majority of animal and plant populations for which these techniques were developed were diploid, sexually reproducing species, several of the assumptions that must be met to apply these techniques were not applicable to haploid, asexual organisms such as *F. oxysporum*. Although multiple bands for a given enzyme may result from post-translational modifications or multiple subunits, as well as different alleles or loci, sexual crosses that would clarify these relationships are not possible with this fungus. Thus, bands can only be treated as putative loci and alleles. When they are analyzed as phenotypic characters, it is possible to quantitatively assess genetic diversity within and among VCGs and races of *F. oxysporum*.

We demonstrated that isolates of *F. o. lycopersici* within a

TABLE 5. Distribution of isolates of *Fusarium oxysporum* f. sp. *lycopersici* by vegetative compatibility group (VCG) and race within each electrophoretic phenotype

	VCG 0030[w]			VCG 0031			VCG 0032			Other VCGs			Other	Total number of
Phenotype	1[x]	2	3	1	2	3	1	2	3	1	2	3	f. spp.	isolates
1	10[y]	15	5	...	...	...	...	1	...	13	2	...	RL,N[z]	49
2	6	2	...	...	...	...	1	...	...	2	1	...	D	13
3	4	...	...	...	...	...	...	1	...	...	1	...	...	6
4	1	...	...	...	...	...	...	...	...	1	...	...	...	2
5	3	1	...	...	...	...	...	...	...	1	1	...	...	6
6	1	...	...	...	...	...	...	...	...	...	...	...	...	1
7	1	...	...	...	...	...	...	...	...	...	...	...	...	1
8	...	2	...	...	...	...	...	1	...	1	1	...	...	5
9	...	...	...	...	2	...	...	...	...	...	...	...	...	2
10	...	...	...	...	2	...	...	...	...	1	1	...	...	4
11	...	...	...	...	1	...	...	...	...	...	...	...	...	1
12	...	...	...	...	1	...	...	...	...	...	...	...	...	1
13	...	...	...	1	1	...	...	...	...	...	...	...	...	2
14	...	...	...	...	...	...	...	...	...	2	...	...	...	2
15	...	...	...	...	...	...	...	...	...	...	...	1	...	1
16	...	...	...	...	...	...	...	...	...	1	...	...	...	1
17	...	...	...	...	...	...	...	...	...	1	...	...	...	1
18	...	...	...	...	...	...	...	...	...	1	...	...	...	1
19	...	...	...	...	...	...	...	...	...	2	...	...	...	2
20	...	...	...	...	...	...	...	...	...	1	...	...	...	1
21	...	...	...	...	...	...	...	...	...	1	...	...	...	1
22	...	...	...	...	...	...	...	...	...	1	...	...	...	1
23	...	...	...	...	...	...	...	...	...	1	...	...	...	1
24	...	...	...	...	...	...	...	...	...	...	...	...	A	1
25	...	...	...	...	...	...	...	...	...	1	...	...	...	1
26	...	...	...	...	...	...	...	...	...	...	1	...	...	1
27	...	...	...	...	...	...	...	...	...	1	...	...	...	1
28	...	...	...	...	...	...	...	...	...	2	...	...	...	2
29	...	...	...	...	...	...	...	...	...	1	1	...	...	2
30	...	...	...	...	...	...	...	...	...	...	1	...	...	1
31	...	...	...	...	...	...	...	...	...	1	...	...	...	1
32	...	...	...	...	...	...	...	...	...	...	...	...	V	1
33	...	...	...	...	...	...	...	...	...	1	...	...	...	1
34	...	...	...	...	...	...	...	...	...	...	...	...	C	1
Total	26	20	5	1	7	0	1	3	0	37	10	1	7	118

[w] VCGs are numbered according to Puhalla (32). VCG 003- is an artificial group containing isolates that are single members of a VCG.

[x] Race designation was determined with the differential tomato cultivars Walter (resistant to races 1 and 2), Supersonic (resistant to race 1), and Fantastic (susceptible to all three races).

[y] Number of isolates.

[z] RL = f. sp. *radicis-lycopersici*; N = f. sp. *niveum*; D = f. sp. *dianthi*; A = f. sp. *asparagi*; V = f. sp. *vasinfectum*; C = f. sp. *cucumerinum*.

