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Classification of *Fusarium oxysporum* f. sp. *asparagi* into Vegetatively Compatible Groups

W. H. Elmer and C. T. Stephens

Research associate and associate professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48823.
Present address of the first author: The Connecticut Agricultural Experiment Station, Box 1106, New Haven 06504.
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ABSTRACT

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Ninety-seven strains of *Fusarium oxysporum* were isolated from asparagus and other crops in Michigan or obtained from collections in the U.S., Europe, and Taiwan. Pathogenicity tests on asparagus (*Asparagus officinalis* L. 'U.C. 157') seedlings revealed that 85 strains (87%) caused root lesions, including strains of *F. oxysporum* f. sp. *cepae*, *F. oxysporum* f. sp. *gladioli*, and *F. oxysporum* f. sp. *apii* race 1. Vegetative compatibility between strains was demonstrated with heterokaryons produced between complementary nitrate-nonutilizing (*nit*) mutants. *Nit* mutants were placed into one of three phenotypic classes (*nit*1, *nit*3, or nitM) by their ability to utilize various nitrogen sources. Mutants with nitM phenotype were recovered from 59% (57 of 97) of the strains, and each one was paired against a mutant with a *nit*1 or *nit*3 phenotype from each of the other strains. Twenty-seven strains of *F. o. asparagi* and one nonpathogenic strain were placed into eight vegetatively compatible groups (VCGs). Thirty-four virulent strains each belonged to a unique VCG. The remaining 24 virulent strains and 11 strains from other formae speciales (that were nonpathogenic on asparagus) were not vegetatively compatible with any other strains, but nitM phenotypes were not recovered from these strains. The largest VCG (1001WE) contained a total of seven strains from Taiwan, Washington state, and three counties in Michigan. Seven VCGs (1002WE-1008WE) contained two to six strains of *F. o. asparagi* from Michigan. No pattern was observed between VCG in *F. o. asparagi* and locality. Most heterokaryons formed between vegetatively compatible strains were slow to develop and lacked robust growth. These findings indicate that strains of *F. o. asparagi* belong to a minimum of 43 VCGs. These data are in contrast to what has been observed in several other formae speciales of *F. oxysporum*.

Fusarium oxysporum (Schlecht.) emend. Snyd. and Hans. f. sp. *asparagi* (Cohen and Heald) is one of two pathogenic Fusaria that cause Fusarium crown and root rot of asparagus (*Asparagus officinalis* L.) (4). The disease is widespread within asparagus plantings in Michigan (23) and within other asparagus-growing regions in the world (4,14,18,20–22). Because highly resistant cultivars are not yet available (40) and effective disease controls are lacking (29,31), Fusarium crown and root rot is the major limiting factor in asparagus production (23).

F. o. asparagi is easily isolated from infected plants (4) and from soil never planted to asparagus (20). No known races of *F. o. asparagi* exist. Isolates of *F. oxysporum* also are recovered in these assays that appear morphologically indistinguishable from isolates of *F. o. asparagi* on carnation-leaf agar (33), but do not cause

disease on asparagus; these isolates are called nonpathogenic. Differentiating isolates of *F. o. asparagi* from nonpathogenic ones is routinely done by means of pathogenicity tests (14,20,21,41). Although useful, pathogenicity tests can be variable in their results and cumbersome to conduct. These obstacles have hindered an accurate assessment of the soil population dynamics of *F. o. asparagi*.

Another method used to distinguish strains of *F. oxysporum* is that of vegetative compatibility (38). Vegetative compatibility in some fungi is mediated by multiple incompatibility loci called *vic* or *het* genes (1,12,30,35). When two fungal strains are vegetatively compatible, their hyphae can make contact, fuse, and produce a heterokaryon that, in most cases, occurs when identical alleles exist at each *vic* or *het* locus (1). Fungi that are vegetatively compatible are in the same vegetative compatibility group (VCG). Evidence suggests that vegetatively compatible strains that reproduce asexually are genetically more similar in certain traits than vegetatively incompatible strains of the same species (3,7,12,16,19).

