Vegetative Compatibility Groups in *Fusarium oxysporum* f. sp. *lycopersici*

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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 and Development Fund.

Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript 89-38-3412.

We thank all contributors for supplying isolates.

Accepted for publication 12 September 1990 (submitted for electronic processing).

ABSTRACT

Elias, K. S., and Schneider R. W. 1991. Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici*. Phytopathology 81:159-162.

A worldwide collection of 115 isolates of *Fusarium oxysporum* f. sp. *lycopersici* from tomato was examined for pathogenicity, colony morphology, and vegetative compatibility. No correlations were found between colony morphology and length of time in culture, race, vegetative compatibility group (VCG), or geographic origin. Vegetative compatibility group 0030 contained all three races of *F. o. lycopersici* and all colony morphology types. Vegetative compatibility groups 0031 and 0032 contained races 1 and 2, but their geographic ranges were somewhat more limited. In addition, 50 isolates of *F. o. lycopersici* were the sole members of a VCG (VCG 003-). Thus, no correlation existed between VCG and race. The occurrence of all three races from diverse locations (Australia, Florida, and California) in the same VCG indicates that several genetic determinants responsible for race specificity may exist within genetically isolated clonal populations (VCGs). While VCG has no known association with pathogenicity, it provides a new trait with which to further characterize fungal strains. More importantly, these previously undefined entities suggest the existence of genetic limits that could govern paths of gene flow related to the origin of races in *F. o. lycopersici*.

Fusarium wilt of tomato (*Lycopersicon esculentum* Miller), caused by the vascular wilt pathogen *Fusarium oxysporum* (Schlechtend.:Fr.) f. sp. *lycopersici* (Sacc.) Snyder & Hans. (36), is a devastating disease that occurs in major tomato-growing regions of the world (3). The three known races of *F. o. lycopersici* are distinguished by their pathogenicity to cultivars with specific dominant resistance genes (23). Race 1 was initially described in 1886 (5). Race 2 was first reported in 1945 in Ohio (1), but did not become economically important until 1961 in Florida (32). Race 3 was reported in Australia in 1978 (15), in Florida in 1982 (35), and finally in California in 1987 (11).

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 parasexual recombination within or between preexisting races of *F. o. lycopersici*, other formae speciales, the nonpathogenic population of *F. oxysporum*, or any combination of these. The genetic pool from which new races might emerge would be very different for each of these scenarios. Spontaneous random mutations may also lead to race development. Vegetative compatibility and heterokaryosis are prerequisites for parasexual recombination. These processes must be more thoroughly documented before testable hypotheses regarding the origin of new races can be proposed (21,31).

In 1985, Puhalla introduced a technique by which he selected spontaneous nitrate metabolism (*nit*) mutants of *F. oxysporum* with no mutagen treatment (29). He obtained two complementary, but uncharacterized, *nit* mutants and used them to assess heterokaryon formation and vegetative compatibility. Growth of heterokaryons was then observed macroscopically. Using this technique, Puhalla (29) proposed an evolutionary model for the origin of formae speciales and races, based on the results from 21 isolates representing 12 formae speciales of *F. oxysporum*. He proposed that when the sexual stage and meiotic recombination of *F. oxysporum* were lost, the loci that determine vegetative compatibility and pathogenicity became fixed in the same thallus. In this way, distinct vegetative compatibility groups (VCGs) with specific virulence genes became genetically isolated, asexual, inbreeding populations. He concluded that confirmation of his model required more extensive analysis.

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In 1987, Correll et al (7) refined Puhalla's system by describing physiological tests for selecting *nit* mutants of known phenotype that are deficient in specific regulatory and structural genes. This system assured that complementary strains were being paired so that false negatives could be minimized. Several investigators used *nit* mutants for a VCG analysis of the following formae speciales: *apii* (8), *asparagi* (14), *conglutinans* (6), *cubense* (28), *melonis* (16), and *vasinfectum* (17). Their objectives were to develop a more rapid race identification technique (8,14,16,17,28), to differentiate pathogens from nonpathogens (8,14,17), or to estimate the diversity within a fungal population (6,14,16,17). In so doing, they also tested the validity of Puhalla's evolutionary model. While the majority of these studies (6,8,16,17) provided support for this model, a strong correlation between VCG and pathotype was not the case with all formae (12–14,28).

