Characterization of a Regional Population of *Fusarium oxysporum* f. sp. *niveum* by Race, Cross Pathogenicity, and Vegetative Compatibility

X. G. Zhou and K. L. Everts

First author: University of Maryland, 27664 Nanticoke Road, Salisbury 21801; and second author: University of Maryland, Salisbury, with joint appointment with the University of Delaware, 16684 County Seat Highway, Georgetown 19947.
Accepted for publication 10 October 2006.

ABSTRACT

Zhou, X. G., and Everts, K. L. 2007. Characterization of a regional population of *Fusarium oxysporum* f. sp. *niveum* by race, cross pathogenicity, and vegetative compatibility. *Phytopathology* 97:461-469.

Eighty-eight isolates of *Fusarium oxysporum* f. sp. *niveum*, collected from wilted watermelon plants and infested soil in Maryland and Delaware, were characterized by cross pathogenicity to muskmelon, race, and vegetative compatibility. Four isolates (4.5%) were moderately pathogenic to ≥ 2 of 18 muskmelon cultivars in a greenhouse test, and one representative isolate also was slightly pathogenic in field microplots. The four isolates all were designated as race 2, and were in vegetative compatibility group (VCG) 0082. Of the 74 isolates to which a VCG could be assigned, 41 were in VCG 0080, the VCG distributed most widely; 27 were in VCG 0082, and were distributed in half of the 20 watermelon fields surveyed; and 6 were in the newly described VCG

0083, and were restricted to three fields. Among the isolates in VCG 0080, 8 were designated as race 0, 21 as race 1, and 12 as race 2. Of the isolates in VCG 0082, 6 were designated as race 0, 11 as race 1, and 10 as race 2. All isolates in VCG 0083 were designated as race 2. Isolates from more than one race within the same VCG or isolates from more than one VCG were recovered from single plants and fields. No differences in aggressiveness on differential watermelon cultivars were observed among isolates from different VCGs of the same race. A diverse association between virulence and VCG throughout the Mid-Atlantic region suggests that the pathotypes of *F. oxysporum* f. sp. *niveum* may be of local origin or at least long existent in the region.

Additional keywords: *Citrullus lanatus*, *Cucumis melo*, cross infection, genetic diversity.

Fusarium oxysporum Schlechtend.:Fr. is a cosmopolitan soilborne plant pathogen that causes vascular wilt and cortical rot diseases of more than 100 agricultural crops of economic importance (5). The fungus is morphologically indistinguishable, but divided into at least 80 different formae speciales, each with a high level of host specificity (3,52).

F. oxysporum f. sp. *niveum* (E. F. Sm.) W. C. Snyder & H. N. Hans. and *F. oxysporum* f. sp. *melonis* W. C. Snyder & H. N. Hans., the causal agents of Fusarium wilt of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and muskmelon (*Cucumis melo* L.), respectively, also show host specialization. Isolates of these formae speciales cause disease only on their respective hosts and not on any related cucurbit species, including cucumber (*C. sativus* L.) or squash (*Cucurbita* spp.) (2). Within the formae specialis *F. oxysporum* f. sp. *niveum*, isolates are further subdivided into three races (races 0, 1, and 2) on the basis of degree of aggressiveness on differential watermelon genotypes that vary in disease resistance (13,35,38,39,43,54). Race 0 only causes wilt on susceptible cultivars with no wilt resistance genes. Race 1 causes severe wilt on susceptible cultivars and slight to moderate wilt on most cultivars that are considered resistant to Fusarium wilt. Race 2 is highly aggressive and causes severe wilt on all known resistant cultivars.

Despite host specificity of the formae specialis, there are numerous reports of cross pathogenicity of individual isolates of *F. oxysporum* within cucurbits (6,14,18,41,42,46,47). An isolate

of *F. oxysporum* f. sp. *niveum* from watermelon in Florida was pathogenic to muskmelon (46,47), while an isolate of *F. oxysporum* f. sp. *cucumerinum* J. H. Owen (Fusarium wilt of cucumber) was pathogenic to both watermelon and muskmelon (46). A race 1 isolate of *F. oxysporum* f. sp. *niveum* from South Carolina was pathogenic to several cultivars of summer squash (41). An isolate of *F. oxysporum* f. sp. *niveum* and an isolate of *F. oxysporum* f. sp. *cucumerinum*, both from the Bahamas, were pathogenic to watermelon, muskmelon, and cucumber (42). Several isolates of *F. oxysporum* from wilted cucumber from the Netherlands also were pathogenic to all three cucurbit species (18). A close genetic relationship between formae speciales in cucurbits also has been demonstrated using restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) (29). These reports indicate that intraspecific variation in virulence may exist within each cucurbit-infecting formae speciales and that they may share common genetic determinants responsible for variation of virulence. However, the phenomenon of cross pathogenicity appears to occur only on inoculated seedlings in the laboratory and greenhouse, and there are no reports of cross pathogenicity under field conditions (19,38). Little is known about the frequency of the occurrence of cross pathogenicity within a given cucurbit-infecting forma specialis population of *F. oxysporum*. This information has a direct bearing on designing more effective crop rotation strategies for management of Fusarium wilt in cucurbits.

Analysis of genetic variation with vegetative compatibility and nucleic acid has become a useful tool to characterize populations of *F. oxysporum* within and between formae speciales or nonpathogenic forms of the fungus. Isolates that are able to form a heterokaryon are vegetatively compatible, and all compatible

Corresponding author: K. L. Everts; E-mail address: keverts@umd.edu

doi:10.1094/PHYTO-97-4-0461

© 2007 The American Phytopathological Society

isolates belong to a unique vegetative compatibility group (VCG). Using this approach, genetic diversity within more than 30 formaes speciales of *F. oxysporum* has been examined and over 125 VCGs identified (32). With the isolates of *F. oxysporum* f. sp. *niveum* characterized to date, three VCGs have been identified (35). All race 2 isolates were contained in the single VCG 0082, whereas race 1 isolates were found in VCG 0080 and VCG 0081. Race 1 isolates from various locations around the world were contained in VCG 0080, whereas only a select group of race 1 isolates from Florida were found to belong to VCG 0081. Analysis of the patterns of RFLP of mtDNA has identified six mtDNA RFLP groups within a total of 50 isolates of *F. oxysporum* f. sp. *niveum* (27). No corresponding relationship between race, geographical distribution, and genetic diversity measured by mtDNA RFLP was observed (26,27).

There is little information available about the geographic origin and evolution of races of *F. oxysporum* f. sp. *niveum*. Several evolutionary models to account for host and race specificity and their correlation to VCG have been proposed for the asexual fungus *F. oxysporum* (10,31). Alterations to host specificity and race could result from genetic mutation, recombination, and gene flow (9). One forma specialis (*F. oxysporum* f. sp. *niveum*) was changed to another forma specialis (*F. oxysporum* f. sp. *melonis*) (6) and a race 2 isolate of *F. oxysporum* f. sp. *niveum* was changed to a race 0 within the same VCG (25) through artificial mutation in the laboratory. A possible genetic exchange between *F. oxysporum* f. spp. *niveum* and *melonis* also has been reported (27). However, in an effort to gain a better understating of the geographic origin of *F. oxysporum* f. sp. *niveum*, it would be very helpful to evaluate the extent of diversity in genetics and virulence among isolates of a given population within the same plant, field, and region (30). A localized population of *F. oxysporum* f. sp. *niveum* exists on the Delmarva Peninsula, a 15,385-km² region comprising Delaware and the parts of Maryland and Virginia that are east of the Chesapeake Bay. Cucurbit crops such as watermelon and muskmelon have been cultivated in the region for at least 300 years (51). A survey of races and population levels of *F. oxysporum* f. sp. *niveum* was conducted in the part of Maryland situated on the peninsula and in Delaware (54). All three races of *F. oxysporum* f. sp. *niveum* were present and widely distributed throughout the region (53,54); all watermelon fields surveyed were infested with the pathogen and a majority of the fields had a range of inoculum densities between 100 to 1,200 CFU/g of soil (54).