Puhalla (38) modified a procedure that was developed by Cove (10) to test for vegetative compatibility in *F. oxysporum* by using nitrate-nonutilizing (*nit*) mutants. *Nit* mutants were selected from rapidly growing chlorate-resistant sectors on a chlorate medium (38). Puhalla (38) used these forced heterokaryons to place 21 strains of *F. oxysporum* into 16 VCGs. A correlation between VCG and formae speciales was observed, so Puhalla proposed a four-digit VCG code to subdivide *F. oxysporum* (38).

Correll et al (5) refined the heterokaryon technique by demonstrating that *nit* mutants of *F. oxysporum* could be subdivided into at least three phenotypic classes of nit mutants (*nit*1, *nit*3, and nitM). These classes could be differentiated by the ability of *nit* mutants to utilize various nitrogen sources (5). Because *F. oxysporum* has no known teleomorph, genetic analysis of nit mutants was hindered. However, Puhalla and Spieth (39) and Klittich and Leslie (27) discovered similar phenotypes among *nit* mutants in *F. moniliforme* (Sheld.) emend. Snyd. and Hans., a closely related species with a teleomorph. Two of these phenotypic classes of *F. moniliforme* were found to be single locus mutations of the gene for nitrate reductase and for the pathway-specific regulatory gene for induction of nitrate reductase and nitrite reductase. These phenotypes were later labeled with the genotype designation *nit*1 and *nit*3, respectively (27,43). The third phenotype discovered in *F. moniliforme* mapped to one of five loci required for the synthesis of the molybdemum cofactor necessary for nitrate reduction and purine dehydrogenase (27,32) that had been previously described in *Aspergillus nidulans* (Eidam) Winter (11). This phenotype was called nitM (27).

Correll et al (5) presumed similar mutations were likely to exist among the classes of *nit* mutants in *F. oxysporum* and suggested similar names. They suggested that future vegetative compatibility tests would be more reliable if a nitM phenotype was included as one of the *nit* mutants in the pairing. In addition, methods were outlined to expedite recovery and selection of *nit* mutants from different media, to classify natural populations of *F. oxysporum* strains into VCGs, and to determine if strains were self-incompatible (5,6,24).

Since Puhalla's (38) report, VCGs in *F. oxysporum* have been compared in other formae speciales (3,7,9,16,24,25,36), races within a formae speciales (7,9,36), and formae speciales and/or races within a geographic locality (24,36). The purpose of this study was to determine if strains of *F. o. asparagi* were vegetatively compatible and to determine if some other formae speciales were virulent on asparagus and/or belong to the same VCGs as strains of *F. o. asparagi*. A preliminary report has been published (17).

MATERIALS AND METHODS

Collection of strains. Isolates of *F. o. asparagi* and other strains of *F. oxysporum* were collected from various locations (Table 1). Fifty-three isolates were collected from infected asparagus plants and from soil from one or two fields in each of four counties in Michigan. The following isolates were sampled from different plants in the same field (F), from soil collected from the same field (S), or from the same plant (P): Isolates MA1–MA9 (F); MA15, MA16 (F); MA22–MA25 (F); MA27–MA29 (P); MA30,MA31 (P); MA32–MA45, MA47–MA56 (F); MA76–MA79 (S); MA80, MA86 (S) and NJB1, NJB2, NJB4, and NJB5 (P). Isolates MA80 and MA86 were recovered from grassland soil with no history of asparagus culture. All other strains were provided by researchers from other areas. Some were reported to us as pathogenic on other crops (Table 1).

Isolations from tissue were conducted by placing pieces of surface-disinfested (0.53% sodium hypochlorite for 1 min) diseased tissue onto Komada's medium (KM) (28). Field soil was collected with a soil probe, air dried, and passed through a 2-mm sieve. Soil dilution plates were prepared by pipetting 0.1-ml aliquots of diluted soil suspensions (1×10^{-3} g of soil per ml) onto KM and incubating the plates for 5–7 days at 25 C. Isolates were sampled from colonies resembling *F. oxysporum* that grew on these plates (28). All strains isolated by us, and those that were collected abroad, were derived from single microconidia (presumably uninucleate) and subcultured onto potato-carrot agar (15) or carnation-leaf agar for species confirmation based on spore morphology (33). Strains were stored in sterile organic soil tubes (33,42) and *nit* mutants originating from single microconidia were stored on silica gel (42). Representative wild-type strains and nitM mutants from each VCG (Table 2) were deposited at the Fungal Genetic Stock Center, University of Kansas Medical Center, Kansas City, KS.