VCG are more genetically similar than those in different VCGs. VCGs 0030 and 0032 are more similar to each other and to members of other formae speciales than to VCG 0031. Our results corroborate those of Bosland and Williams (4) and Katan et al (22). Bosland and Williams (4), who examined isolates of *F. oxysporum* pathogenic on crucifers, found enzyme polymorphisms for three of 18 enzymes examined, and the polymorphisms were correlated with formae speciales and VCGs but not races. Katan et al (22) found polymorphisms for one of eight enzymes examined; these were found in a majority of isolates in one of the VCGs of *F. o. radicis-lycopersici*.

We propose the following scenario to account for the clustering of isolates of *F. o. lycopersici* by VCGs rather than races. It is likely that vegetative compatibility genes are very stable, and evolutionary events such as mutations at virulence loci led to the occurrence of multiple races within VCGs. If such were the case, the remainder of the genome should be quite similar to the ancestral progenitor if there has been relatively little genetic drift and fixation. Our results support this evolutionary model because most of the members of a VCG were genetically very similar, if not electrophoretically identical, regardless of race or geographic origin. In addition, we propose that mutations at vegetative incompatibility loci led to the occurrence of single-member VCGs. Again, if such were the case, the remainder of the genome should be quite similar to the parent VCG. Our results support this evolutionary model because most of the single-member VCGs are distributed with the two VCG clusters. The obvious exceptions are EPs 15, 19, and 23. These populations should be closely monitored because they may be sources of pathogenic diversity not found in other strains.

Furthermore, our data provide strong support for the independent evolution of races. In the dendrogram, race 3 occurs only in two EPs (EPs 1 and 15) that are widely separated and probably do not share a recent common ancestor. In contrast, races 1 and 2 are found scattered throughout the dendrogram with no discernible pattern of distribution. Because race 1 is found in all parts of the dendrogram, even in the most genetically distant groups (EPs 19 and 23), it is probable that it has existed for the longest time. Race 2 probably developed subsequent to the divergence of these EPs. If we suppose that race 1 alone was the ancestral progenitor, it follows that race 2 and race 3 are of more recent origin and have arisen multiple times in several VCGs. The ability to overcome resistance in the host can be readily detected, and probably one or very few pathogenicity-related genes are involved (25,40,41). These results suggest that the host exerts the major selection pressure on the pathogen population, and there is rapid mutation and fixation of pathogenicity genes in the absence of electrophoretically measurable variation.

The clustering of VCGs provides persuasive evidence that, in *F. o. lycopersici*, the parasexual cycle does not contribute significantly to genetic variation in nature. However, a low frequency of parasexual genetic recombination would be difficult to detect because of the limited genetic diversity within VCGs. Thus, non-detectable genetic introgression (16) could be occurring and account for the apparent rapid spread of new races. This question remains open. In addition, the apparent genetic homogeneity of isolates within EP 1, isolates of diverse race and geographic origin, suggests that a large portion of the population of *F. o. lycopersici* arose from a common progenitor that subsequently became widely distributed. In view of this genetic uniformity, it seems unlikely that the progenitor population was a widely distributed soil inhabitant. Rather, it may have been associated with undomesticated plant species that are closely related to tomato. There are numerous weed species that are members of the Solanaceae (15) with a worldwide distribution. This hypothesis could be tested by conducting VCG and isozyme analyses of isolates of *F. oxysporum* obtained from such weed species as well as species of *Lycopersicon* in the center of origin of tomato.

It is thought that formae speciales of *F. oxysporum* undergo not only a parasitic life cycle in association with specific host plants but also a saprophytic cycle in the soil (6). A successful saprophyte must possess a diverse array of metabolic enzymes to utilize various substrates under a wide range of soil conditions such as pH, chemical composition, temperature, microbial antagonists, etc. If this were the case, quite different selection pressures over many generations would be expected to cause an

