The Probable Center of Origin of *Fusarium oxysporum* f. sp. *lycopersici* VCG 0033

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ABSTRACT

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Isolates of the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*, predominantly from commercial tomato fields in Florida and southwestern Georgia, were characterized using vegetative compatibility grouping (VCG), nuclear restriction fragment length polymorphism (RFLP), and virulence. All field isolates that could be grouped into VCG belonged to VCG 0033. This VCG was first described by Marlatt et al. in 1996 for isolates from northern Florida, Arkansas, and North Carolina. This study demonstrates that VCG 0033 is also widespread in central and southern Florida, in addition to southwestern Georgia, and also was found to be present in Puerto Rico. Population genetic and phylogenetic analyses of 121 isolates indicated that molecular diversity among VCG 0033 isolates was by far the highest in Manatee County, FL, suggesting it to be the probable center of origin of this relatively newly described VCG. Virulence tests with a subset of isolates identified all VCG 0033 isolates as race 3, although differences in aggressiveness were observed among tested isolates, independent of resistance genes in the differential cultivars. The widespread VCG 0030 of *F. oxysporum* f. sp. *lycopersici* was not present in our field collections. This was unexpected, as strains from Florida isolated prior to 1990 were predominantly VCG 0030. This would suggest that VCG 0033 has replaced VCG 0030 in recent years in commercial tomato fields of Florida and southwestern Georgia.

Fusarium wilt of tomato has been known since the beginning of the twentieth century from many tomato growing regions and has been labeled as "one of the classical vascular wilt diseases in plants" (29). Consequently, its causal agent, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans, is relatively well studied. At present, three races are known for *F. oxysporum* f. sp. *lycopersici*. Race 1 was prevalent at the beginning of the last century. After the discovery of single-gene (*I*) resistance (3) and its subsequent use in many tomato cultivars, isolates were found overcoming this resistance (race 2) (1). Race 2 has become widespread in tomato growing areas since 1961, after initial reports from two Florida counties, Palm Beach (24) and Manatee (15). Florida was also among the first locations where race 3, overcoming resistance gene I_2, was reported (28). Race 3 is currently present in the United States (California, Florida, Georgia, Arkansas, North Carolina, Tennessee), Australia, and Mexico (4,6,8,13,18,27).

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Isolates of *F. oxysporum* f. sp. *lycopersici* have also been examined using morphological and molecular markers. A worldwide collection of over 100 isolates comprising all three races was grouped with regard to vegetative compatibility (10), isozyme patterns (11), and restriction fragment length polymorphism (RFLP) (12). A major vegetative compatibility group (VCG) was identified (VCG 0030), in addition to two minor ones, VCG 0031 and VCG 0032. Vegetatively self-incompatible isolates or "single-member VCGs" generally were molecularly similar or identical to isolates belonging to VCG 0030 or VCG 0031.

VCG 0030 currently includes isolates of races 1, 2, and 3, while VCG 0031 and 0032 include races 1 and 2 only (10,18). More recent analysis of race 3 isolates from the southern United States demonstrated the existence of the previously undescribed VCG 0033, which differed from VCG 0030 in mitochondrial RFLP patterns (18). VCG 0033 isolates in that study originated from northern Florida, Arkansas, and North Carolina. All VCG 0033 isolates tested so far have been race 3 (6,18). Regarding its source, it was speculated that VCG 0033 isolates may have been introduced into Arkansas from Florida by seasonal workers (18).

A recent examination of isolates from diseased tomato plants from central and southern Florida exhibiting crown and root rot symptoms yielded an unexpectedly high number of *F. oxysporum* f. sp. *lycopersici* isolates in addition to *F. oxysporum* f. sp. *radicis-lycopersici* (22). We suggested that under certain (unknown) conditions, the symptoms of these two Fusarium diseases may overlap, making it difficult to differentiate them. All *F. oxysporum* f. sp. *lycopersici* examined for VCG were of VCG 0033, which increased the known area of distribution of this particular VCG to include southern and central Florida.

The objectives of the present study were to further characterize isolates of *F. oxysporum* f. sp. *lycopersici* from central and southern Florida using molecular markers and virulence characteristics, and to compare them with a hitherto uncharacterized collection of *F. oxysporum* f. sp. *lycopersici* from northern Florida and southwestern Georgia. Specific questions addressed in this study were: (i) Can we identify a center of diversity (and therefore probably the center of origin) of *F. oxysporum* f. sp. *lycopersici* VCG 0033 (northern Florida/southwestern Georgia versus central and southern Florida)? (ii) Are all isolates of VCG 0033 race 3? (iii) Are VCGs other than VCG 0033 present in tomato fields in Florida or southwestern Georgia? Previous analyses placed 18 out of 19 isolates from Florida into VCG 0030, either directly or based on their isozyme or RFLP patterns (10–12). Therefore, we expected to find at least some isolates of *F. oxysporum* f. sp. *lycopersici* from Florida belonging to VCG 0030.