The objectives of this study were to further characterize isolates of *F. oxysporum* f. sp. *niveum* from this region by cross pathogenicity to muskmelon, race, and vegetative compatibility. We wished to gain a better understanding of (i) cross pathogenicity of *F. oxysporum* f. sp. *niveum* to muskmelon under greenhouse and simulated field (microplots) conditions; (ii) VCG diversity and its correlation with race and geographic distribution at the levels of plant, field, and region; and (iii) the possible geographic origin of races within this forma specialis. A preliminary report of this research has been published (55).

MATERIALS AND METHODS

Fungal strains. In all, 100 isolates of *F. oxysporum* f. sp. *niveum* were used in this study. Eighty-eight isolates were recovered from infested soil and symptomatic watermelons grown throughout the major area of watermelon production in Maryland and Delaware, and all were single-spore cultured (54). The race of these isolates was identified and reported in a previous study (54). Nine additional isolates of *F. oxysporum* that were nonpathogenic to watermelon also were included. The origin of each isolate from plant, field, county, and state was recorded. Three reference isolates of *F. oxysporum* f. sp. *niveum* were included for virulence

and VCG comparison. A reference isolate (TX-471) of race 0 of *F. oxysporum* f. sp. *niveum*, which originated from Texas, was obtained from B. D. Bruton of the United States Department of Agriculture Southern Agriculture Research Laboratory in Lane, OK; and a reference isolate of each of race 1 (ATCC44293) from California (44,45) and race 2 (TX-X1D) from Texas (37) was obtained from The American Type Culture Collection (ATCC) in Manassas, VA. Race identity for these reference isolates was confirmed in a previous study (54), and the VCGs of ATCC44293 and TX-X1D were identified by Larkin et al. (35). VCG identity, race designation, and geographic origin of each isolate are listed in Table 1.

Cross-pathogenicity tests. Cross-pathogenicity tests were performed in the greenhouse using a root-dip technique (36,39). All isolates initially were tested for pathogenicity on the susceptible muskmelon cv. Topmark (susceptible to all known races of *F. oxysporum* f. sp. *melonis*) to separate pathogenic from non-pathogenic isolates to muskmelon. The pathogenic isolates then were tested for pathogenicity on 18 muskmelon cultivars (Table 2), representing a wide range of resistance. They also were tested on the three differential watermelon cvs. Sugar Baby, Charleston Gray, and Calhoun Gray (38,39). Inoculum was prepared from each isolate by harvesting microconidia from 10-day-old cultures grown in a liquid mineral salts medium (43) on a rotary shaker (80 rpm). Seedlings of each cultivar, when the first true leaf appeared, were root dipped for 30 s in an inoculum suspension of approximately 1.5×10^6 microconidia/ml and replanted in pots (7.5 by 7.5 cm) containing a pasteurized vermiculite/peat moss (2:3, vol/vol mixture) growth medium. Seedlings of each cultivar dipped in diluted, sterile mineral salts medium served as non-inoculated controls. There were three replicate pots of 13 plants each for each isolate-cultivar combination. Plants were maintained in a greenhouse at 20 to 31°C. The number of seedlings showing symptoms of Fusarium wilt (yellowing, stunting, or wilting) was counted 3 weeks later. Because stem colonization bioassay is a useful means to quantify watermelon resistance to Fusarium wilt (56,57), pathogen colonization in the lower stem tissue of inoculated seedlings also was evaluated. Three plants were randomly sampled from each pot 7 days after inoculation, and their lower stems (5 cm from the crown) were excised, collected, surface disinfected for 30 s in 5.5% NaOCl, and homogenized on a blender for 1 min. The homogenates were filtrated through eight layers of cheesecloth, diluted with sterile water, and plated on Komada's medium (34). There were two plates for each replicate pot and six plates for each isolate-cultivar combination. Colonies on the plates were counted 6 days later and counts converted to CFU/g of stem fresh weight. The experiment was conducted twice.

To evaluate cross pathogenicity to muskmelon under simulated field conditions, two representative isolates, F063-1 and F17B-R-2, were used to test for pathogenicity to muskmelon and watermelon in field microplots in summer 2003. The former was shown to be pathogenic to both muskmelon and watermelon in greenhouse tests, whereas the later was pathogenic only to watermelon. However, both were identified as race 2 and in VCG 0082. Nitrate-nonutilizing (*nit*) mutants of each isolate were generated and tested for pathogenicity to muskmelon and watermelon, their growth in soil, and their growth on potato dextrose agar (PDA). One representative mutant strain from each of the two isolates with characteristics similar to their respective wild-type progenitor isolates was used for infesting microplot soil. The field soil (Norfolk "A" loamy sand, pH 6.7) used in the microplots (76 cm in diameter and 61 cm deep) was fumigated with methyl bromide at ≈ 50 g/m² under black plastic film. The film was removed after 4 days, and the plots were left for aeration by turning the soil with a rake twice a week for 4 weeks before infesting with inoculum. Inoculum for each isolate was prepared as described earlier and added to the top 20 cm of soil. After 4 weeks, when inoculum

density in the soil stabilized at $\approx 1,032$ CFU/g soil as determined by soil plating on MMCPA selective medium (minimal medium amended with KClO_3 at 7.5 g/liter and supplemented with pentachloronitrobenzene [0.5 g a.i./liter], chloramphenicol [250 mg/liter], and 85% lactic acid [0.6 ml/liter]) for growth of chlorate-resistant (*nit*) strains (20), five 4-week-old plants of the wilt-susceptible muskmelon cv. Banana were transplanted in one-half of each microplot. Five 4-week-old plants of the wilt-susceptible watermelon cv. Sugar Baby were transplanted in the other half. Microplots not infested with any isolate inoculum served as controls. Four replicate microplots for each isolate-cultivar combination were arranged in a randomized complete block design. Fifteen days after transplanting, the identical experiment was repeated in additional adjacent microplots. Development of *Fusarium* wilt of muskmelon and watermelon was monitored weekly and the number of plants showing symptoms of *Fusarium* wilt (yellowing, stunting, or wilting) was counted 8 weeks after transplanting.

Pathogenicity of isolates recovered from infected plants in the greenhouse and microplots was determined. Representative *Fusarium* colonies on Komada's medium growing from infected stem tissue from greenhouse tests, and colonies on MMCPA medium from microplot-grown, diseased root and stem tissues were assayed for their pathogenicity on the muskmelon cv. Banana and the watermelon cv. Sugar Baby in the greenhouse as described previously (54).

