Local and Regional Variation in Populations of *Fusarium oxysporum* from Agricultural Field Soils

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ABSTRACT

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Populations of *Fusarium oxysporum* were sampled from two agricultural fields in Maryland. Among 197 isolates, 56 vegetative compatibility groups (VCGs) and 25 mitochondrial DNA (mtDNA) haplotypes were identified. Fifty-four VCGs, representing 97% of the VCG diversity and 92% of the mtDNA haplotypes, were found among 78 isolates that were not pathogenic to muskmelon. The remaining two VCGs included 115 isolates of *F. o. melonis*: 40 isolates in VCG 0131 (races 0, 1, and 2) and 75 in 0134 (races 1 and 1,2). This is the first report from North America of race 1,2, for which there is presently no resistance in muskmelon. The two VCGs associated with virulent isolates also included four nonpathogenic isolates. Only one VCG and four mtDNA haplotypes overlapped between the two locations sampled. Sixty nonpathogenic VCGs of *F. oxysporum* previously identified from the San Joaquin Valley of California, were paired with representative isolates of the 54 VCGs from Maryland; only one VCG was shared between these populations. Based on parsimony analysis of the mtDNA data, the Maryland nonpathogens were more closely related to *F. o. melonis* than to the California nonpathogens. One nonpathogen shared the mtDNA haplotype of VCG 0131 but lacked pathogenicity to muskmelon. The other nonpathogen within VCG 0131 and two in VCG 0134 differed in mtDNA haplotype from the pathogens within these groups and were not closely related to them, except for their shared vegetative compatibility phenotype. Two nonpathogen VCGs from Maryland and eight from California shared the same mtDNA haplotype as VCG 0131, but none shared the mtDNA haplotype associated with VCG 0134. This result may indicate that VCG 0131 has resided longer in North America than VCG 0134. Based on mtDNA, both pathogen VCGs were more closely related to a nonpathogen VCG than to each other. Therefore, isolates of *F. o. melonis* do not form a distinct phylogenetic group as the forma specialis designation implies. However, the recent appearance of the new races in North America is most likely the result of an introduction from another continent and not the result of evolution of isolates associated with VCG 0131 or indigenous nonpathogens into new races of *F. o. melonis*.

Additional keywords: Fusarium wilt.

Fusarium oxysporum Schlechtend.:Fr. is a soilborne fungus that includes both nonpathogenic strains and economically important plant pathogens. Nonpathogenic strains colonize the cortex of plant roots without causing disease symptoms and can survive in nonliving organic matter. Pathogenic strains of *F. oxysporum* colonize roots of nonhosts in competition with nonpathogenic strains (8,12) but also can move past the cortical tissue and invade the vascular tissue of susceptible hosts, causing wilting. Possibly due to competition, nonpathogenic strains reduce disease severity caused by pathogenic strains (1,33). The distribution of *F. oxysporum* is cosmopolitan, but most pathogenic strains have more limited distributions. Agricultural practices likely play a major role in the dispersal of pathogenic strains. Despite the lack of observed sexual reproduction, extensive genetic diversity exists within *F. oxysporum*, especially among the nonpathogens.

Although *F. oxysporum* has a wide host range, individual strains are specialized parasites on a limited number of host species, and specialized race relationships are known (3). Host range has been used to subdivide this species into host-specific groups designated formae speciales. Other genetic markers such as isozymes (4), vegetative compatibility groups (VCGs) (6,14, 15,28), polymorphisms in mitochondrial DNA (mtDNA) (16,18, 19), and nuclear DNA polymorphisms (7,20,34) have provided further support for some formae speciales designations. Within certain formae speciales, including *F. o. cubense* (29), *F. o. lycopersici* (6), and *F. o. melonis* (14,15), race may not be correlated with VCG or mtDNA haplotype. Wilt diseases caused by *F. oxysporum* are often controlled through the use of resistant cultivars. For this reason, a better understanding of the dynamics of pathogen populations may enhance our ability to manage Fusarium wilt diseases.

The subject of the present study is *F. o. melonis* W.C. Snyder & H.N. Hansen, a wilt pathogen of muskmelon (*Cucumis melo* L.). Fusarium wilt of muskmelon was first described in North America in 1938 (23). Based on two disease resistance genes, *Fom1* and *Fom2*, four races have been described (0, 1, 2, and 1,2) (32). Eight VCGs, each associated with one or more races, have been described for this forma specialis (14,17). Race 2 alone was found in North America until 1985 when race 1 was reported in Maryland (5) and race 0 in Texas (26).