MATERIALS AND METHODS

Fungal collections. In 1995 and 1996, diseased tomato plants with signs of infection by *Fusarium oxysporum* were collected in nine counties of central and southern Florida. Fungal cultures were established and maintained as previously described (22). Vegetative compatibilities were determined for these isolates. In five of the nine counties sampled (Collier, Dade, Martin, St. Lucie, Manatee), isolates of *F. oxysporum* f. sp. *lycopersici* VCG 0033 were detected by vegetative-compatibility grouping, using complementation tests between nitrate-nonutilizing (*nit*) mutants (22; T. Katan, *unpublished*). In 1996, isolates suspected of being *F. oxysporum* f. sp. *lycopersici* were also

obtained from counties in northern Florida (Gadsden) and southwestern Georgia (Seminole and Decatur). Additionally, we also received a collection of *F. oxysporum* isolates that had been compiled in 1991 by D. Chellemi, then at Florida Research & Education Center, Quincy. The approximate geographic origins of *F. oxysporum* f. sp. *lycopersici* isolates from Florida and Georgia are depicted in Figure 1.

Four isolates of *F. oxysporum* f. sp. *lycopersici* from San Isabel, Puerto Rico, (plant samples obtained in 1995) were also included in this study. Four isolates that have been analyzed previously (18) were kindly provided by J. Correll, University of Arkansas. These included two isolates from Arkansas, MM2 (VCG 0033, race 3) and MM 59 (VCG 0032, race 2), in addition to DC1 and DC2 (both VCG 0033) from northern Florida, isolated in 1991 by D. Chellemi. Previously characterized isolates were also part of this study. VCG 0031 was represented by OSU451 (race 2) (10). Three isolates belonged to VCG 0030 (19): SC548 (race 2), isolated at Bradenton (Manatee County) in 1964; SC626 (race 1), isolated in Italy in the 1960s (7); and I5397=SC761=BE-1 (race 3), isolated at Bradenton probably around 1982.

Generation of RFLP data and analysis. Suitable probes for population genetic analysis of *F. oxysporum* f. sp. *lycopersici* isolates were selected from genomic libraries of *F. oxysporum* f. sp. *radicis-lycopersici* (22). For analyzing isolates in this study, probes pRLs 82.2, 172, 50, and 162 were found to be useful. pRLs 82.2 and 172 were repetitive probes and generated fingerprint patterns, whereas pRLs 50 and 162 were low-copy probes. These probes were individually hybridized to nylon membranes containing the *Eco*RV-digested DNA of all isolates. Restriction fragments revealed by each probe were considered distinct loci, and their presence and absence were scored as 1 and 0, respectively. Multilocus RFLP haplotypes were generated from these data.

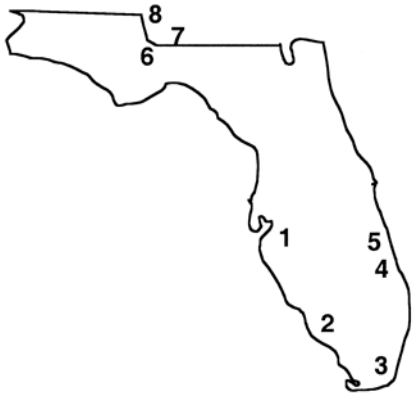

Fig. 1. Map of Florida to indicate counties from which samples of *Fusarium oxysporum* f. sp. *lycopersici* were obtained. 1, Manatee; 2, Collier; 3, Dade; 4, Martin; 5, St. Lucie; 6, Gadsden; 7, Decatur (GA); 8, Seminole (GA).

Isolates that were determined to be VCG 0033 were arranged according to their county of origin and subjected to analysis at a population level using Arlequin ver. 2.000 (S. Schneider, D. Roessli, and L. Excoffier, Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Switzerland). Molecular diversity indices were computed, and tests for the presence of population structure were conducted as previously described (22). Phylip version 3.6a2.1 (J. Felsenstein, Department of Genetics, University of Washington, Seattle) was used to help disclose additional evolutionary tendencies among the various RFLP haplotypes of VCG 0033 isolates and their phylogenetic relationship to haplotypes not belonging to VCG 0033. First, the option SEQBOOT was used for bootstrapping to generate 100 data sets of our binary data (additional data sets were not supported by computational facilities used). The bootstrapped data were then subjected to Wagner parsimony (9,17) using the MIX option. The input order was randomized using the Jumble option. A consensus tree was constructed by using CONSENSE. The unrooted consensus tree was visualized using Treeview (21).