Vegetative compatibility. Vegetative compatibility tests were performed following the method developed by Puhalla (49) and Correll et al. (12). *Nit* mutants were generated on both minimal salts medium (MM) and PDA containing 1.5% KClO_3 . When isolates were not restricted with 1.5% KClO_3 , the concentration of KClO_3 was increased to 3 or 5% (22). Portions of the fast-growing sectors around colonies were plated on MM and PDA. Those that produced thin expansive growth with no aerial mycelium on MM but showed wild-type growth with aerial mycelium on PDA were considered to be *nit* mutants. *Nit* mutants

TABLE 1. Isolates of *Fusarium oxysporum* f. sp. *niveum* from the Mid-Atlantic region and other areas used in this study

Isolate ^w	VCG ^x	Race ^y	Origin			Isolate ^w	VCG ^x	Race ^y	Origin		
			Field	County	State ^z				Field	County	State ^z
TX-471	80	0	...	...	TX	F005-1	82	0	1	Wicomico	MD
F034-1	80	0	6	Wicomico	MD	F029-2	82	0	5	Wicomico	MD
F053-1	80	0	8	Dorchester	MD	F088-2	82	0	16	Wicomico	MD
F053-6	80	0	8	Dorchester	MD	F104-1	82	0	20	Sussex	DE
F060-1	80	0	9	Dorchester	MD	F121-2	82	0	25	Caroline	MD
F081-1	80	0	14	Wicomico	MD	F17B-010	82	0	26	Wicomico	MD
F095-1	80	0	18	Sussex	DE	F016-1	82	1	4	Wicomico	MD
F116-1	80	0	23	Caroline	MD	F017-1	82	1	4	Wicomico	MD
F117-1	80	0	24	Caroline	MD	F019-1	82	1	4	Wicomico	MD
ATCC44293	80	1	...	...	CA	F021-3	82	1	4	Wicomico	MD
F014-1	80	1	4	Wicomico	MD	F022-2	82	1	4	Wicomico	MD
F015-1	80	1	4	Wicomico	MD	F024-1	82	1	4	Wicomico	MD
F021-1	80	1	4	Wicomico	MD	F028-1	82	1	5	Wicomico	MD
F021-2	80	1	4	Wicomico	MD	F086-1	82	1	16	Wicomico	MD
F022-1	80	1	4	Wicomico	MD	F088-1	82	1	16	Wicomico	MD
F027-1	80	1	5	Wicomico	MD	F107-1	82	1	21	Sussex	DE
F027-2	80	1	5	Wicomico	MD	F17B-R-5	82	1	26	Wicomico	MD
F029-1	80	1	5	Wicomico	MD	TX-X1D	82	2	...	...	TX
F029-3	80	1	5	Wicomico	MD	F014-2	82	2	4	Wicomico	MD
F030-1	80	1	5	Wicomico	MD	F061-2	82	2	10	Dorchester	MD
F049-1	80	1	7	Dorchester	MD	F063-1	82	2	10	Dorchester	MD
F049-2	80	1	7	Dorchester	MD	F063-2	82	2	10	Dorchester	MD
F079-1	80	1	13	Wicomico	MD	F070-1	82	2	10	Dorchester	MD
F080-1	80	1	13	Wicomico	MD	F097-2	82	2	19	Sussex	DE
F083-2	80	1	15	Wicomico	MD	F100-2	82	2	19	Sussex	DE
F099-2	80	1	19	Sussex	DE	F17B-R-6	82	2	26	Wicomico	MD
F100-1	80	1	19	Sussex	DE	F17B-R-8	82	2	26	Wicomico	MD
F120-3	80	1	25	Caroline	MD	F17B-R-2	82	2	26	Wicomico	MD
F17B-1-38	80	1	26	Wicomico	MD	F085-1	83	2	15	Wicomico	MD
F17B-1-6	80	1	26	Wicomico	MD	F085-2	83	2	15	Wicomico	MD
F17B-R-3	80	1	26	Wicomico	MD	F099-1	83	2	19	Sussex	DE
F032-1	80	2	6	Wicomico	MD	F17B-R-7	83	2	26	Wicomico	MD
F083-1	80	2	15	Wicomico	MD	F17B-1-25	83	2	26	Wicomico	MD
F17BR-004	80	2	26	Wicomico	MD	F17B-1-29	83	2	26	Wicomico	MD
F17B-1-3	80	2	26	Wicomico	MD	F013-1	NA	NP	3	Wicomico	MD
F17B-1-30	80	2	26	Wicomico	MD	F031-1	NA	NP	6	Wicomico	MD
F17BR-005	80	2	26	Wicomico	MD	F090-1	NA	NP	17	Sussex	DE
F17B-R-4	80	2	26	Wicomico	MD	F109-1	NA	NP	21	Sussex	DE
F17B-1-20	80	2	26	Wicomico	MD	F109-2	NA	NP	21	Sussex	DE
F17B-1-22	80	2	26	Wicomico	MD	F114-1	NA	NP	23	Caroline	MD
F17B-1-18	80	2	26	Wicomico	MD	F119-1	NA	NP	24	Caroline	MD
F17B-1-10	80	2	26	Wicomico	MD	F121-1	NA	NP	25	Caroline	MD
F17BR-006	80	2	26	Wicomico	MD	F17BS-008	NA	NP	26	Wicomico	MD

^w Three reference isolates of *F. oxysporum* f. sp. *niveum* were TX-471 (race 0) obtained from B. D. Bruton of the United States Department of Agriculture in Lane, OK; ATCC44293 (race 1, vegetative compatibility group [VCG] 0080) from the American Type Culture Collection (ATCC) in Manassas, VA; and TX-X1D (race 2, VCG 0082) from ATCC in Manassas, VA. Nine isolates were nonpathogenic to watermelon. Among 88 isolates of *F. oxysporum* f. sp. *niveum* collected from the Mid-Atlantic region, 14 isolates from which no nitrate-nonutilizing mutants could be generated were not listed in the table.

^x VCGs were numbered according to Puhalla (49), Larkin et al. (35), and Kistler et al. (32). NA = not assigned to a VCG because no compatibility reactions with all testers of VCGs established in the study were observed.

^y Race designation of the pathogen was based on a previous study (54). NP = no pathogenicity to watermelon.

^z TX = Texas, MD = Maryland, DE = Delaware, and CA = California.

were generated from each isolate until two complementary *nit* mutants were obtained. Complementary mutants were recognized by the robust growth at the interface of the two colonies (a visual indication of heterokaryon formation) when paired on MM under a daily 12-h fluorescent light for 5 to 14 days at 22 to 25°C. Mutants used for testers or those that initially could not be assigned to a known VCG were characterized for physiological phenotypes (*nit1*, *NitM*, and *nit3*) on the basis of their growth on MM supplemented with different nitrogen sources (12). *Nit* mutant testers for VCGs 0080 and 0082 were generated from the reference isolates ATCC44293 and TX-X1D (35), respectively. Eight mutants were recovered from each of the two isolates, and three *nit* mutants, *nit1*, *NitM*, and *nit3*, from each of the isolates were chosen as testers.