The objectives of this research were to characterize the relationships between pathogenic races of *F. o. melonis* and co-occurring nonpathogenic strains of *F. oxysporum* in two agricultural fields in Maryland and to compare the nonpathogen population from Maryland to the nonpathogen population from California. Pathogenicity, vegetative compatibility, and polymorphisms in mtDNA were used to characterize isolates and analyze their interrelationships. A preliminary report on this research has been published (2).

MATERIALS AND METHODS

Soil isolation. *F. oxysporum* populations were sampled in 1989 from soil collected at two locations in Maryland separated by approximately 75 km. These fields were chosen because multiple races of *F. o. melonis* were known to occur there. One location was a commercial field (Caroline County, MD) where muskmelon was grown in rotation with other crops. The other location was a University of Maryland Experiment Station (Wicomico County) where muskmelon was cropped on an annual basis. The experiment station was known to have high levels of *F. o. melonis* and was used to screen muskmelon varieties for resistance to

Fusarium wilt. At each location, 10 2- to 3-kg soil samples were randomly selected at a depth of 10–25 cm in an area approximately 100 m^2. A total of 20 soil samples were air-dried and stored at 10 C (11).

F. oxysporum isolates were obtained directly from each soil sample by dilution plating onto Komada's selective medium (21) and incubating for 2 wk (9). Five discrete colonies, tentatively identified as *F. oxysporum*, were replated onto Komada's selective medium. Single-spore subcultures were subsequently confirmed as *F. oxysporum* on carnation-leaf agar (27).

Five additional isolates from each sample were collected from the roots of melon plants (*C. melo* cv. Top Mark) grown in the collected soil. Plants were grown for 2 wk in a controlled-environment growth chamber with a 14 h of light/10 h of dark cycle and a temperature of 25:18 C (light/dark). After 2 wk, roots from these plants were harvested, washed, and plated onto Komada's selective medium (12); colonies were selected and identified as previously described.

Pathogenicity testing. All isolates were tested for pathogenicity on melon by a root-dip assay (14). Eleven-day-old muskmelon seedlings (Top Mark) were transplanted after trimming roots longer than 2 cm and dipping the injured roots into a conidial suspension of *F. oxysporum* (5×10^5 spores per milliliter) for 1 min. Water-dipped controls also were included in this test. Plants were rated for chlorosis and wilt symptoms after 1 and 2 wk in the greenhouse. Isolates pathogenic to melon cultivar Top Mark were retested on two other muskmelon cultivars (Perlita and CM17-187) to identify the race (32).

Vegetative compatibility. Nitrate utilization (*nit*) mutants were generated from all isolates, and phenotypes were determined as described by Gordon and Okamoto (9), a variation of the standard procedure originally described for *F. oxysporum* (30). Complementary mutants were paired on a minimal medium containing nitrate as the sole source of nitrogen. Pairing reactions were rated as positive if aerial mycelium was produced on the border between two complementing mutants (25). As isolates were assigned to VCGs, complementary mutants were selected from each group to serve as tester pairs for screening the remaining isolates.

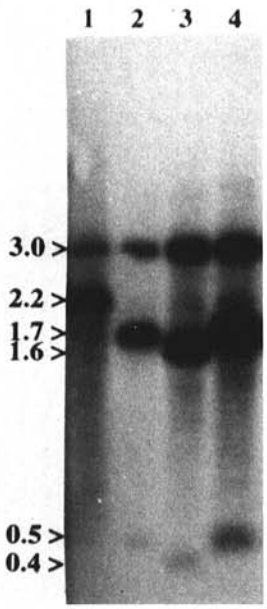

Fig. 1. An autoradiograph showing total DNA digested with *Hae*III and probed with a 2.9-kb fragment (pFOM-5) cloned from the mitochondrial genome of *Fusarium oxysporum* f. sp. *melonis* (lane 1). Lanes 2–4 contain DNA from nonpathogenic *F. oxysporum* isolated from Maryland soils. The probe hybridized with a 3.0-kb fragment in all isolates shown. Relative to the *F. o. melonis* reference strain, isolates in lanes 2–4 have an additional *Hae*III site in the 2.2-kb fragment. Additional differences are evident for the isolate shown in lane 3; they were interpreted as independent 0.1-kb deletions from each new fragment.