Pathogenicity tests. Pathogenicity tests were performed using the differential tomato cultivars Manapal (resistant to race 1) and Horizon (resistant to races 1 and 2) to determine race composition of VCG 0033. Thirty-five VCG 0033 isolates (representing a variety of RFLP haplotypes) were tested for pathogenicity and virulence. Some isolates were also tested for virulence to cultivar Fla. 7547 (resistant to races 1, 2, and 3) (23). As positive and negative controls, we included several reference isolates of *F. oxysporum* f. sp. *lycopersici*, belonging to races 1, 2, and 3, as well as a nonpathogenic isolate of *F. oxysporum* from tomato (HE-5) and a standard water control.

Fungal strains were streaked out from long-term storage (at –80°C in 50% glycerol) onto half-strength potato dextrose agar (19.5 g of potato dextrose broth and 15 g of agar per liter) and subcultured after 1 week onto plates containing Czapek-Dox Agar (35 g of Czapek-Dox broth and 15 g of agar per liter). One-week-old fungal cultures were flooded with about 10 ml of sterile distilled water, and conidia were dislodged with a cell spreader. Conidia were filtered through cheesecloth, then counted with a hemacytometer and the concentration adjusted to 10^6 conidia per ml.

Before inoculation, seedlings were grown in vermiculite. After most seedlings had emerged (after 1 week), they were fertilized once with a 20-18-18 NPK fertilizer. Inoculations were done 14 days after sowing, at which time the first true leaves had emerged. Excess vermiculite was removed from the roots, and the roots were cut to about 2.5 cm. Roots of three seedlings per cultivar were then dipped for 1 min in the conidial suspension before planting them in a single pot (8.5 cm diameter) that contained commercial potting mix (PrimeGro #33, Therm-O-Rock East, Inc., New Eagle, PA). Inoculated seedlings were kept in a Conviron PGW36 growth chamber (Controlled Environments Inc., Pembina, ND) at a constant 28°C with 14 h light (light intensity: 960 μmol/m^2/s). Disease severity was assessed 3 weeks after inoculation using a 1 to 5 scale previously described by Marlatt et al. (18) (1 = no symptoms; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting; 4 = severe chlorosis, wilting, or stunting; and 5 = dead plant). A rating of 2.5 or more has previously been considered to constitute a susceptible rating (18).

RESULTS

RFLP data analysis and vegetative compatibility groupings. Four polymorphic probes (pRLs 82.2, 172, 50, and 162) were used to generate RFLP haplotypes for the *Eco*RV-digested DNA of all isolates. Each unique combination of 1's and 0's at 115 loci was considered a different RFLP haplotype. RFLP haplotypes of 121 isolates were similar or identical to profiles of previously known members of VCG 0033, and differing multilocus RFLP haplotypes were serially numbered (1 to 41). Most of the 41 haplotypes were represented by only one or two isolates, and 46 isolates were organized into 39 haplotypes. Only two haplotypes, i.e., haplotypes 32 and 30, contained more isolates, with 68 and 7 isolates, respectively.

VCG analysis of 120 of these isolates (one isolate could not be revived for VCG testing) confirmed them as being vegetatively compatible with testers of VCG 0033. We also determined the multilocus RFLP haplotypes for representative isolates of VCGs 0030, 0031, and 0032 of *F. oxysporum* f. sp. *lycopersici* and three isolates of *F. oxysporum* f. sp. *radicis-lycopersici* VCG 0094. Twelve isolates of *F. oxysporum*, although isolated from diseased tomato tissue, displayed RFLP patterns greatly different from the various VCGs of *F. oxysporum* f. sp. *lycopersici,* and vegetative compatibility analysis did not place them into any known VCG of *F. oxysporum* f. sp. *lycopersici* or *F. oxysporum* f. sp. *radicis-lycopersici*. These 12 isolates were designated with letters (A to L) and subjected to pathogenicity testing using the universally susceptible tomato cultivar Bonny Best. The 12 isolates, along with 43 additional *F. oxysporum* isolates nonassigned to VCG (22), did not incite disease symptoms under our experimental conditions (L. R. Gale, *unpublished*) and were therefore assumed to be nonpathogenic *F. oxysporum*.

For population genetic analysis, we only considered RFLP haplotypes 1 to 41 of

VCG 0033, organized into county or region of origin (Table 1). For these analyses, we removed those RFLP loci with a score of 0 for all VCG 0033 isolates, thereby reducing the loci under consideration from 115 loci (used for phylogenetic analysis described below) to 79 loci. Molecular diversity indices of RFLP haplotypes, i.e., mean number of pairwise differences and average gene diversity, were calculated using all data and clone-corrected data, where each haplotype is considered only once (Table 2). The same analyses were also conducted to compare diversity of the Manatee population with the pooled population from northern Florida/southwestern Georgia (Table 3). As sample size was low for most counties or regions, population structure analysis was only conducted to evaluate gene flow between isolates from the counties of northern Florida/southwestern Georgia and between the Manatee population and the combined sample from northern Florida/southwestern Georgia.

A phylogenetic approach was used to further visualize phylogenetic relationships among all 63 haplotypes of *F. oxysporum* disclosed by this study. The unrooted consensus tree of the various haplotypes is displayed in Figure 2.