Mutants of uncharacterized isolates were paired with each of complementary *nit* mutant testers from each established VCG and with each other. If a mutant formed a heterokaryon with at least one of the three testers of the same VCG, the isolate was placed in the corresponding VCG. If isolates with mutants failed to form a heterokaryon with any of the established VCG testers, the isolates

were kept for further examination. Up to 14 additional mutants from each of these isolates were generated and phenotype determined. If mutants from an isolate formed a heterokaryon with one another, but not with all established VCG testers, the isolate was assigned a new VCG. The complementary *nit* mutants of the new VCG consisting of all three phenotypes were used as new testers for further complementation tests. Each pairing in all combinations with VCG testers was conducted at least three times.

RESULTS

Cross-pathogenicity tests. Of the 88 isolates of *F. oxysporum* f. sp. *niveum* in the Mid-Atlantic collection, only 4 (4.5%) were pathogenic to two or more of the 18 muskmelon cultivars evaluated in the greenhouse (Table 2). All four isolates were shown to be moderately pathogenic (43 to 57% wilt) to the muskmelon cvs. Banana and Topmark, while 1 (F063-1) also was slightly pathogenic (30% wilt) to cv. Hale's Best No. 36. All other muskmelon cultivars, including all of the honeydew type, had 17% or less wilt and were considered resistant to these isolates.

TABLE 2. Cross pathogenicity of four isolates of *Fusarium oxysporum* f. sp. *niveum* from the Mid-Atlantic region to 18 cultivars of muskmelon in greenhouse seedling bioassays^y

Host, cultivar	F061-2		F063-1		F097-2		F100-2		F17B-R-2 (control)	
	Wilt (%)	Stem	Wilt (%)	Stem	Wilt (%)	Stem	Wilt (%)	Stem	Wilt (%)	Stem
Watermelon										
Sugar Baby	100	235	100	325	100	356	100	265	100	317
Charleston Gray	100	210	100	240	100	310	93	265	93	274
Calhoun Gray	83	97	87	121	80	79	83	123	77	51
Muskmelon										
Banana	47	54	53	76	57	86	47	72	0	0
Topmark	43	50	47	65	50	76	43	56	0	0
Hale's Best No. 36	0	0	30	78	0	0	0	0	0	0
Tam Uvalde	0	0	17	12	0	0	0	0	0	0
Rocky Ford	0	0	10	5	0	0	0	0	0	0
Burpee Hybrid	0	0	3	1	0	0	0	0	0	0
Jumbo Hale's Best	7	1	3	2	0	0	13	6	0	0
Ananas	0	0	0	0	0	0	0	0	0	0
Honeydew Greenflesh	0	0	0	0	0	0	0	0	0	0
Honeydew Orangeflesh	0	0	0	0	0	0	0	0	0	0
Imperial 4-50	0	0	0	0	0	0	0	0	0	0
Israeli (Old Original)	0	0	0	0	0	0	0	0	0	0
Mildew Resistance No. 45	0	0	0	0	0	0	0	0	0	0
Planters Jumbo	0	0	0	0	17	5	0	0	0	0
Saticoy	0	0	0	12	0	0	7	4	0	0
Superstar	7	4	0	0	0	0	0	0	0	0
Tam Dew (Honey Dew)	0	0	0	0	0	0	0	0	0	0
Yellow Canary	0	0	0	0	0	0	0	0	0	0
LSD ($P = 0.05$) ^z	6	12	7	29	5	11	6	16	3	22

^y Wilt: percentage of plants showing symptoms of *Fusarium* wilt was the means of three replicated pots, each with 10 plants. Stem: internal lower stem colonization (CFU/g of fresh tissue $\times$ 100) was assayed by placing serial diluted, homogenized tissues on Komada's medium.

^z Fisher's protected least significant difference (LSD) test at $P = 0.05$, using the GLM procedure of the Statistical Analysis System (SAS, version 8.0; SAS Institute, Cary, NC). Prior to analysis, wilt data were arcsine-square root-transformed, and colonization data were transformed to $\log_{10}$ (CFU/g + 1). All noninoculated control plants remained healthy and were not included in the table. All data were from a single representative experiment.

TABLE 3. Classification of *Fusarium oxysporum* f. sp. *niveum* from the Mid-Atlantic region by vegetative compatibility group (VCG), race, and cross pathogenicity to muskmelon

VCG ^x	Race ^y	Cross pathogenicity ^z	No. of isolates (total = 74)	Origin (county, state)
0080	0	—	8	Caroline, Dorchester, Wicomico, MD; Sussex, DE
	1	—	21	Caroline, Dorchester, Wicomico, MD; Sussex, DE
	2	—	12	Wicomico, MD
0082	0	—	6	Caroline, Wicomico, MD; Sussex, DE
	1	—	11	Wicomico, MD; Sussex, DE
	2	—	6	Dorchester, Wicomico, MD
0083		+	4	Dorchester, MD; Sussex, DE
	2	—	6	Wicomico, MD; Sussex, DE

^x VCGs were numbered according to Puhalla (49), Larkin et al. (35), and Kistler et al. (32).

^y Race designation of the pathogen was based on a previous study (54).

^z Cross pathogenicity to certain cultivars of muskmelon (— = no pathogenicity and + = pathogenicity) according to the results of greenhouse seedling bioassays in this study.

Stem colonization by these isolates was detected in all cultivars exhibiting wilt symptoms, and the degree of colonization corresponded to the level of wilt incidence. All four isolates and the control isolate (F17B-R-2) caused high levels of wilt incidence and stem colonization in the three differential watermelon cultivars, indicating that they were race 2.

In the field microplot tests, wilt incidence on muskmelon (cv. Banana) in plots infested with the cross-pathogenic isolate was 35 and 20% in the first and second experiments, respectively. The corresponding wilt incidence on watermelon (cv. Sugar Baby) was 90 and 100%, respectively. In contrast, the control isolate did not cause any symptoms of *Fusarium* wilt on muskmelon in either experiment; however, wilt incidence of watermelon caused by this control isolate was 85 and 95% in the first and second experiments, respectively. Plants in noninfested plots remained healthy.

Symptoms of stunting and chlorosis usually appeared on infected muskmelon plants 9 to 15 days after inoculation, and were followed by wilting of the leaves and seedling death in the greenhouse or microplot tests. Vascular discoloration occurred in some of the root and crown sections of the affected plants. The pathogen was reisolated from root and stem sections of symptomatic muskmelon plants, and the cultures recovered on Komada medium, MMCPA, and PDA were visually and microscopically similar to the original isolates and were virulent to seedlings of both muskmelon and watermelon in greenhouse inoculations.

Vegetative compatibility and its relationship with race. *Nit* mutants were obtained readily from 86 isolates. Fourteen isolates failed to produce *nit* mutants even when up to 62 mycelium agar blocks for each isolate were plated on either induction medium and when the concentration of KClO_3 added in the media was increased to a level two or three times higher than the normal concentration. All isolates pathogenic to watermelon were self compatible and were not compatible with any of the nine non-pathogenic isolates examined. All isolates within a VCG were compatible with all other isolates of the same VCG, and no isolates were compatible with isolates from more than one VCG.