Pathogenicity tests. Methods for conducting pathogenicity tests with asparagus seedlings have been described before (41). Disease severity was rated 3–4 wk later as the percentage of root area with lesions and was based on a modified scale of 1–5 (14), in which 1 = no disease, 2 = lesions present on 0–25% of the root system, 3 = lesions on 25–50% of the root system, 4 = lesions on 50–75% of the root system, and 5 = lesions on 75–100% of the root system. Pathogenicity tests were carried out three times with distilled water treatments as controls. Strains that were considered to be nonpathogenic were those that received mean disease ratings of less than 2.0.

Selection and characterization of *nit* mutants. Details for preparing the media used in selecting and characterizing *nit* mutants have been described before (5). Complementary *nit* mutant pairs were selected from each strain, and each *nit* mutant was placed into one of three phenotypes (*nit*1, *nit*3, or nitM) based on its ability to utilize various nitrogen sources (5) (Table 1). Phenotype labels were the same as before (5) and were based on the nomenclature assigned to *nit* mutants in *F. moniliforme* (27). An effort was made to recover a nitM phenotype and *nit*1 phenotype from each strain, but this was not always possible (Table 1). The intensity of growth of the heterokaryon formed between complementary *nit* pairs on nitrate media was rated as weak or strong after 10–12 days at 25 C (Table 1, Fig. 1).

Vegetative compatibility tests. Strains from which a nitM was derived were paired against other complementary *nit* mutants. Five agar plugs (5 × 5 mm) colonized by different *nit* mutants were positioned equidistantly on a minimal nitrate agar (MM) around a nitM phenotype from a different strain and incubated at 20–25 C under cool-white fluorescent lights for 16-hr photoperiods. Plates were examined weekly for heterokaryon development. Interstrain pairings that failed to produce heterokaryons after 2 wk were considered vegetatively incompatible only when a nitM phenotype was included as one of the testers. These pairings were done at least twice. Because weak complementary growth from interstrain pairings could be the result of cross feeding between *nit* mutants and not from heterokaryosis, autoclaved cellophane (Dupont 193 PUDO) strips (3 cm) were occasionally placed between *nit* mutants from these strains. Cellophane prevents hyphal contact, but does not restrict nutrients from diffusing through.

Four digit numbers, followed by the first author's initials, were assigned to identify VCGs consisting of two or more strains that formed heterokaryons (Table 2). These codes will serve as temporary designations for the VCGs until comparisons with other known VCGs (5) can be made.

RESULTS

Pathogenicity tests. Seedling tests revealed that 85 of 97 strains incited lesions on cultivar U.C. 157 asparagus seedlings roots (Table 1). Extent of root lesion development per strain varied from less than 10–100%. Strains reported as pathogenic on onion (FO17, F110A); gladiolus (FO16, FO23, FO24); and self-blanching celery cultivars (A8) also were pathogenic on asparagus seedlings. Other strains pathogenic on cabbage (FO18, FO22), green celery cultivars (FA3), chrysanthemum (FO10), cotton (FO25, FO26), cucumber (FO27), melon (FO8), radish (FO19), sweet potato (FO30), watermelon (FO29), and one (MA49) that

TABLE 1. Origin, source, disease ratings, complementary *nit* mutant phenotypes, and heterokaryon ratings of strains of *Fusarium oxysporum* f. sp. *asparagi* and *F. oxysporum*