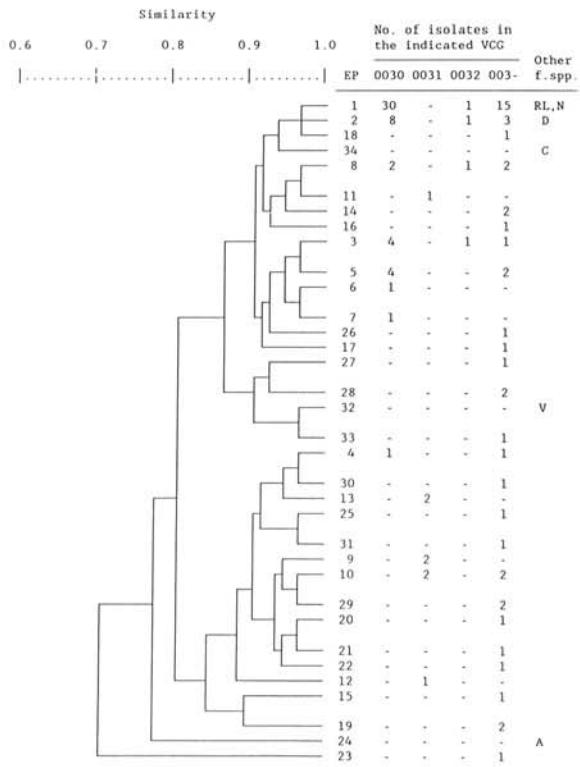

Fig. 1. A dendrogram generated by the unweighted paired-group method with arithmetic averaging (UPGMA) cluster analysis of simple matching coefficients of similarity by use of the 34 electrophoretic phenotypes (EP) that represent 118 isolates of *Fusarium oxysporum*. VCG = vegetative compatibility group; other f. spp. are RL = *radicis-lycopersici*, N = *niveum*, D = *dianthi*, C = *cucumerinum*, V = *vasinfectum*, and A = *asparagi*.

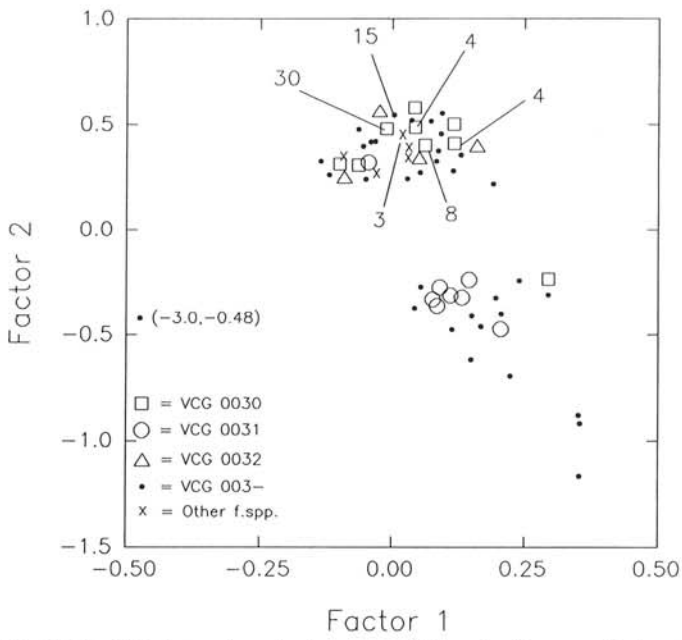

Fig. 2. Plot of 34 electrophoretic phenotypes (EP) on the first two principal axes. Factors 1 and 2 account for 29.6 and 23.2% of the variability, respectively. Except where indicated otherwise, each symbol represents one fungal isolate. A numbered symbol indicates the number of isolates represented by that symbol. Numbers in parentheses indicate coordinates for an isolate that was off the scale.

increase in genetic diversity both within an inbreeding population (VCG) and among genetically isolated populations or VCGs (5). Yet, the entire collection of 111 isolates of *F. o. lycopersici* was tightly clustered. Because our isolates came from sites with diverse climatic and edaphic conditions, we conclude that the host plant exerts the primary selection pressure, and *F. o. lycopersici* does not undergo a significant saprophytic phase in its life history. This conclusion is particularly significant as it relates to strategies for biological control. For example, Alabouvette and his coworkers (1) proposed that nonpathogenic strains of *F. oxysporum* compete saprophytically in the soil with pathogenic formae for limiting substrates as opposed to parasitic competition within the host (36).

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