Three VCGs were identified in this study (Table 1). Two VCGs (0080 and 0082) have been described previously (35); the reference isolates TX-417 and ATCC44293 both belonged to

VCG 0080, and the reference isolate TX-X1D belonged to VCG 0082. The third VCG was the previously undescribed VCG (designated VCG 0083). Of the 74 isolates of *F. oxysporum* f. sp. *niveum* from the Mid-Atlantic region, 41 (55%) were identified as VCG 0080 and were distributed in 15 of the 20 fields and in all four counties surveyed in Maryland and Delaware; 27 (36%) were identified as VCG 0082, and occurred in 10 fields and in all the counties; and 6 (8%) were identified as VCG 0083, and were limited to two fields in Maryland and one in Delaware (Table 3). The association between race, cross pathogenicity to muskmelon, and VCG was complex. Among the isolates in VCG 0080, eight were designated as race 0 according to race determinations made previously (54), 21 were designated as race 1, and 12 were designated as race 2. Of the isolates in VCG 0082, 6 were designated as race 0, 11 were designated as race 1, and 10 were designated as race 2 that included four isolates cross-pathogenic to muskmelon. All six isolates in VCG 0083 were designated as race 2.

Distribution of isolates of *F. oxysporum* f. sp. *niveum* by VCG and race within single fields (Table 4) and watermelon plants (Table 5) was characterized. Among the 12 fields, each with two or more isolates recovered (Table 4), 6 fields (fields 4, 5, 6, 15, 16, and 26) contained two or three races of the same VCG, accounting for 30% of all the fields sampled. Field 26 contained the greatest diversity, with two races (races 1 and 2) within VCG 0080 and all three races within VCG 0082. Six fields (fields 4, 5, 15, 19, 25, and 26) also contained isolates from two or all three VCGs. Among the 14 single watermelon plants from which two or more isolates were recovered (Table 5), three (plants 8, 10, and 14) contained two different races within the same VCG, accounting for 21% of all plants assayed, whereas seven (plants 1, 2, 3, 5, 11, 12, and 14) contained two or three VCGs. Isolates from plant 14 comprised all three VCGs, with VCGs 0080 and 0082 each containing two different races (races 1 and 2).

Comparisons of wilt data by race and VCG on the differential watermelon cultivars indicated that there were no significant differences in aggressiveness toward watermelon among isolates from different VCGs of the same race (Table 6). Within race 0 or race 1, isolates from VCG 0080 and VCG 0082 produced a comparable level of disease incidence on each of the six cultivars, with an identical mean of percent wilt across all cultivars. Within

TABLE 4. Distribution of isolates of *Fusarium oxysporum* f. sp. *niveum* by vegetative compatibility group (VCG) and race within each of 20 watermelon fields surveyed in the Mid-Atlantic region

Field ^y	Number of isolates ^z									Total
	VCG 0080			VCG 0082			VCG 0083			
	Race 0	Race 1	Race 2	Race 0	Race 1	Race 2	Race 0	Race 1	Race 2	
1	...	...	...	1	...	...	...	...	...	1
4	...	5	...	...	6	1	...	...	...	12
5	...	5	...	1	1	...	...	...	...	7
6	1	...	1	...	...	...	...	...	...	2
7	...	2	...	...	...	...	...	...	...	2
8	2	...	...	...	...	...	...	...	...	2
9	1	...	...	...	...	...	...	...	...	1
10	...	...	...	...	...	4	...	...	...	4
13	...	2	...	...	...	...	...	...	...	2
14	1	...	...	...	...	...	...	...	...	1
15	...	1	1	...	...	...	...	...	2	4
16	...	...	...	1	2	...	...	...	...	3
18	1	...	...	...	...	...	...	...	...	1
19	...	2	...	...	...	2	...	...	1	5
20	...	...	...	1	...	...	...	...	...	1
21	...	...	...	...	1	...	...	...	...	1
23	1	...	...	...	...	...	...	...	...	1
24	1	...	...	...	...	...	...	...	...	1
25	...	1	...	1	...	...	...	...	...	2
26	...	3	10	1	1	3	...	...	3	21
Total	8	21	12	6	11	10	0	0	6	74

^y Fields 1 through 25 were commercial watermelon fields, and field 26 was a research watermelon field (54).

^z VCGs were numbered according to Puhalla (49), Larkin et al. (35), and Kistler et al. (32). Race designation of the pathogen was based on a previous study (54).

race 2, isolates from VCGs 0080, 0082, and 0083 produced no significant differences ($P \leq 0.05$) in percent wilt on all but one cultivar (Dixielee), with a similar mean of percent wilt of all six cultivars.

DISCUSSION

The results of this study demonstrated that variation in virulence beyond the three described races exists among a small portion (<5% of total isolates) of the population of *F. oxysporum* f. sp. *niveum* obtained from the Mid-Atlantic region. The number of muskmelon cultivars infected and the degree of disease incidence and stem colonization in these cultivars caused by the cross-infecting isolates were significantly reduced compared with those obtained with the original watermelon host. The four isolates were moderately pathogenic to the two muskmelon cvs. Banana and Topmark, and one isolate was slightly pathogenic on cv. Hale's Best No. 36. A similar observation is found in a greenhouse inoculation study by Owen (46), who reported that an isolate of *F. oxysporum* from wilted watermelon was slightly cross pathogenic to Banana and Hale's Best No. 36. In addition, our field microplot tests also demonstrated that an isolate cross-pathogenic to greenhouse-grown muskmelon seedlings was still pathogenic to muskmelon transplants under simulated field conditions; however, severity of Fusarium wilt caused by this isolate

was significantly decreased. Watermelon is the most extensively planted cucurbit crop in Maryland and Delaware, and muskmelon often is grown as a rotation crop in the same fields. Our results suggest that the potential impact of the cross-infection population of *F. oxysporum* f. sp. *niveum* to muskmelon production in the region is limited. However, because these cross-infecting isolates belong to race 2, and race 2 populations can be selectively increased by widely deployed race 1-resistant genes in watermelon (21), the impact may become greater over time. Thus, based on our results, as well as other previous cross-infection reports (6,14, 18,41,42,46,47), we suggest that cross infection of *F. oxysporum* in cucurbits should be monitored and crop rotation guidelines adjusted, if necessary.

Pathogenicity of *F. oxysporum* f. sp. *niveum* to muskmelon and watermelon was established by symptoms of Fusarium wilt and internal stem colonization in plants. No stem colonization was observed where the incompatible interactions between muskmelon and *F. oxysporum* f. sp. *niveum* isolate occurred. However, high levels of stem colonization were observed where compatible interactions took place. This indicated that isolates of *F. oxysporum* f. sp. *niveum* that were not cross pathogenic to muskmelon may lack the ability to colonize the stems of plants and that muskmelon resistance to infection of *F. oxysporum* f. sp. *niveum* was directly related to the degree of pathogen colonization.

Difficulties in classification of strains of *F. oxysporum* f. sp. *niveum* into races have been recognized for many years (2,35,37).