Representative isolates of all Maryland VCGs were paired with 60 tester pairs representing nonpathogenic strains of *F. oxysporum* from California.

Mitochondrial DNA. DNA was extracted from the *F. oxysporum* isolates by the method of Lee et al (24), as modified by Jacobson and Gordon (16). Total DNA was digested with either *Hae*III or *Pst*I restriction enzymes and probed sequentially with each of eight cloned *Pst*I fragments, representing approximately 95% of the mitochondrial genome of *F. o. melonis* (16). Differences in mtDNA were identified by comparison to the reference strain, from which the cloned DNA was obtained. Differences (as shown in Fig. 1) were explained by the minimum number of changes that could account for the observed patterns. Each character consisted of a postulated restriction site or length change. Presence or absence of a character was designated by 1 or 0, respectively for each group. Data were analyzed by parsimony analysis (PAUP 3.0; D. L. Swofford, Illinois Natural History Survey, Champaign) to describe relationships among these isolates.

RESULTS

Isolate collections. A total of 197 *F. oxysporum* isolates were collected from two agricultural fields. The commercial field collection included a total of 50 isolates sampled directly from the soil and 49 isolates from the roots. In the experiment station collection, 48 isolates were from soil, and 50 isolates were from roots. Both root and soil samples were taken to avoid a possible sampling bias in the event that some strains were restricted either to the roots or to the soil.

Pathogenicity. Of 197 isolates of *F. oxysporum* tested for pathogenicity on muskmelon, 115 (58%) were identified as *F. o. melonis*. Among these melon pathogens, all known races were represented (0, 1, 2, and 1,2) (Table 1). The other 82 (42%) isolates failed to induce disease after 2 wk and were regarded as nonpathogenic.

Vegetative compatibility. Based on pairing complementary *nit* mutants, 56 VCGs were identified among 197 isolates of *F. oxysporum*. The two largest VCGs represented the pathogen *F. o. melonis*, VCG 0134 (77 isolates) and VCG 0131 (42 isolates), both described previously (14,15). Within each pathogen VCG, we paired 697 (VCG 0131) and 2,294 (VCG 0134) combinations of *nit* mutants. Some isolates did not pair with all other isolates in the same VCG or did so in a weak manner. Similar infidelity of vegetative compatibility within a VCG has been reported elsewhere (29). This could be due to a lack of complementation between certain combinations of mutants (10), or it may reflect

TABLE 1. Isolates of *Fusarium oxysporum* f. sp. *melonis* from Maryland soil

	Commercial field[b]		Experiment station[c]			
VCG[a]	Root	Soil[f]	Root	Soil	mtDNA[d] haplotype	Pathogenicity and race[e]
0131	0	0	17	19	MO	Race 2
	0	0	0	1	MR	Race 1
	0	0	0	3	MO	Race 0
	0	0	0	1	MO	Nonpathogen
	1	0	0	0	MY	Nonpathogen
0134	24	16	23	10	MR	Race 1
	0	2	0	0	MR	Race 1,2
	0	1	0	0	ML	Nonpathogen
	0	0	1	0	MP	Nonpathogen

[a] Vegetative compatibility group.
[b] Caroline County, MD.
[c] University of Maryland, Wicomico County.
[d] Each mitochondrial DNA (mtDNA) haplotype represents a unique combination of characters (postulated length or site changes) relative to the *F. o. melonis* reference strain.
[e] Pathogenicity and race were determined using three differential cultivars in a root-dip assay.
[f] Isolates were collected directly from soil dilution plates or from the roots of muskmelon plants grown in this soil for 2 wk.

as yet undescribed complexities in the genetic basis for vegetative compatibility.

The distribution of the pathogen with respect to VCG and race was uneven between the two fields (Table 1). In the experiment station field, where melon was grown every year, *F. o. melonis* represented 64% of the population and included representatives from both VCGs and races 0, 1, and 2. In contrast, only 36% of the isolates found in the commercial field were pathogenic to muskmelon. All *F. o. melonis* isolates from this field were race 1 or 1,2 and associated with VCG 0134. Two nonpathogenic isolates that were vegetatively compatible with isolates in pathogen VCGs were found in each field.