The objective of this study was to examine a worldwide collection of all races of *F. o. lycopersici* for vegetative compatibility groups to gain a better understanding of the origin of races within this forma specialis. Preliminary reports have been published (12,13).

MATERIALS AND METHODS

Fungal strains. One hundred and nine isolates reported to be *F. o. lycopersici* were 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. Six isolates of *F. o. lycopersici* were collected from infested tomato fields in Louisiana. Isolates were single-spored on potato-dextrose agar (PDA) and then stored on silica gel at 4 C (26). Segments of stem or taproot that showed obvious vascular discoloration were surface-sterilized by being dipped in 70% ethanol for 5 sec; then, they were flamed. Tissue was plated on Komada's medium (20) and incubated for 5–10 days at room temperature. Uniform colonies of *F. oxysporum* grew out of all diseased tissue, and one colony was selected from each plate, single-spored, and stored on silica gel (26).

Pathogenicity tests. To confirm the forma specialis and race identity of all putative strains of *F. o. lycopersici*, greenhouse pathogenicity tests were performed using the differential tomato cultivars Fantastic (no resistance), Supersonic (resistant to race

1), and Walter (resistant to races 1 and 2). Fungal strains were grown in Czapek's solution (34) for 5 days on an orbital shaker (100 rpm) at room temperature. The culture was then filtered through cheesecloth to obtain a spore suspension containing about 1×10^6 spores per milliliter. Seedlings were grown in sterile peat moss potting-soil mix for 10 days until the first true leaf began to emerge. The root-dip method of inoculation (37) was used as described below. Seedlings were shaken to remove excess soil. The roots of six seedlings per cultivar were dipped into spore suspensions of each isolate for 30 sec and then transplanted to plastic cell trays that contained a sterile sand/soil (1:1) mix with one seedling per cell. Other seedlings were dipped in sterile Czapek's solution or inoculum of known virulent isolates of each race and were planted as negative and positive controls, respectively. The susceptibility of tomato cultivars to a particular isolate of *F. o. lycopersici* was recorded after 14 days. Pathogenicity tests were conducted at least twice for each isolate.

Colony morphology. All isolates were grown on acidified PDA (pH 4.0) under light for 12 hr at 25 C and in darkness for 12 hr at 20 C for 10 days, as suggested by Nelson et al (24). Isolates were then grouped according to similarity in colony color and type.

Selection, characterization, and pairing of *nit* mutants. Nitrate metabolism (*nit*) mutants were selected by the method of Puhalla (29). Three- to four-day-old colonies were transferred from a minimal salts medium (MM) to potato-sucrose agar (KPS) containing 1.5% $KClO_3$. Wild-type cells took up the chlorate ion, an analog of nitrate, and converted it to chlorite, which is toxic. Mutant cells that cannot metabolize chlorate or nitrate emerged from restricted colonies as fast-growing sectors, usually within 10–14 days. These *nit* mutants produced very thin expansive growth on MM, which contained nitrate as the sole nitrogen source.

The phenotypes of all *nit* mutants were determined by the method outlined by Correll et al (7). *Nit* mutants and the wild-type parents were transferred to MM containing either nitrate, nitrite, hypoxanthine, ammonium, or uric acid as the nitrogen source. The plates were incubated at room temperature, and colony growth was scored relative to the wild-type parent after 4 days. Assignment of *nit* mutants to biochemical phenotypes was based on nitrate metabolic pathways in *Aspergillus nidulans* (9,10), *Neurospora crassa* (22), and *Fusarium moniliforme* (19). Several complementary nitM and *nit1* mutants (7) were saved from each isolate and stored on silica gel at 4 C. A *nit1* and a nitM mutant of the 115 isolates of *F. o. lycopersici* were then paired in all possible combinations in petri plates containing MM. The plates were incubated at room temperature under fluorescent room light. Five to 7 days later, complementary, vegetatively compatible isolates were recognized by the robust growth at the interface of the two colonies. All pairings were made at least twice.