TABLE 5. Distribution of some of the isolates of *Fusarium oxysporum* f. sp. *niveum* by vegetative compatibility group (VCG) and race within each of 14 symptomatic watermelon plants sampled in the Mid-Atlantic region

		Number of isolates ^y									
		VCG 0080			VCG 0082			VCG 0083			
Plant	Field ^z	Race 0	Race 1	Race 2	Race 0	Race 1	Race 2	Race 0	Race 1	Race 2	Total
1	4	...	1	...	...	...	1	...	...	...	2
2	4	...	2	...	...	1	...	...	...	...	3
3	4	...	1	...	...	1	...	...	...	...	2
4	5	...	2	...	...	...	...	...	...	...	2
5	5	...	2	...	1	...	...	...	...	...	3
6	7	...	2	...	...	...	...	...	...	...	2
7	10	...	...	...	...	...	2	...	...	...	2
8	15	...	1	1	...	...	...	...	...	...	2
9	15	...	...	...	...	...	...	...	...	2	2
10	16	...	...	...	1	1	...	...	...	...	2
11	19	...	1	...	...	...	...	...	...	1	2
12	19	...	1	...	...	...	1	...	...	...	2
13	26	...	...	3	...	...	...	...	...	...	3
14	26	...	1	1	...	1	3	...	...	1	7
Total	...	0	14	5	2	4	7	0	0	4	36

^y VCGs were numbered according Puhalla (49), Larkin et al. (35), and Kistler et al. (32). Race designation of the pathogen was based on a previous study (54).

^z Field 26 was a research watermelon field, and all others were commercial watermelon fields (54).

TABLE 6. Comparison in mean percent wilt (%) of differential watermelon cultivars inoculated with isolates of *Fusarium oxysporum* f. sp. *niveum* from various vegetative compatibility groups (VCGs) within each of three races from the Mid-Atlantic region

<i>F. oxysporum</i> f. sp. <i>niveum</i>			Differential watermelon cultivar ^w						
Race ^x	VCG ^y	No. of isolates ^z	Calhoun Gray	Dixielee	Allsweet	Charleston Gray	Crimson Sweet	Sugar Baby	Mean
0	0080	8	0 a	0 a	1 a	0 a	2 a	45 a	8
	0082	6	0 a	0 a	0 a	4 a	2 a	45 a	8
1	0080	21	15 a	24 a	21 a	50 a	78 a	99 a	48
	0082	11	15 a	19 a	30 a	57 a	76 a	100 a	49
2	0080	12	66 a	73 a	72 a	75 a	92 a	100 a	80
	0082	10	66 a	57 b	69 a	86 a	91 a	99 a	78
	0083	6	73 a	74 a	77 a	86 a	95 a	100 a	84

^w This analysis was based on the results of a previous study (54). Values in column followed by the same letter within each race represent no significant difference according to Fisher's protected least significant difference test at $P = 0.05$, using the GLM procedure of the Statistical Analysis System (SAS, version 8.0, SAS Institute, Cary, NC). Prior to analysis, wilt data were arcsine-square root-transformed to normalize variance.

^x Race designation of the pathogen was based on a previous study (54).

^y VCGs were numbered according to Puhalla (49), Larkin et al. (35), and Kistler et al. (32).

^z Number of isolates (total = 74).

Unlike some formae speciales of *F. oxysporum* in which races are determined by virulence on differential cultivars with complete resistance genes, designated races of *F. oxysporum* f. sp. *niveum* are determined based on varying degree of aggressiveness on cultivars of varying levels of resistance (35). Thus, there frequently is a continuum in disease reaction of differential cultivars to strains (especially race 1) of *F. oxysporum* f. sp. *niveum*, ranging from highly resistant to susceptible (35,54). This variability, along with factors associated with virulence test conditions (different inoculation methods, inoculum level, age of tested plants, and environmental conditions) (36,40), has resulted in difficulties with consistent race identification. Because of this, the distinction between race 0 and race 1 was questioned (2,35,38,39). Race 0 was not separated from the race 1 isolates of *F. oxysporum* f. sp. *niveum* within a VCG (VCG 0080) in a previous study (35), although some variability in disease reaction on differential cultivars did exist. In this study, there were sufficient differences in aggressiveness among isolates within each of VCGs 0080 and 0082 to differentiate them into races 0 and 1. However, it should be noted that multiple races of *F. oxysporum* f. sp. *niveum* within each VCG may not indicate the same magnitude of genetic differences as would races of other formae speciales, because they may not be defined by a specific pathogenicity gene corresponding to a specific resistance gene (gene-for-gene theory).

Three VCGs were identified from the Mid-Atlantic populations of *F. oxysporum* f. sp. *niveum*. VCGs 0080 and 0082 were the two most common groups and occurred throughout the region surveyed. This is in agreement with the results of Larkin et al. (35) that these VCGs also are the most frequently occurring groups of all three VCGs identified from their collection of *F. oxysporum* f. sp. *niveum*. The third VCG is a new, previously undescribed group, and we named it VCG 0083 according to the numbering system for VCG in *F. oxysporum* (32,35,49). This group is considered to be different from VCG 0081 established in the study of Larkin et al. (35), although no direct comparisons were made because the reference isolates were unavailable. In their study, VCG 0081 consisted solely of race 1 of *F. oxysporum* f. sp. *niveum*, and distribution was limited to a pathogen-infested research field in Florida. In contrast, in our study, VCG 0083 consisted solely of race 2 isolates and was isolated from a limited number of commercial fields in Maryland and Delaware. The status of the additional 14 isolates from which no *nit* mutants could be generated is unclear. The inability to generate *nit* mutants has been reported by other investigators (11,22). Collectively, four VCGs to date have been described in *F. oxysporum* f. sp. *niveum*, which is the smallest number of VCGs that potentially exists for each of formae speciales in *F. oxysporum* based on the theory of vegetative incompatibility (*vic*) genes mediating VCGs (two alleles by two *vic* loci) (33,49). VCG 0080 is the most widely distributed group, followed by VCG 0082; VCGs 0081 and 0083 have a more limited geographic distribution. VCG 0081 is reported only in Florida, whereas VCG 0083 is reported in Maryland and Delaware.

The results of this study indicate a complex relationship between virulence (race or cross pathogenicity) and VCG among isolates of *F. oxysporum* f. sp. *niveum* obtained from the region. VCGs 0080 and 0082 each included all three races, and race 2 was found in all three VCGs. VCG 0083 consisted solely of race 2 isolates, and the cross-infecting isolates all were VCG 0082 and race 2. The known geographic range for isolates from VCG 0083 and for the cross-infecting isolates is more limited. Our results corroborate those of Kim et al. (27) who, while examining a worldwide collection of 50 isolates of *F. oxysporum* f. sp. *niveum*, found that there was no correlation between mtDNA RFLP pattern, race, and geographic origin, and that the two most common patterns of RFLP each contained all three races from different locations. Complex relationships between genetic diversity and

virulence also have been reported for numerous other formae speciales, such as *F. oxysporum* f. spp. *melonis* (24), *lycopersici* (16), *cubense* (4), and *asparagi* (17). Although VCG cannot be used to distinguish races of *F. oxysporum* f. sp. *niveum*, it is useful in distinguishing pathogenic isolates from nonpathogenic isolates of *F. oxysporum*, and in characterizing genetic diversity among isolates of *F. oxysporum* f. sp. *niveum*.

The origin of races of *F. oxysporum* f. sp. *niveum* in the Mid-Atlantic region is unknown, but evolutionary relationship or mechanisms involved in the development of a new race may be hypothesized.