The race structure within pathogen VCGs was complex (Table 1). All known races of *F. o. melonis* have been found in VCG 0134 (14,15), but the present collection of this VCG included only race 1 and 1,2 isolates. This represents the first report of race 1,2 in North America. Previously, race 2 alone was associated with VCG 0131, so it was significant to find race 0 and 1 isolates also associated with this VCG.

Of the 56 VCGs in our collection, 54 included only nonpatho-

TABLE 2. Mitochondrial DNA (mtDNA) haplotypes representative of the *Fusarium oxysporum* populations from California and Maryland used in the phylogenetic analysis

Clone[a]	Character[b]	mtDNA haplotype[c]												
		A	C	N	K	MC	ME	MG	MK	ML	MO	MR	MP	MY
pFOM-1	No change	0	0	1	1	0	1	1	0	1	1	1	0	0
pFOM-1	+*Hae*III site A	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-1	+*Pst*I site	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-1	+*Hae*III site B	0	1	0	0	0	0	0	0	0	0	0	0	0
pFOM-1	+*Hae*III site C	0	0	0	0	0	0	0	1	0	0	0	1	1
pFOM-1	+*Hae*III site D	0	0	0	0	1	0	0	0	0	0	0	0	0
pFOM-2a	No change	0	0	0	0	0	1	0	0	1	1	0	0	1
pFOM-2a	+0.2 kb insert	1	1	1	1	0	0	0	0	0	0	0	0	0
pFOM-2a	+*Pst*I site A	1	1	0	1	0	0	0	0	0	0	0	0	0
pFOM-2a	+3.7 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-2a	−3.25 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-2a	+0.7 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-2a	+1.5 kb	0	1	1	1	0	0	0	0	0	0	0	0	0
pFOM-2a	+1.1 kb	0	0	1	0	1	0	0	0	0	0	0	0	0
pFOM-2a	+1.2 kb	0	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-2a	+*Hae*III site	0	0	0	0	0	0	0	0	0	0	1	0	0
pFOM-2a	+0.9 kb	0	0	0	0	0	0	1	1	0	0	0	1	0
pFOM-2a	+0.1 kb	0	0	0	0	0	0	1	1	0	0	0	1	0
pFOM-2a	+*Pst*I site B	0	0	0	0	0	0	0	0	0	0	0	1	0
pFOM-2a	+*Pst*I site C	0	0	0	0	0	0	1	0	0	0	0	1	0
pFOM-2b	No change	0	0	0	0	0	0	0	0	0	1	0	0	0
pFOM-2b	−0.4 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-2b	+*Pst*I site	1	0	0	0	0	0	0	0	1	0	0	0	0
pFOM-2b	−1.0 kb	0	1	0	1	0	0	0	0	0	0	0	0	0
pFOM-2b	+1.1 kb	0	1	0	1	0	0	0	0	0	0	0	0	0
pFOM-2b	−*Hae*III site	0	0	0	0	0	1	1	1	1	0	1	1	1
pFOM-2b	+*Hae*III site	0	0	0	0	0	0	1	1	0	0	0	1	0
pFOM-2b	+1.0 kb	0	0	0	0	0	0	1	0	0	0	0	1	0
pFOM-3	No change	0	1	0	1	0	1	0	1	1	1	1	1	1
pFOM-3	+0.15 kb	1	0	1	0	0	0	0	0	0	0	0	0	0
pFOM-3	+0.25 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-3	−0.3 kb	0	0	1	0	0	0	0	0	0	0	0	0	0
pFOM-3	+14 kb	0	0	1	0	0	0	0	0	0	0	0	0	0
pFOM-3	+7.6 kb	0	0	1	0	1	0	1	0	0	0	0	0	0
pFOM-3	+*Pst*I site	1	0	1	0	0	0	0	0	0	0	0	0	0
pFOM-4	No change	0	1	1	1	0	1	0	1	1	1	1	1	1
pFOM-4	+*Pst*I site	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-4	−1.35 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-4	MC4-1S	0	0	0	0	1	0	0	0	0	0	0	0	0
pFOM-4	MC9-8S	0	0	0	0	0	0	1	0	1	0	0	0	0
pFOM-5	No change	0	0	0	0	0	0	0	0	0	1	0	0	1
pFOM-5	−*Pst*I site	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-5	−0.35 kb	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-5	+*Hae*III site A	0	1	0	1	0	0	0	0	0	0	0	0	0
pFOM-5	+*Hae*III site B	0	1	0	1	0	0	0	0	0	0	0	0	0
pFOM-5	−0.1 kb	0	0	0	0	0	0	1	1	0	0	0	0	0
pFOM-5	−0.1 kb	0	0	0	0	0	0	1	1	0	0	0	0	0
pFOM-5	+*Hae*III site C	0	0	0	0	0	1	1	1	1	0	1	1	0
pFOM-5	+*Pst*I site	0	0	0	0	0	0	1	0	0	0	0	0	0
pFOM-6	No change	0	1	0	1	0	1	0	0	1	1	1	1	1
pFOM-6	+*Hae*III site	1	0	0	0	0	0	0	0	0	0	0	0	0
pFOM-6	+1.0 kb	0	0	0	0	1	0	1	0	0	0	0	0	0
pFOM-6	−0.1 kb	0	0	0	0	0	0	0	1	0	0	0	0	0
pFOM-7	No change	0	1	0	1	0	1	0	1	1	1	0	1	1
pFOM-7	−0.1 kb	1	0	0	0	0	0	1	0	0	0	1	0	0
pFOM-7	+*Pst*I site	0	0	0	0	0	0	0	0	0	0	1	0	0