Pathogenicity tests. VCG 0033 isolates were scored for disease symptoms on the differential cultivars Manapal (resistant to race 1) and Horizon (resistant to races 1 and 2). The two scores were added and an average was computed. Most isolates displayed medium to high virulence on both Manapal and Horizon, with 29 out of 35 isolates tested producing an average rating of 3.0 or more. If only the disease rating for Horizon was considered, 33 isolates generated a disease severity of 3.0 or higher, which would classify them as race 3. Two isolates, MN-44 and MN-47, only had an average rating of 2.0 and 2.3, respectively, on Horizon. As their disease ratings were low on Manapal also, race could not be assigned to these two isolates. In a second experiment, the eight VCG 0033 isolates with the lowest average scores were retested. For comparison, several isolates with moderate to high scores in the first experiment were included, as well as the standard controls (water and a nonpathogenic *F. oxysporum* isolate). I 5937 (VCG 0030), a race 3 isolate not tested in the first experiment, was also included. In this experiment, isolates were inoculated onto Bonny Best (no resistance), Manapal (resistance to race 1), E335 (resistance to race 2), and Horizon (resistance to races 1 and 2). In addition to the root dipping method, about 5 ml of inoculum was added to each plant immediately after planting. Scoring and data analysis were done as described previously. Disease ratings in the second experiment were generally higher for most isolates tested, which was probably due to the increased level of inoculum used. MN-44 and MN-47 both had an average disease rating of 3.0 on Horizon, therefore confirming them too as race 3 isolates. Even with the increased inoculum level, three isolates had an average score of <3 across all four cultivars used. While our experiments demonstrated that all 35 isolates tested were in fact race 3 isolates, they also indicated that variation in general aggressiveness might be present within VCG 0033. Future studies, specifically addressing the level of aggressiveness, need to be conducted to quantitatively address this issue further.

Table 1. Geographic distribution of multilocus restriction fragment length polymorphism (RFLP) haplotypes among VCG 0033 isolates of *Fusarium oxysporum* f. sp. *lycopersici*

Origin[a]	Sample size[b]	Multilocus RFLP haplotype[c]
Manatee	32	4, 5, 6, 7, 8, 9, 10, 15*[d], 16, 19, 20, 21, 22, 23, 24 (2), 30* (5), 32* (9), 36, 37
Gadsden	29	18*, 28, 32* (24), 35, 40, 41
Gadsden (91, 92)[e]	9	15*, 17, 27, 30*, 32* (4), 33
Decatur (GA)	29	1, 2, 11 (2), 12, 13, 18*, 25, 26, 31, 32* (19)
Seminole (GA)	7	3, 29*, 32* (4), 34
Collier	1	32*
Dade	2	14, 32*
Martin	6	32* (2), 38 (2), 39 (2),
St. Lucie	2	29*, 30*
Arkansas (state)	1	32*
Puerto Rico (commonwealth)	3	32* (3)

[a] Florida counties, except when indicated otherwise.
[b] Number of isolates examined.
[c] Haplotypes of 121 VCG 0033 isolates were generated using four RFLP probes (pRLs 82.2, 172, 50, and 162) and serially numbered from 1 to 41.
[d] Numbers followed by * indicate haplotypes that were shared among counties or regions. Numbers in parentheses indicate number of individuals displaying a particular haplotype, if more than one.
[e] Isolates collected in 1991/1992 by D. Chellemi.

Table 2. Mean number of pairwise differences and average gene diversity of restriction fragment length polymorphism (RFLP) haplotypes for isolates of *Fusarium oxysporum* f. sp. *lycopersici* VCG 0033

	Sample size		Polymorphic	Mean number of pairwise differences[c]		Gene diversity[d]	
Origin[a]	All data	c.c.[e]	loci[b]	All data	c.c.	All data	c.c.
Manatee	32	19	47	7.974	11.404	0.101	0.144
Gadsden	38	11	15	0.939	3.164	0.012	0.040
Decatur (GA)	29	10	33	2.714	7.333	0.034	0.093
Seminole (GA)	7	4	11	3.143	5.500	0.040	0.070
Collier	1	1	0	N/A	N/A	N/A	N/A
Dade	2	2	3	3.000	3.000	0.038	0.038
Martin	6	3	7	3.733	4.667	0.047	0.059
St. Lucie	2	2	2	2.000	2.000	0.025	0.025
Arkansas (state)	1	1	0	N/A	N/A	N/A	N/A
Puerto Rico (commonwealth)	3	1	0	0.000	N/A	0.000	N/A

[a] Florida counties, except when indicated otherwise.
[b] Among a total of 79 RFLP loci.
[c] According to Tajima (26).
[d] According to Nei (20) and Tajima (25).
[e] Clone-corrected data.