Strain/origin	Source	Path. test[v]	Phenotype[w] *nit*1	*nit*3	nitM	Intensity of het.[x]
MA1/Van Buren, MI	W. Elmer	4.7	**			W
MA2/Van Buren, MI	W. Elmer	4.6	*		*	S
MA3/Van Buren, MI	W. Elmer	3.0	*		*	S
MA4/Van Buren, MI	W. Elmer	4.4	*		*	S
MA5/Van Buren, MI	W. Elmer	4.3	*	*		W
MA6/Van Buren, MI	W. Elmer	2.6	*		*	S
MA7/Van Buren, MI	W. Elmer	4.1	*		*	S
MA8/Van Buren, MI	W. Elmer	3.4	*		*	S
MA9/Van Buren, MI	W. Elmer	4.0	*		*	S
MA15/Oceana, MI	W. Elmer	3.0	*		*	S
MA16/Oceana, MI	W. Elmer	2.3	*		*	S
MA21/Oceana, MI	W. Elmer	2.7	*		*	S
MA22/Oceana, MI	W. Elmer	2.3	*	*		S
MA23/Oceana, MI	W. Elmer	4.6	*		*	S
MA24/Oceana, MI	W. Elmer	2.6	*		*	S
MA25/Oceana, MI	W. Elmer	4.2	*		*	S
MA27/Clinton, MI	W. Elmer	4.8	*		*	S
MA28/Clinton, MI	W. Elmer	4.9	*		*	S
MA29/Clinton, MI	W. Elmer	4.8	*	*		S
MA30/Lapeer, MI	W. Elmer	5.0	*		*	S
MA31/Lapeer, MI	W. Elmer	4.8	*		*	S
MA32/Ingham, MI	W. Elmer	4.5	**			W
MA33/Ingham, MI	W. Elmer	2.3	*		*	S
MA34/Ingham, MI	W. Elmer	4.8	*		*	S
MA35/Ingham, MI	W. Elmer	4.0	**			W
MA36/Ingham, MI	W. Elmer	2.4	*		*	S
MA37/Ingham, MI	W. Elmer	5.0	*		*	S
MA38/Ingham, MI	W. Elmer	4.3	*		*	S
MA39/Ingham, MI	W. Elmer	4.0	*		*	S
MA40/Ingham, MI	W. Elmer	4.6	*	*		W
MA41/Ingham, MI	W. Elmer	4.6	*		*	S
MA42/Ingham, MI	W. Elmer	2.0	**			W
MA43/Ingham, MI	W. Elmer	3.0	*		*	S
MA44/Ingham, MI	W. Elmer	4.5	**			S
MA45/Ingham, MI	W. Elmer	3.1	*	*		S
MA47/Ingham, MI	W. Elmer	5.0	*		*	S
MA48/Ingham, MI	W. Elmer	4.5	*		*	S
MA49/Ingham, MI	W. Elmer	1.0	*		*	S
MA50/Ingham, MI	W. Elmer	5.0	*		*	S
MA51/Ingham, MI	W. Elmer	4.7	**			W
MA52/Ingham, MI	W. Elmer		*	*		W
MA53/Ingham, MI	W. Elmer	4.6	**			W
MA54/Ingham, MI	W. Elmer	5.0	*		*	S
MA55/Ingham, MI	W. Elmer	4.0	**			W
MA56/Ingham, MI	W. Elmer	4.0	**			W
MA76/Oceana, MI	W. Elmer	4.3	**			W
MA77/Oceana, MI	W. Elmer	3.0	*	*		W
MA79/Oceana, MI	W. Elmer	3.0	*		*	S
MA80/Oceana, MI	W. Elmer	2.3	*		*	S
MA86/Oceana, MI	W. Elmer	3.0	*		*	S
MA306/Oceana, MI	W. Elmer	4.2	*		*	S
F-10/Oceana, MI	W. Elmer	4.5	*		*	S
F-11/Oceana, MI	W. Elmer	4.6	*		*	S
NJB1/New Jersey[y]	W. Elmer	3.0	**			W
NJB2/New Jersey[y]	W. Elmer	3.7	*		*	S
NJB4/New Jersey[y]	W. Elmer	3.7	*	*		S
NJB5/New Jersey[y]	W. Elmer	4.0	*		*	S
NJP1/New Jersey[y]	W. Elmer	3.0	*		*	S
FMD/Maryland	T. Toussoun	2.1	*		*	S
WFO2/Washington	D. Johnson	4.6	*		*	S
WFO3/Washington	D. Johnson	4.6	*		*	S
MAAS2/ Massachusetts	K. Elias	3.6	*		*	S
MAAC3/ Massachusetts	K. Elias	4.5	*		*	S
QFO2/Quebec	M. Caron	2.5	*	*		S
QFO5/Quebec	M. Caron	2.7	*	*		W
QFO8/Quebec	M. Caron	3.2	**			W
QFO11/Quebec	M. Caron	2.9	**			W
QFO25/Quebec	M. Caron	3.7	**			W
T-29/Taiwan	C. Tu	5.0	*		*	S
T-143/Taiwan	C. Tu	3.6	**			W
T-190/Taiwan	C. Tu	4.6	*	*		W
T-207/Taiwan	C. Tu	5.0	*	*		W
T-236/Taiwan	C. Tu	3.3	*		*	S
F-61/France	P. Molot	3.6	**			W
F-84/France	P. Molot	2.0	*		*	S
F-cp3/France	P. Molot	2.1	*		*	S
F-cp5/France	P. Molot	2.1	*		*	S
F-nd4/France	P. Molot	2.5	**			W
FI-1100/Italy	M. Fantino	3.3	*		*	S
SFOE1/Spain	M. Castanon	2.0	*		*	S
A8[z]/France	J. Puhalla	3.8	*		*	S
FA3[z]/Michigan	W. Elmer	1.0	*		*	S
FO8[z]/Michigan	W. Elmer	1.0	*	*		S
FO10[z]/Michigan	T. Isakeit	1.0	**			W
FO16[z]/Michigan	G. Adams	4.3	*		*	S
FO17[z]/Michigan	W. Elmer	4.6	*		*	S
F110[z]/New York	M. Lacy	4.7	*		*	S
FO18[z]/Wisconsin	P. Bosland	1.0	*	*		S
FO19[z]/Wisconsin	P. Bosland	1.0	*	*		S
FO22[z]/California	P. Bosland	1.0	**			S
G1[z]/Italy	P. D. Lenna	2.2	*		*	S
G2[z]/Italy	P. D. Lenna	2.5	**			W
FO25[z]/Texas	K. Elias	1.0	*	*		W
FO26[z]/Louisiana	K. Elias	1.0	*	*		W
FO28[z]/Texas	K. Elias	1.0	*	*		W
FO29[z]/Texas	K. Elias	1.0	**			W
FO30[z]/Louisiana	K. Elias	1.0	**			W