[a] Each clone corresponds to a different *Pst*I fragment, representing 95% of the mitochondrial genome of a California isolate of *F. o. melonis*.
[b] Each character state represents a postulated restriction site or length change relative to the reference strain.
[c] Each mtDNA haplotype represents a unique combination of characters (postulated length or site changes) relative to the *F. o. melonis* reference strain.

genic isolates, and these accounted for 97% of the VCG diversity but only 40% of the total population. The greatest number of isolates associated with a nonpathogen VCG was five; 39 VCGs, the majority, were represented by single isolates. Nineteen (32%) of the nonpathogen VCGs were found in the experiment station field, whereas 40 nonpathogen VCGs (70%) were found in the commercial field. Only one nonpathogen VCG was common to both fields (separated by approximately 75 km). Populations were compared on a continental scale by pairing the 60 nonpathogen VCGs from California with the 54 nonpathogen VCGs from Maryland. Two mutant combinations were tested for each pair of VCGs. Only one VCG was shared between these two populations. Therefore, 114 nonpathogen VCGs have been described from California and Maryland.

mtDNA. Representative isolates from each VCG were examined for polymorphisms in mtDNA relative to a reference strain of *F. o. melonis* (16). Postulated insertions, deletions, or site changes in the mtDNA relative to the reference strain were treated as characters and scored as present (1) or absent (0). A total of 33 characters was described for isolates from the Maryland population. Isolates with the same combination of characters were regarded as the same mtDNA haplotype; a total of 25 haplotypes was identified. Using the character state data, relationships among the 25 mtDNA haplotypes from Maryland and four haplotypes from California were described by parsimony analysis. Fifty-six characters were applicable to the combined data sets (Table 2).

The most parsimonious unrooted phylogenetic tree had a length of 97. A bootstrap 50% majority-rule consensus tree of relationships among the 29 mtDNA haplotypes is shown in Figure 2. The length of each branch is proportionate to the number of characters that change between the nodes and (or) termini to which the branch is connected. Isolates within the boxed region of the tree (Fig. 2) were separated by branches with bootstrap values less than 50%. Seventeen mtDNA haplotypes associated with nonpathogens found within the boxed region of the tree were left out for clarity (Fig. 2). Overall, the nonpathogens from Maryland were clustered near the *F. o. melonis* haplotypes and were not closely related to the California nonpathogens.