Table 3. Mean number of pairwise differences and average gene diversity of restriction fragment length polymorphism (RFLP) haplotypes for isolates of *Fusarium oxysporum* f. sp. *lycopersici* VCG 0033 originating from Manatee County (FL) and from northern Florida/southwestern Georgia

	Sample size	Polymorphic	Pairwise differences[b]	Gene diversity[c]
Locality	All data [c.c.][d]	loci[a]	All data [c.c.]	All data [c.c.]
Manatee	32 [19]	47	7.974 [11.404]	0.101 [0.144]
N. Florida/ S.W. Georgia[e]	74 [22]	48	1.845 [5.801]	0.023 [0.073]

[a] Among a total of 79 RFLP loci.
[b] Mean number according to Tajima (26).
[c] According to Nei (20) and Tajima (25).
[d] Clone-corrected data.
[e] Includes samples from the counties of Gadsden (FL), Decatur (GA), and Seminole (GA).

DISCUSSION

Before the development of resistant tomato cultivars, *F. oxysporum* f. sp. *lycopersici* was a serious disease in Florida, nearly destroying tomato production in parts of Florida in 1899 and causing $500,000 worth of damage in 1903 (references in 29). Although a race 2 isolate was isolated from a greenhouse in Ohio as early as 1945 (1), the very first instance of race 2 causing economic losses in tomato cultivars having the *I* gene was described from Delray Beach (Palm Beach County) in Florida (24). Four years later, race 2 was also present in Manatee County (15). Individual crop losses in Florida fields associated with race 2 were estimated to be as high as 74%. In Florida, race 3 was first observed in 1982 (28). Surveys during that year detected wilt symptoms in 25 out of 91 commercial fields grown with cultivars with race 1 and 2 resistance in the counties of Manatee and the neighboring Hillsborough. Due to the documented widespread distribution in 1982, emergence of race 3 of *F. oxysporum* f. sp. *lycopersici* probably occurred prior to 1982. As disease incidence in some affected fields was high (28), one could have expected that race 3, too, was to become a widespread and serious impediment to tomato production in Florida. This was not the case, and incidence of tomato wilt caused by race 3 remained low.

An accurate assessment of the incidence of tomato wilt is difficult because disease symptoms caused by the predominant Florida lineage of the crown rot *F. oxysporum* f. sp. *radicis-lycopersici* VCG 0094 and *F. oxysporum* f. sp. *lycopersici* can be similar (22) and both pathogens can be present in the same field. In our study, for example, multiple plants with signs of *F. oxysporum* infection were obtained from 13 Florida tomato fields. Both pathogens were encountered in four of them (31%). These fields were located in the counties of Martin, St. Lucie, and Manatee.

A relatively large proportion of *F. oxysporum* isolates within the forma specialis *lycopersici* have been reported to belong to "single-member VCGs". Among 115 isolates of *F. oxysporum* f. sp. *lycopersici,* 50 isolates could not be grouped with other isolates and were classified either as single-member VCGs or as self-incompatible (10). Most of these isolates were later determined to be molecularly indistinguishable from isolates of VCG 0030 (11,12). Thus, it has been concluded that for *F. oxysporum* f. sp. *lycopersici*, VCG analysis may not be the best method to relate unknown field isolates to known groups (19). While this may hold true for VCGs 0030 to 0032, our data would suggest that VCG testing is currently rather straightforward and reliable for members of VCG 0033. All 120 isolates (one isolate could not be revived for VCG testing) that were molecularly affiliated with known members of VCG 0033 also were heterokaryon-compatible with testers DA-1/7 from parent DA-1 or MN-27/9 from parent MN-27 (22) and therefore were placed into VCG 0033. Single-member VCGs or self-incompatible isolates of *F. oxysporum* f. sp. *lycopersici* were not encountered in this study.

If we assume that VCG 0030 has been associated with tomato for an extended period of time, then the presence of molecularly related but incompatible isolates may be an indication of a certain "age" and a consequent divergence among isolates belonging to this clonal lineage. Divergence as an indication of evolution also becomes obvious when examining the evolutionary relationship between VCG 0030 and VCG 0032. These two VCGs are phylogenetically closely related (5,12,19), and the hypothesis that these two VCGs originated from a common ancestor has been validated by two studies (5,19). In both studies, limited vegetative compatibility between these two VCGs was observed if co-cultured for an extended period of time. Additional evidence for a relatively long association of VCG 0030 and tomato may be inferred by the fact that all three races are known from VCG 0030, indicative of adaptation of the fungal population to changes in the resistance spectrum of the host. Also, there is evidence that race 3 of VCG 0030 emerged independently in several locations. Independent emergence of race 3 in California was recently demon-