RESULTS

Pathogenicity tests. Race determination was straightforward in that all or none of the seedlings of a particular cultivar showed symptoms 10–14 days after inoculation. In all cases in which donated strains of *F. o. lycopersici* were received with a race designation, the race identity was confirmed in the pathogenicity tests. In summary, 67, 42, and 6 strains were determined to be races 1, 2, and 3, respectively (Tables 1 and 2).

Colony morphology. There were no correlations between colony morphology or color and length of time in culture, race, VCG, or geographic origin. Sixty-two isolates produced orange colonies; within this group, 50 had fluffy wild-type growth, while 12 were characterized by appressed growth. Forty-eight isolates produced purple and orange colonies with 21 and 27 of these showing fluffy and appressed growth, respectively. Three isolates produced purple and white colonies, all of which had fluffy growth. Finally, one isolate (IK-6) produced white fluffy growth. Rings of alternating colony color and aerial growth were observed, probably because of the alternating light and dark regimes.

Selection, characterization, and pairing of *nit* mutants. One to 28 *nit* mutants were obtained from each isolate of *F. o. lycopersici*. Although sectoring frequency per isolate was not recorded, there were great differences in both the number and phenotype ratio of *nit* mutants recovered. Sectoring frequency of *F. moniliforme* on chlorate has been shown to be heritable and to vary among isolates (18).

All 115 isolates of *F. o. lycopersici* were assigned to VCGs (Tables 1 and 2). The VCG numbering system used in this study was introduced by Puhalla (29) and designated VCG 0030 for *F. o. lycopersici* and included FRC 0-1078 as the tester strain. No correlation between VCG and race was observed. Vegetative compatibility group 0030 (53 isolates) contained all colony morphology types as well as all three races of *F. o. lycopersici* from throughout the world. Vegetative compatibility group 0031 contained eight isolates, included races 1 and 2, and was limited to the United States. VCG 0032, with four members, included races 1 and 2, and was limited to Louisiana. In addition, 50 isolates of *F. o. lycopersici* were the sole members of a VCG (VCG 003-). Isolates within a VCG were not vegetatively compatible with isolates from other VCGs. Self-incompatibility was not observed between complementary *nit* mutants recovered from single isolates of *F. o. lycopersici* (16).

DISCUSSION

The forma specialis classification in *F. oxysporum* is based on the host species in which a fungal strain is pathogenic, while race is determined by the ability of a fungal strain to cause disease on particular cultivars of the host species. Associating phenotypic traits, such as colony morphology, with physiological characters, such as pathogenicity, is customary. In this way, colony morphological characteristics have become valuable tools that offer important taxonomic information (8,25). However, laboratory culture-induced variation in colony morphology is a well-documented phenomenon in *Fusarium* spp. (4,5,24). We have very little information regarding the length of time in culture or method of storage employed for the majority of cultures donated by other investigators. Thus, the morphological variation observed among isolates of *F. o. lycopersici* in this study was of little value as a phenotypic marker.

The occurrence of all three races from diverse locations (Australia, Florida, and California) in the same VCG indicates that the genetic determinants responsible for host specificity exist within genetically isolated clonal populations (VCGs). The genetic determinants for development of new races probably already exist within the endemic clonal populations of *F. o. lycopersici*, but are not detectable as we can only identify those races for which specific resistance genes have been incorporated into the host. The observed race diversity within VCGs implies that new races could arise independently at different locations as well as at a center of origin, followed by long-distance dispersal. In contrast, the observed distribution of races among several VCGs could indicate past mutations in vegetative incompatibility (*vic*) loci (29) resulting in an accumulation of multiple VCGs with common virulence loci. These scenarios are speculative because not enough is known about the stability of either *vic* loci or virulence loci in pathogenic formae of *F. oxysporum*.