The *F. o. melonis* mtDNA haplotypes described previously were conserved within each VCG (16), but this was not true for the Maryland population. Nonpathogenic isolates associated with the pathogen VCGs did not share the mtDNA haplotype of the pathogens in that VCG (Table 1). Two nonpathogens that had closely related mtDNA haplotypes (ML and MY) (Fig. 2) were each associated with a different pathogen VCG. One nonpathogenic isolate that had mtDNA haplotype MP was associated with VCG 0134 but was equally unrelated to both pathogen VCGs based on differences in mtDNA (Fig. 1). Another isolate was linked to *F. o. melonis* by shared VCG and mtDNA haplotype (MO), but it lacked pathogenicity to muskmelon. Only a single race 1 isolate was associated with VCG 0131 (Table 1); this isolate did not have the MO mtDNA haplotype typical of VCG 0131 and instead shared the MR mtDNA haplotype of race 1 isolates associated with VCG 0134.

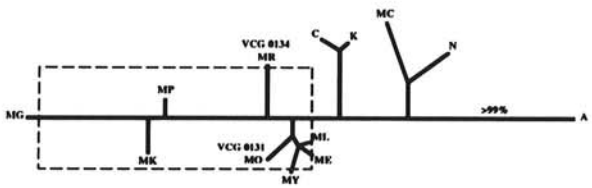

Fig. 2. An unrooted phylogenetic tree describing the relationships among 29 mitochondrial DNA (mtDNA) haplotypes of *Fusarium oxysporum*. Letters correspond to the haplotypes described in Table 2. The length of each branch is proportionate to the number of characters that change between the nodes and termini to which the branch is connected. Haplotypes designated A, C, K, and N correspond to isolates from California, and all other haplotypes correspond to isolates from Maryland. Isolates shown within the boxed region of the tree were separated by branches with bootstrap values less than 50%. Seventeen other mtDNA haplotypes not shown also were found within the boxed region but were left out of the tree for clarity.

Of the 25 mtDNA haplotypes found in Maryland, 23 were associated with nonpathogenic *F. oxysporum* (Table 3). The most common mtDNA haplotype (ME) represented 20 isolates in 10 VCGs and was found in both fields. All other mtDNA haplotypes were associated with less than eight isolates and fewer than six VCGs. Twelve mtDNA haplotypes were uniquely associated with a single isolate and VCG. Nonpathogenic isolates shared the same mtDNA haplotype as pathogens associated with VCG 0131, but they were not vegetatively compatible with isolates in this VCG. No nonpathogens shared the same mtDNA haplotype as pathogens associated with VCG 0134.

mtDNA haplotypes associated with nonpathogens were distributed unevenly between the two fields sampled. More mtDNA haplotype diversity was evident in the commercial field (14 haplotypes) than in the experiment station field (5 haplotypes). Four nonpathogen mtDNA haplotypes occurred in both fields. Thus, some mtDNA haplotypes were widely distributed in this region, relative to the more localized distribution of VCGs.

DISCUSSION

Isolates pathogenic to muskmelon represented 60% of the 197 isolates collected for this study, but nonpathogenic isolates accounted for 92% of the VCG diversity and 86% of the diversity in mtDNA haplotypes. Among the nonpathogens, only one VCG and four mtDNA haplotypes were common to both Maryland fields. Therefore, excluding the pathogens, these fields supported highly differentiated populations of *F. oxysporum*. Whether this difference can be attributed to the distance separating the two fields or differences in cropping practices cannot be determined. However, previous studies of *F. oxysporum* populations from both native and agricultural soils in California suggest that isolation by distance has an important effect on population structure in this species (11,13). The soilborne nature of this fungus probably limits long-distance dispersal of conidia, restricting gene flow and contributing to the development of discrete populations of the nonpathogens. In contrast, pathogenic strains have worldwide distributions probably due to dispersal with soil and seed and intense selection for virulent phenotypes.

There was overlap of only one nonpathogen VCG between Maryland and California, indicating we have identified a total of 114 VCGs in *F. oxysporum* (9–11), excluding those known to be pathogenic. Assuming that vegetative compatibility follows the one locus and two allele system found in *F. moniliforme* (31), a minimum of seven vegetative compatibility loci are necessary to account for the 114 VCGs already found in *F. oxysporum*.

Virulence diversity among isolates associated with the same VCG has previously been documented in *F. o. melonis* (14,15) as well as in other formae speciales of *F. oxysporum* (6,22,29). In Europe, all four known races of *F. o. melonis* are associated with VCG 0134. All four races also are now known to occur in North America. The recent appearances of races 1 and 1,2 are most likely the result of introductions from Europe, because both are associated with VCG 0134, which is common in France and relatively rare in North America.