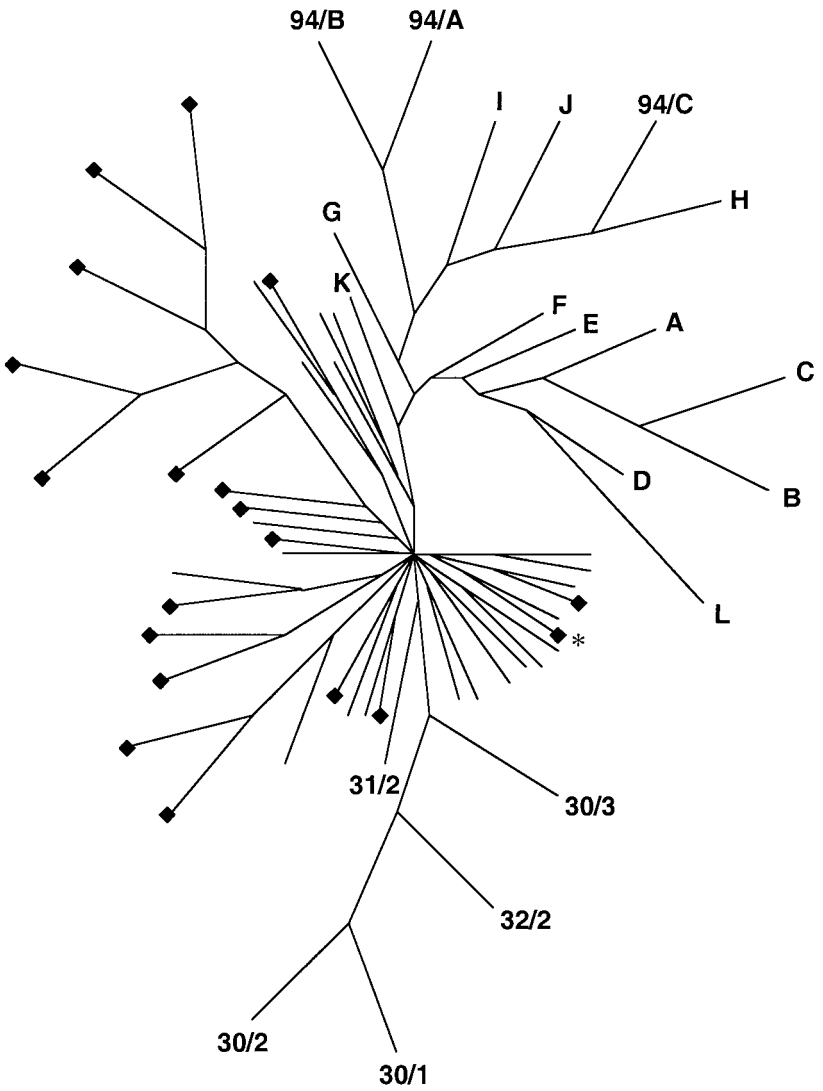

Fig. 2. Unrooted consensus tree based on restriction fragment length polymorphism (RFLP) haplotypes of *Fusarium oxysporum* f. sp. *lycopersici* isolates and other isolates of *F. oxysporum*. PHYLIP 3.6a2.1 (J. Felsenstein, Department of Genetics, University of Washington, Seattle) was used to generate 100 data sets. Bootstrapped data were then subjected to Wagner parsimony. The unrooted consensus tree was visualized using TREEVIEW (21). Haplotypes of VCG 0033 are not labeled, except for haplotypes identified from Manatee County (◆). The predominant haplotype 32 is indicated by *. Isolates of other VCGs of *F. oxysporum* f. sp. *lycopersici* are labeled with their VCG affiliation (without 00) and their race indicated after a slash. VCG 0094 of *F. oxysporum* f. sp. *radicis-lycopersici* is represented by three isolates, 94/A to 94/C. Nonpathogenic *F. oxysporum* isolates are labeled with letters A to L.

strated by Cai et al. (5). Historic race 3 isolates from Florida, while being members of VCG 0030, cluster in phylogenetic analyses with isolates from VCG 0032 (this study and 5). Mexican and Australian race 3 isolates also appear to be forming independent phylogenetic branches (5).

In contrast to these phenotypic and molecular characteristics displayed by VCG 0030, VCG 0033 appears to be of a more recent origin. All VCG 0033 isolates examined in this study were compatible with the tester strains, which would indicate a low level of divergence at loci determining vegetative compatibility. Also, races 1 and 2 so far have not been identified among VCG 0033 isolates (6,18). While our virulence tests identified all isolates as race 3, some isolates were apparently reduced in overall pathogenicity even with high levels of inoculum. This issue should be further researched using an increased number of replications in future experiments.