Studies of other fungi (2,27,30) have indicated that a single mutation at a *vic* locus could bring about a change from compatibility to incompatibility or vice versa. Although almost nothing is known about the genetics of virulence in *F. o. lycopersici*, a single gene-for-gene relationship has been implicated (33), and it is plausible that a single mutation at a virulence locus could cause a change in virulence, i.e., development of a new race. The VCG may be a very stable character, while virulence genes must evolve quickly and become fixed because of intense host selection pressure. In contrast, virulence genes that determine race could be very stable, while *vic* genes could be influenced by some unknown selection pressure. Mutation of genes that affect both of these characteristics is likely to be taking place concurrently. Answers to these questions can be obtained by correlating

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

Isolate[w]	Race[x]	Source[y]	Origin
VCG 0030[z]			
LSU-3	1	a	Louisiana
LSU-5	1	a	Louisiana
LSU-6	1	a	Louisiana
FDA-2(FTCC855)	2	b	Florida
FRC 0-1078	2	c	Florida
FRC 0-1079	2	c	Florida
A-22068	1	d	Australia
A-18947	2	d	Australia
A-19156	2	d	Australia
A-21990	3	d	Australia
A-21991	3	d	Australia
OSU-409	1	e	Ohio
OSU-460	2	e	Ohio
UM-1(FRC 0-1118)	1	f	California
L-37	1	g	Michigan
HMS-2(#66)	1	h	California
HMS-3(#74)	1	h	California
HMS-6(#80)	2	h	California
NK-1	2	i	Florida
NK-2	2	i	Florida
NK-3	2	i	Florida
NK-4	2	i	Florida
JBF-4(548-4-10)	2	j	Florida
JBF-5(8)	2	j	Florida
JBF-6(761-Sp6)	3	j	Florida
JBF-7(761-Sp4)	3	j	Florida
BFOL-53	2	k	Louisiana
BFOL-65	2	k	Louisiana
PS-5	3	l	California
IK-1(FOL-I)	1	m	Israel
IK-2(FOL-I-MX)	1	m	Israel
IK-3(FOL-R)	2	m	Israel
IK-4(FOL-649)	1	m	Israel
IK-5(FOL-650)	1	m	Israel
IK-7(FOL-1295)	1	m	Israel
MA-1(E7C)	1	n	Morocco
MA-2(OE2)	1	n	Morocco
MA-3(MH197)	1	n	Morocco
MA-4(MEXII)	1	n	Morocco
MA-5(KE1)	1	n	Morocco
MA-6(FK3)	1	n	Morocco
MA-7(MB6)	1	n	Morocco
MA-8(M6)	1	n	Morocco
MA-9(F1V1)	1	n	Morocco
MA-10(MVH2)	1	n	Morocco
FA-4(FOL15)	2	n	Tunisia
FA-7(FOL27)	2	n	France
FA-8(FOL28)	2	n	France
FA-9(FOL29)	1	n	France
FA-11(FOL31)	2	n	France
FA-13(FOL33)	2	n	France
IA-7(FOL77)	1	n	Italy
IA-11(FOLV)	1	n	Italy
VCG 0031			
BFOL-51	1	k	Louisiana
OSU-451	2	e	Ohio
HMS-4(65)	2	h	California
HMS-5(67)	2	h	California
PS-2	2	l	California
PS-3	2	l	California
UCD-2(1776)	2	o	California
CIC-2	2	p	California
VCG 0032			
LSU-2	2	a	Louisiana
LSU-4	1	a	Louisiana
LSU-7	2	a	Louisiana
BFOL-70	2	k	Louisiana
VCG 003-			
PS-1	1	l	California
PS-4	2	l	California
CIC-1	1	p	California
JBF-1(626-6B)	1	j	Florida
JBF-2(626K-1)	1	j	Florida
JBF-3(626K-2)	1	j	Florida
FDA-1(FTCC854)	1	b	Florida
FDA-3(FTCC979)	3	b	Florida
FRC 0-1081	1	c	Florida
FRC 0-1082	1	c	Florida
UCD-1(1775)	1	o	California
OSU-415	1	e	Ohio
OSU-416	2	e	Ohio
FOL 84-1590	1	g	Michigan
FO-14	1	q	Michigan
T-1	1	r	Taiwan
R5-6	1	g	Wisconsin
HMS-1(60)	1	h	California
F1E	1	s	Maryland
F22(701-2)	2	s	Florida
F23	2	s	Maryland
F34	1	s	Maryland
F37	2	s	Maryland
BFOL-54	1	k	Louisiana
BFOL-56	1	k	Arkansas
BFOL-57	2	k	Arkansas
BFOL-63	1	k	Louisiana
BFOL-64	2	k	Louisiana
BFOL-67	1	k	Louisiana
BFOL-69	1	k	Louisiana
BFOL-75	2	k	Louisiana
PHW-554	1	t	Wisconsin
PHW-555	2	t	Wisconsin
IK-6(FOL-835)	1	m	Israel
FA-3(FOL8)	1	n	France
FA-5(FOL24)	1	n	France
FA-6(FOL26)	1	n	France
FA-10(FOL30)	1	n	France
FA-12(FOL32)	1	n	France
FA-14(FOL62)	1	n	Senegal
IA-1(FOLAL)	1	n	Italy
IA-2(FOL82)	1	n	Italy
IA-3(FOLBari)	1	n	Italy
IA-4(FOL81)	1	n	Italy
IA-5(FOL74)	1	n	Italy
IA-6(FOL84)	1	n	Italy
IA-8(FOLD77)	1	n	Italy
IA-9(FOL1)	2	n	Italy
IA-10(FOL2)	2	n	Italy
IA-12(FOLVA)	1	n	Italy