In previous collections from Maryland (14,15), race 1 was associated only with VCG 0134, as were the majority of the race 1 isolates described in the present study (73/74). The single race 1 isolate associated with VCG 0131, may reflect an independent origin of this virulence phenotype. However, the race 1 isolate associated with VCG 0131 has a mtDNA haplotype that differs from the race 2 and 0 isolates in this VCG but is identical to isolates associated with VCG 0134. One possible explanation is that race 1 and 2 isolates are associated with VCG 0131 through convergence rather than common descent. That is, a race 1 isolate associated with VCG 0134, through somatic mutation, might have acquired the vegetative compatibility (VC) phenotype of 0131. Similarly, a mutation at a VC locus may have occurred in a California isolate of *F. o. melonis*, causing the conversion of VCG 0131 into VCG 0130; race and mtDNA haplotype are identical in each VCG, but the VC phenotype differs (16). However, the relatively small number of known pathogen VCGs

suggests that if mutations at VC loci do generate new VC phenotypes, it must be a relatively rare event. The asexual nature of *F. oxysporum* makes it difficult to address these questions, but finer measures of relatedness, such as genomic DNA fingerprinting or sequence data from a variable region of the nuclear or mitochondrial genome, could be examined to determine if they confirm a close relationship between isolates in VCGs 0131 and 0134.

Both VC and mtDNA indicate that some nonpathogens are closely related to isolates pathogenic to muskmelon. Overall, the nonpathogens found in Maryland are more closely related to the pathogen groups than to the nonpathogens from California. One nonpathogen, associated with both the same VCG and mtDNA haplotype as the pathogen, may be an avirulent mutant of *F. o. melonis* VCG 0131. Gaining pathogenicity appears to be rare in this population because a limited number of discrete pathogen groups have been identified, but loss of pathogenicity may occur more frequently.

Where nonpathogens are vegetatively compatible with pathogens but have different mtDNA, VCG may be shared due to convergence, as suggested for the race 1 isolate associated with VCG 0131. Alternatively, two isolates might have identical alleles at all loci affecting vegetative compatibility by coincidence, reflecting past sexual reproduction (30). A common mtDNA haplotype between pathogenic and nonpathogenic isolates that are vegetatively incompatible with each other has been reported previously (10). Shared mtDNA haplotypes would be expected in a population in which a significant proportion of the diversity could be directly traced to past sexual reproduction. Progeny from outcrossing would share the maternal mtDNA haplotype but generally would have different VC genotypes. If the genetic determinants of virulence to muskmelon are simple, it would not be surprising to find that some of the progeny were pathogens and some were not. Consequently, our data may indicate that sexual reproduction has occurred fairly recently in *F. oxysporum*. Because nonpathogens shared the same mtDNA haplotype as VCG 0131, but not VCG 0134, this might imply a longer residence time for VCG 0131 in North America, whereas isolates associated with VCG 0134 may have been introduced recently.

Based on the apparently close relationship between nonpathogenic strains of *F. oxysporum* and the pathogens associated with VCG 0131, we might conclude this VCG originated in the populations we have sampled and may have had an independent origin as a melon pathogen separate from the origin of VCG 0134. Alternatively, perhaps the nonpathogens from Maryland with mtDNA haplotypes closely related to *F. o. melonis* were introduced along with the pathogen from some ancestral location. However, in this case it would be difficult to explain why the