The observation that VCG 0033 seemingly consists only of race 3 would suggest that VCG 0033 came into existence at a time when tomato cultivars with race 1 and 2 resistance already were widely used. Still, it could be argued that the exclusive recovery of race 3 of VCG 0033 in this study could be related to sampling strategy. All samples of diseased plant material were obtained from commercially grown large-fruited tomato cultivars. Contemporary cultivars in general are resistant to races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* (14). While plum tomato cultivars generally also have this resistance spectrum, the prevalent Florida cherry tomato cultivars (e.g., Mountain Belle, Cherry Grande) are only resistant to race 1. As our collection did not include isolates from cherry tomatoes, there is a possibility that races other than 3 of VCG 0033 may be present in these production areas. Also, VCG 0030, which was not detected in our sample, may still be present, as either race, in tomato production areas without race 2 resistance. Examples of alternative production segments include production of heirloom tomatoes or private gardens. In fact, races other than race 3 recently have been identified in a study that isolated Fusaria from one conventional and one organic tomato field located close to Fort Pierce, St. Lucie County (2; D. Flavell, *personal communication*). Virulence testing of suspected *F. oxysporum* f. sp. *lycopersici* on differential cultivars determined 24 isolates as race 3, two as race 1, and three as race 2 (D. Flavell, *personal communication*). These isolates should be examined at least for VCG to further elucidate the population dynamics of *F. oxysporum* f. sp. *lycopersici* in Florida.

Our RFLP data analyses strongly support the origin of VCG 0033 of *F. oxysporum* f. sp. *lycopersici* as Manatee County. Not only was *F. oxysporum* f. sp. *lycopersici* VCG 0033 highly prevalent in our sample from Manatee, but also this population was by far the most diverse in all parameters measured here (polymorphic loci, pairwise differences between haplotypes, and gene diversity). Also, assuming that the ubiquitous haplotype 32 constitutes the basal, original haplotype of VCG 0033, from which all other haplotypes have been derived, and assuming that specific environmental factors did not differentially contribute to variability, then we can reason that a more diverse population would have been in existence longer than less diverse populations. Specifically, the population from Manatee had the lowest percentage of haplotype 32. Only 28.1% of isolates were classified as haplotype 32, while 76.4% of the remaining isolates in our collection pooled together were of this haplotype. Accordingly, the population differences in diversity measurements between the Manatee population and those from other counties are especially pronounced when all data are considered. But even when data analyses are based on clone-corrected data, which removes the effect of repeat sampling of haplotype 32, we still observe a substantial difference in diversity between the Manatee population and samples from other areas. An indication that the Manatee population has had more time to evolve is also obvious from the unrooted phylogenetic tree. While most haplotypes do not appear to be much genetically differentiated, several more evolved branches could be visualized. Figure 2 illustrates that haplotypes forming these more evolved branches are mostly from isolates from Manatee.

Tests for the presence of population structure were conducted using Arlequin ver. 2000 and F_{st} values were calculated that determine the level of population differentiation. These tests indicated a close relationship between isolates of the three northernmost counties (Gadsden, Seminole, and Decatur), with F_{st} values between 0.0024 and 0.0781 for all data and between –0.0324 and 0.0193 for clone-corrected data. Additional analyses compared population differentiation between the combined North population and the Manatee population. With all data considered, F_{st} was 0.1750, while the value of F_{st} for clone-corrected data was 0.1126. The low levels of population differentiation between the northern counties may be the result of recent colonization of the entire area by a single inoculum source of VCG 0033, but is more likely explained by aerial dissemination of inoculum at a regional level. It has been shown (16) that *F. oxysporum* f. sp. *lycopersici* can sporulate on stem surfaces, which would allow for aerial dissemination and therefore for migration of *F. oxysporum* f. sp. *lycopersici* VCG 0033 between tomato production fields. The moderate level of population differentiation between Manatee and the northern counties may indicate that frequent migration over this distance (ca. 450 km) does not take place.

In summary, Florida appears to be a center of origin and diversity and an evolutionary hotspot for *F. oxysporum* on tomato. A previous study examining *F. oxysporum* f. sp. *radicis-lycopersici* on tomato (22) identified two previously unknown VCGs, 0098 and 0099. In addition, population genetic analysis of VCG 0094, which was known previously only from northwestern Europe, implied that VCG 0094 originated in Florida. In *F. oxysporum* f. sp. *lycopersici*, race 2 was first observed in the state of Florida in the early 1960s in the counties of Palm Beach (24) and Manatee (15). Florida was also among the first locations where race 3 has been reported (28). In this study, we suggest that VCG 0033 of *F. oxysporum* f. sp. *lycopersici* originated in Manatee County and eventually moved to northern Florida, southwestern Georgia, Arkansas, and North Carolina. Its presence among samples from Puerto Rico suggests further and ongoing dissemination.