It is presumed that VCGs generally represent good predictors of genetic similarity or clonal lineage (19,30). The occurrence of all three races within the same VCG (VCG 0080 or VCG 0082) suggests that the genes that determine the races are present within genetically isolated clonal populations and that one pathogenic race may give rise to another within a clonal population. Mutation at a single gene may lead to a change in virulence, resulting in the development of a new race. It has been demonstrated that transformational mutagenesis of a race 2 isolate of *F. oxysporum* f. sp. *niveum* resulted in a change to race 0 without a concomitant change in the VCG trait in that isolate (25). In addition, a recent example of one race arising from another in a local population within an evolutionary lineage (VCG) has been reported in *F. oxysporum* f. sp. *lycopersici* in California (8).

Based on the observed diversity in geographic range for the races of *F. oxysporum* f. sp. *niveum* in this study, it is proposed that race 0 may be the ancestral progenitor, and that race 2 may be of more recent origin. Evidence to support this is the common distribution of race 0 isolates throughout the region (54), which contrasts with the rare occurrence of the race reported for other areas (35,39). Race 0 may serve as the base population of *F. oxysporum* f. sp. *niveum* for the subsequent development of other races due to the wide use of races 0- or 1-resistant cultivars. Race 0 was first reported in 1963 in Florida (13) whereas race 2 was not observed in the United States until it was found in 1985 in Texas (37). Race 2 has since been reported in only five states: Delaware (53), Florida (39), Maryland (53), Indiana (15), and Oklahoma (7). Race 2 was first reported in 1973 in Israel (44). Race 1 is currently the most frequently occurring race (35,38,39,54). It is anticipated that, with evolution, the populations of race 0 will continue to decline while those of race 2 increase. This can be illustrated from the frequency of distribution of different races observed in a research field (Table 4, field 26) in this study and a similar study of a research field in Florida (21). High similarity in DNA profiles among all three races of *F. oxysporum* f. sp. *niveum* reported in previous studies (27,28) provides genetic evidence to support this hypothesis. All races were closely related and shared common patterns of mtDNA RFLP (27) and common sequences of chromosomal DNA (28).

The observed distribution of races among VCGs, especially the occurrence of race 2 in all three VCGs, implies the possibility of the derivation of one VCG from the other as a result of a mutation in a *vic* gene. Single mutation in a *vic* gene resulting in a change from compatibility to incompatibility or vice versa has been demonstrated in other fungi (1,48,50). A mutation to alter vegetative compatibility also has been suggested to explain the origin of two different VCGs with identical virulence (race) in *F. oxysporum* f. sp. *melonis* (24). Similarity in mtDNA polymorphism between VCGs of *F. oxysporum* f. sp. *niveum* found by Kim et al. (27) appears to support this evolutionary model.

Our isolate distribution data may provide additional support for the evolution of races and VCGs of *F. oxysporum* f. sp. *niveum* in the region. The high frequency of co-presence of one race with another from the same VCG or co-occurrence of one VCG with another within the same plants and fields indicates the possibility of one race arising from another or one VCG from another. However, the introduction of the pathotypes from foreign loca-

tions cannot be excluded. If this were the case, such introduction likely did not occur recently.

F. oxysporum f. sp. *niveum* in the Mid-Atlantic region appears to be a less complex, genetically diverse group compared with other formae speciales such as *F. oxysporum* f. sp. *melonis* (22–24) and *F. oxysporum* f. sp. *asparagi* (17), in which there are numerous VCGs and complex relationships with race. Each race of *F. oxysporum* f. sp. *niveum* represents a genetically heterogeneous group of isolates. However, such a regional population can be unified by the common characteristic of virulence to watermelon, with a few unique isolates showing a weak cross pathogenicity to muskmelon. The pathotypes of *F. oxysporum* f. sp. *niveum* may be of local origin or at least long existent in the region. The results of further work using DNA molecular techniques may provide a more detailed understanding of the geographic origin and evolution of the forma specialis and races in *F. oxysporum* f. sp. *niveum*.

ACKNOWLEDGMENTS

We thank B. D. Bruton for supplying isolates of *F. oxysporum* f. sp. *niveum*, J. F. Leslie for providing some advice during this investigation, and anonymous referees for critical review that improved the text.