[w]Original strain number in parentheses.

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

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

[z]Vegetative compatibility groups (VCG) are numbered according to Puhalla (29). VCG 003- is an artificial group containing isolates that are single members of a VCG.

biochemical and molecular estimates of genetic diversity with such characteristics as geographic origin, formae speciales, race, and VCG.

The large number of single-member VCGs (VCG 003-) is perplexing. They may have been members of major VCGs that underwent a simple mutation at one or more of the *vic* loci such that they can no longer form heterokaryons with the other members. In contrast, they may have been members of other

TABLE 2. Categorization of *Fusarium oxysporum* f. sp. *lycopersici* by vegetative compatibility groups (VCG)

VCG[x]	Race[y]	Number of isolates	Origin[z]
0030	1	27	LA, OH, CA, MI, Australia, France, Israel, Italy, Morocco
	2	21	LA, FL, OH, CA, Australia, France, Israel, Tunisia
	3	5	CA, FL, Australia
0031	1	1	LA
	2	7	CA, OH
0032	1	1	LA
	2	3	LA
003-	1	38	AK, CA, FL, LA, MD, MI, OH, WI, France, Israel, Italy, Senegal, Taiwan
	2	11	AK, CA, FL, LA, MD, OH, WI, Italy
	3	1	FL

[x]Vegetative compatibility groups (VCG) are numbered according to Puhalla (29). VCG 003- is an artificial group containing isolates that are single members of a VCG.

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

[z]LA = Louisiana; OH = Ohio; CA = California; MI = Michigan; FL = Florida; AK = Arkansas; MD = Maryland; and WI = Wisconsin.

formae speciales or nonpathogenic populations that underwent mutations at virulence loci. Biochemical or molecular measures of genetic diversity should provide explanations for the existence of the many single-member VCGs.

The VCG analysis of *F. o. lycopersici* described in this study is the most clear-cut exception to Puhalla's evolutionary model, i.e., a correlation between VCG and pathotype (race). These results are particularly noteworthy because of the large size of the collection (115 isolates), the global distribution of its members, and the well-characterized loci responsible for resistance in the host. Puhalla's discovery and elucidation of VCGs in *F. oxysporum* were essential to the qualitative analysis of the development of races within *F. o. lycopersici.*

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