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LITERATURE CITED

1. Alexander, L. J., and Tucker, C. M. 1945. Physiological specialization in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici*. J. Agric. Res. 70:303-313.
2. Bao, J., Fravel, D., Lazarovitz, G., Chellemi, D., van Berkum, P., and O'Neill, N. 2001. Population structure of *Fusarium oxysporum* in tomato production in Florida. (Abstr.) Phytopathology 91:S5.
3. Bohn, G. W., and Tucker, C. M. 1939. Immunity to Fusarium wilt in the tomato. Science 89:603-604.
4. Bost, S. C. 2001. First report of *Fusarium oxysporum* f. sp. *lycopersici* race 3 on tomato in Tennessee. Plant Dis. 85:802.
5. Cai, G., Gale, L. R., Schneider, R. W., Kistler, H. C., Davis, R. M., Elias, K. S., and Miyao, E. M. Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. Phytopathology 93:1014-1022.
6. Chellemi, D. O., and Dankers, H. A. 1992. First report of *Fusarium oxysporum* f. sp. *lycopersici* race 3 on tomato in northwest Florida and Georgia. Plant Dis. 76:861.
7. Cirulli, M., and Alexander, L. J. 1966. A comparison of *Fusarium oxysporum* f. sp. *lycopersici* and different sources of resistance. Phytopathology 56:1301-1304.
8. Davis, R. M., Kimble, K. A., and Farrar J. J. 1988. A third race of *Fusarium oxysporum* f. sp. *lycopersici* identified in California. Plant Dis. 72:453.
9. Eck, R. V., and Dayhoff, M. O. 1966. Atlas of Protein Sequence and Structure. National

Biomedical Research Foundation, Silver Spring, MD.
10. Elias, K. S., and Schneider, R. W. 1991. Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici*. Phytopathology 81:159-162.
11. Elias, K. S., and Schneider, R. W. 1992. Genetic diversity within and among races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici* as determined by isozyme analysis. Phytopathology 82:1421-1427.
12. Elias, K. S., Zamir, D., Lichtman-Pleban, T., and Katan, T. 1993. Population structure of *Fusarium oxysporum* f. sp. *lycopersici*: Restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility is an indicator of evolutionary origin. Mol. Plant-Microbe Interact. 6:565-572.
13. Grattidge, R., and O'Brien, R. G. 1982. The occurrence of a third race of Fusarium wilt of tomato in Queensland. Plant Dis. 66:165-166.
14. Hochmuth, G. J., Maynard, D. N., Vavrina, C. S., Stall, W. M., Kucharek, T. A., Stansly, P. A., Taylor, T. G., Smith, S. A., and Webb, S. E. 2001. Tomato production in Florida. Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Online, publication HS 739.
15. Jones, J. P., and Litrell, R. H. 1965. Another appearance in Florida of a wilt Fusarium pathogenic to race 1-resistant tomato varieties. Plant Dis. Rep. 49:536-537.
16. Katan, T., Shlevin, E., and Katan, J. 1997. Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. Phytopathology 87:712-719.
17. Kluge, A. G., and Farris, J. S. 1969. Quantitative phyletics and the evolution of anurans. Systematic Zool. 18:1-32.
18. Marlatt, M. L., Correll, J. C., and Kaufmann, P. 1996. Two genetically distinct populations of *Fusarium oxysporum* f. sp. *lycopersici* race 3 in the United States. Plant Dis. 80:1336-1342.
19. Mes, J. J., Weststeijn, E. A., Herlaar, F., Lambalk, J. J. M., Wijbrandi, J., Haring, M. A., and Cornelissen, B. J. C. 1999. Biological and molecular characterization of *Fusarium oxysporum* f. sp. *lycopersici* divides race 1 isolates into separate virulence groups. Phytopathology 89:156-160.
20. Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
21. Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comp. Appl. Biosci. 12:357-358.
22. Rosewich, U. L., Pettway, R. E., Katan, T., and Kistler, H. C. 1999. Population genetic analysis corroborates dispersal of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida to Europe. Phytopathology 89:623-630.
23. Scott, J. W., and Jones, J. P. 1995. Fla. 7547 and Fla. 7481 tomato breeding lines resistant to *Fusarium oxysporum* f. sp. *lycopersici* races 1, 2, and 3. HortScience 30:645-646.
24. Stall, R. E. 1961. Development of *Fusarium* wilt on resistant varieties of tomato caused by a strain different from race 1 isolates of *Fusarium oxysporum* f. sp. *lycopersici*. Plant Dis. Rep. 45:12-15.
25. Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437-460.
26. Tajima, F. 1993. Measurement of DNA polymorphism. Pages 37-59 in: Mechanisms of Molecular Evolution. Introduction to Molecular Paleopopulation Biology. N. Takahata and A. G. Clark, eds. Sinauer Associates, Sunderland, MA.
27. Valenzuela-Ureta, J. G., Lawn, D. A., Heisey, R. F., and Zamudio-Guzman, V. 1996. First report of Fusarium wilt race 3, caused by *Fusarium oxysporum* f. sp. *lycopersici*, of tomato in Mexico. Plant Dis. 80:105.
28. Volin, R. B., and Jones, J. P. 1982. A new race of Fusarium wilt of tomato in Florida and sources of resistance. Proc. Fla. State Hortic. Soc. 95:268-270.
29. Walker, J. C. 1971. *Fusarium* Wilt of Tomato. Monogr. 6. American Phytopathological Society, St. Paul, MN.