LITERATURE CITED

- Anagnostakis, S. L. 1982. Genetic analyses of *Endothia parasitica*: Linkage data for four single genes and three vegetative compatibility types. *Genetics* 102:25-28.
- Armstrong, G. M., and Armstrong, J. K. 1978. Formae speciales and races of *Fusarium oxysporum* causing wilts of the Cucurbitaceae. *Phytopathology* 68:19-28.
- Armstrong, G. M., and Armstrong, J. K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt disease. Pages 391-399 in: *Fusarium: Disease, Biology, and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park.
- Bentley, S., Pegg, K. G., Moore, N. Y., Davis, R. D., and Buddenhagen, I. W. 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cubense* in Banana. *Phytopathology* 88:1283-1293.
- Both, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.
- Bouhot, D. 1981. Some aspects of the pathogenic potential in formae speciales and races of *Fusarium oxysporum* on Cucurbitaceae. Pages 318-326 in: *Fusarium: Disease, Biology, and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park.
- Bruton, B. D., Patterson, C. L., and Martyn, R. D. 1988. Fusarium wilt (*F. oxysporum* f. sp. *niveum* race 2) of watermelon in Oklahoma. *Plant Dis.* 72:734.
- Cai, G., Gale, L. R., Schneider, R. W., Kistler, H. C., Davis, R. M., Elias, K. S., and Miyao, E. M. 2003. Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. *Phytopathology* 93:1014-1022.
- Carlile, M. J., and Watkinson, S. C. 1994. The Fungi. Academic Press, London.
- Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology* 81:1061-1064.
- Correll, J. C. 1992. Genetic, biochemical, and molecular techniques for the identification and detection of soilborne plant-pathogenic fungi. Pages 7-16 in: *Methods for Research on Soilborne Phytopathogenic Fungi*. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. The American Phytopathological Society, St. Paul, MN.
- Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1604-1646.
- Crall, J. M. 1963. Physiological specialization in *Fusarium oxysporum* f. sp. *niveum*. (Abstr.) *Phytopathology* 53:873.
- Davis, D. 1966. Cross-infection in Fusarium wilt diseases. *Phytopathology* 56:825-828.
- Egel, D. S., Harikrishnan, R., and Martyn, R. D. 2005. First report of *Fusarium oxysporum* f. sp. *niveum* race 2 as causal agent of Fusarium wilt of watermelon in Indiana. *Plant Dis.* 89:108.
- Elias, K. S., and Schneider, R. W. 1991. Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici*. *Phytopathology* 81:159-162.
- Elmer, W. H., and Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. *Phytopathology* 79:88-93.
- Gelragh, M., and Blok, W. J. 1988. *Fusarium oxysporum* f. sp. *cucurbitacearum* n. f. embracing all formae speciales of *F. oxysporum* attacking cucurbitaceous crops. *Neth. J. Plant Pathol.* 94:17-31.
- Gordon, T. R., and Martyn, R. D. 1997. The evolutionary biology of *Fusarium oxysporum*. *Annu. Rev. Phytopathol.* 35:111-128.
- Hadar, E., Katan, J., and Katan, T. 1989. The use of nitrate-nonutilizing mutants and a selective medium for studies of pathogenic strains of *Fusarium oxysporum*. *Plant Dis.* 73:800-803.
- Hopkins, D. L., Lobinske, R. J., and Larkin, R. P. 1992. Selection for *Fusarium oxysporum* f. sp. *niveum* race 2 in monocultures of watermelon cultivars resistant to Fusarium wilt. *Phytopathology* 82:290-293.
- Jacobson, D. J., and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. *Phytopathology* 78:668-672.
- Jacobson, D. J., and Gordon, T. R. 1990. Further investigation of vegetative compatibility within *Fusarium oxysporum* f. sp. *melonis*. *Can. J. Bot.* 68:1245-1248.
- Jacobson, D. J., and Gordon, T. R. 1991. *Fusarium oxysporum* f. sp. *melonis*: A case study of diversity within a forma specialis. *Phytopathology* 81:1064-1067.
- Kim, D. H., Magill, C. W., and Martyn, R. D. 1995. Transformation of the plant pathogenic fungus, *Fusarium oxysporum* f. sp. *niveum*, to hygromycin B resistance and altered pathogenicity. *Mol. Cells* 5:658-667.
- Kim, D. H., Martyn, R. D., and Magill, C. W. 1991. Mitochondrial DNA restriction fragment length polymorphisms in *Fusarium oxysporum* f. sp. *niveum*. *Phytoparasitica* 19:211-223.
- Kim, D. H., Martyn, R. D., and Magill, C. W. 1992. Restriction fragment length polymorphism groups and physical map of mitochondrial DNA from *Fusarium oxysporum* f. sp. *niveum*. *Phytopathology* 82:346-353.
- Kim, D. H., Martyn, R. D., and Magill, C. W. 1993. Chromosomal polymorphism in *Fusarium oxysporum* f. sp. *niveum*. *Phytopathology* 83:1209-1216.
- Kim, D. H., Martyn, R. D., and Magill, C. W. 1993. Mitochondrial DNA (mtDNA) - relatedness among formae speciales of *Fusarium oxysporum* in the Cucurbitaceae. *Phytopathology* 83:91-97.
- Kistler, H. C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology* 87:474-479.
- Kistler, H. C. 2001. Evolution of host specificity in *Fusarium oxysporum*. Pages 70-82 in: *Fusarium: Paul E. Nelson Memorial Symposium*. B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden, and L. W. Burgess eds. The American Phytopathological Society, St. Paul, MN.
- Kistler, H. C., Alabouvette, C., Baayen, R. P., Bentley, S., Brayford, D., Coddington, A., Correll, J., Daboussi, M. J., Elias, K., Fernandez, D., Gordon, T. R., Katan, T., Kim, H. G., Leslie, J. F., Martyn, R. D., Migheli, Q., Moore, N. Y., O'Donnell, K., Ploetz, R. C., Rutherford, M. A., Summerell, B., Waalwijk, C., and Woo, S. 1998. Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. *Phytopathology* 88:30-32.
- Klei, K. K., and Correll, J. C. 2001. Vegetative compatibility group diversity in *Fusarium*. Pages 83-96 in: *Fusarium: Paul E. Nelson Memorial Symposium*. B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden, and L. W. Burgess, eds. The American Phytopathological Society, St. Paul, MN.
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-124.
- Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1990. Vegetative compatibility within *Fusarium oxysporum* f. sp. *niveum* and its relationship to virulence, aggressiveness, and race. *Can. J. Microbiol.* 36:352-358.
- Latin, R. X., and Snell, S. J. 1986. Comparison of methods for inoculation of muskmelon with *Fusarium oxysporum* f. sp. *melonis*. *Plant Dis.* 70:297-300.
- Martyn, R. D. 1987. *Fusarium oxysporum* f. sp. *niveum* race 2: A highly aggressive race new to the United States. *Plant Dis.* 71:233-236.
- Martyn, R. D. 1996. Fusarium wilt of watermelon. Pages 13-14 in: *Compendium of Cucurbit Diseases*. Zitter, T. A., Hopkins, D. L., and Thomas, C. E. eds. The American Phytopathology Society, St. Paul, MN.
- Martyn, R. D., and Bruton, B. D. 1989. An initial survey of the United States for races of *Fusarium oxysporum* f. sp. *niveum*. *HortScience* 24:696-698.

40. Martyn, R. D., and McLaughlin, R. J. 1983. Effects of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f. sp. *niveum*. Plant Dis. 67:493-495.
41. Martyn, R. D., and McLaughlin, R. J. 1983. Susceptibility of summer squash to the watermelon wilt pathogen (*Fusarium oxysporum* f. sp. *niveum*). Plant Dis. 67:263-266.
42. McMillan, R. T. 1986. Cross pathogenicity studies with isolates of *Fusarium oxysporum* from either cucumber or watermelon pathogenic to both crop species. Ann. Appl. Biol. 109:101-105.
43. Netzer, D. 1976. Physiological races and soil population level of *Fusarium wilt* of watermelon. Phytoparasitica 4:131-136.
44. Netzer, D., and Dishon, I. 1973. Screening for resistance and physiological specialization of *Fusarium oxysporum* in watermelon and muskmelon. (Abstr. 941) Second Int. Congr. Plant Pathol. Minneapolis, MN.
45. Netzer, D., and Weintall, C. 1980. Inheritance of resistance in watermelon to race 1 of *Fusarium oxysporum* f. sp. *niveum*. Plant Dis. 64:853-854.
46. Owen, J. H. 1955. *Fusarium wilt* of cucumber. Phytopathology 45:435-439.
47. Owen, J. H. 1956. Cucumber wilt, caused by *Fusarium oxysporum* f. *cucumerinum* n. f. Phytopathology 46:153-157.
48. Perkins, D. D., Radford, A., Newmeyer, D., and Bjorkman, M. 1982. Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. 46:426-570.
49. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative incompatibility. Can. J. Bot. 63:179-183.
50. Puhalla, J. E., and Spieth, P. T. 1983. Heterokaryosis in *Fusarium moniliforme*. Exp. Mycol. 7:328-335.
51. Scott, S. 1991. Between Ocean and Bay: A Natural History of Delmarva. Tidewater Publishers, Centreville, MD.
52. Snyder, W. C., and Hansen, H. N. 1940. The species concept in *Fusarium*. Am. J. Bot. 27:64-67.
53. Zhou, X. G., and Everts, K. L. 2001. First report of the occurrence of *Fusarium oxysporum* f. sp. *niveum* race 2 in commercial watermelon production areas of Maryland and Delaware. Plant Dis. 85:1291.
54. Zhou, X. G., and Everts, K. L. 2003. Races and inoculum density of *Fusarium oxysporum* f. sp. *niveum* in commercial watermelon fields in Maryland and Delaware. Plant Dis. 87:692-698.
55. Zhou, X. G., and Everts, K. L. 2004. Characterization of a regional population of *Fusarium oxysporum* f. sp. *niveum* by cross pathogenicity, race, and vegetative compatibility. (Abstr.) Phytopathology 94:S116.
56. Zhou, X. G., and Everts, K. L. 2004. Quantification of root and stem colonization of watermelon by *Fusarium oxysporum* f. sp. *niveum* and its use in evaluating resistance. Phytopathology 94:832-841.
57. Zhou, X. G., and Everts, K. L. 2006. Suppression of *Fusarium wilt* of watermelon enhanced by hairy vetch green manure and partial cultivar resistance. Online. Plant Health Progress doi:10.1094/PHP-2006-0404-01-RS.