TABLE 3. Nonpathogenic isolates of *Fusarium oxysporum* from Maryland soils

VCG[a]	Isolate	Commercial field[b]		Experiment station[c]		mtDNA haplotype[d]	VCG[a]	Isolate	Commercial field[b]		Experiment station[c]		mtDNA haplotype[d]
		Roots	Soil	Roots	Soil[e]				Roots	Soil	Roots	Soil[e]	
1	MC1-4S	0	1	0	0	MA	27	D2-7R	1	0	0	0	MO
2	MC2-7S	0	1	0	0	MB	28	D3-4R	1	0	0	0	MN
3	MC4-1S	0	1	0	0	MC	29	D4-6R	1	0	0	0	MN
4	MC6-2S	0	1	0	0	MD	30	D5-6R	1	0	0	0	MN
5	MC6-10S	0	1	0	0	MB	31	D7-1R	1	0	0	0	MH
6	MC7-4S	0	1	0	0	MA	32	D7-7R	1	0	0	0	ML
7	MC7-8S	0	1	0	0	MA	33	D8-4R	1	0	0	0	MP
8	MC8-7S	0	1	0	0	ME	34	D9-5R	1	0	0	0	MJ
9	MC9-1S	0	1	0	0	MF		D4-1R	1	0	0	0	MJ
10	MC9-8S	0	1	0	0	MG	35	D2-4R	1	0	0	0	MH
11	MC9-9S	0	1	0	0	MI		D9-4R	1	0	0	0	MH
12	MC10-2S	0	1	0	0	MA	36	MC2-1S	0	1	0	0	MQ
13	MC10-3S	0	1	0	0	MK		MC10-8S	0	1	0	0	MQ
14	MC10-7S	0	1	0	0	ME		D8-8R	1	0	0	0	MQ
15	MC4-4S	0	1	0	0	MA		B7-7S	0	0	0	1	MQ
	MC5-4S	0	1	0	0	MA	39	B5-7S	0	0	0	1	ME
16	MC3-2S	0	1	0	0	MJ		B5-10S	0	0	0	1	ME
	MC8-1S	0	1	0	0	MJ	40	B10-4S	0	0	0	1	ME
	MC8-6S	0	1	0	0	MJ		B5-6S	0	0	0	1	ME
	MC5-9S	0	1	0	0	MJ		B10-2S	0	0	0	1	ME
17	MC3-5S	0	1	0	0	ME	41	B1-1S	0	0	0	1	ME
	MC5-10S	0	1	0	0	ME		B6-8S	0	0	0	1	ME
	MC6-1S	0	1	0	0	ME	42	B4-2S	0	0	0	1	MQ
	D3-8R	1	0	0	0	ME	43	B4-8S	0	0	0	1	MO
	D9-1R	1	0	0	0	ME	44	B5-8S	0	0	0	1	MJ
18	MC1-10S	0	1	0	0	MK	45	B7-2S	0	0	0	1	ME
	D8-9R	1	0	0	0	MK	46	B10-3S	0	0	0	1	ME
19	MC9-3S	0	1	0	0	MD	47	C5-3R	0	0	1	0	ME
	D7-2R	1	0	0	0	MD		C5-4R	0	0	1	0	ME
20	MC6-5S	0	1	0	0	ML		C5-5R	0	0	1	0	ME
	D6-5R	1	0	0	0	ML	48	C8-8R	0	0	1	0	MJ
21	MC8-4S	0	1	0	0	ML	49	C1-9R	0	0	1	0	MS
	D3-9R	1	0	0	0	ML	50	C8-2R	0	0	1	0	MT
22	MC1-5S	0	1	0	0	MJ	51	B3-6S	0	0	0	1	MU
	D9-2R	1	0	0	0	MJ	52	MC4-7S	0	1	0	0	MV
23	D1-3R	1	0	0	0	MA	53	MC5-6S	0	1	0	0	MQ
24	D1-5R	1	0	0	0	ME	54	C1-10R	0	0	1	0	MW
25	D1-8R	1	0	0	0	MM	55	C7-4R	0	0	1	0	MX
26	D2-6R	1	0	0	0	MN	56	C4-1R	0	0	1	0	MQ

[a]Vegetative compatibility group.
[b]Caroline County, MD.
[c]University of Maryland, Wicomico County.
[d]Each mitochondrial DNA (mtDNA) haplotype represents one or more isolates with a unique combination of characters (postulated length or site changes) relative to the *F. o. melonis* reference strain.
[e]Isolates were sampled directly from soil dilution plates or from the roots of muskmelon plants grown in the collected soil for 2 wk.

introduced nonpathogenic strains became such a prominent component of the population from Maryland.

The close relationship between pathogenic and nonpathogenic strains of *F. oxysporum* indicates that the forma specialis designation is not entirely congruent with genetic relationships in *F. o. melonis*. Nevertheless, virulence does appear to be restricted to a limited number of VCGs. We have no evidence that the appearance of *F. o. melonis* races in new locations is the result of the recent evolution of new pathogens from local populations of nonpathogenic strains. Rather, these occurrences most likely result from introductions of previously recognized pathogenic races from other locations or possibly the derivation of a new race from a preexisting race of the same forma specialis (17).

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