[v] Values represent the mean of three pathogenicity tests on asparagus seedlings (cv. U.C. 157; three seedlings per test); disease was based on a 1–5 scale in which 1 = no disease, 2 = lesions present on 0–25% of the root system, 3 = lesions on 25–50% of the root system, 4 = lesions on 50–75% of the root system, and 5 = lesions on 75–100% of the root system.

[w] Phenotypes of the complementary *nit* mutant pair selected from each strain are designated with asterisks; *nit*1 = mutation in the locus for the structural nitrate reductase enzyme, *nit*3 = mutation in the pathway-specific regulatory locus for nitrate assimilation, and nitM = mutation in one of five loci for assemblage of the Mo-containing cofactor (27,32).

[x] Intensity of the heterokaryon: W = weak; faint or broken line of aerial mycelium at the point of anastomosis; S = strong; intense robust mycelial growth at the point of anastomosis.

[y] Strains from New Jersey were isolated from diseased asparagus crowns provided by Steve Johnston, Rutgers University.

[z] These strains were reported by their sources as the following formae speciales: A8 = *apii* race 1 (designated as belonging to VCG 0011 [6,38]); FA3 = *apii* race 2 (ATCC 52626, designated as belonging to VCG 0010 [6,38]); FO8 = *melonis* race 2; FO10 = *chrysanthemi*; FO16, G1, G2 = *gladioli*; FO17, F110 = *cepae*; FO18 = *conglutinans* race 1 (ATCC 52557 [3]); FO22 = *conglutinans* race 2 (ATCC 58385 [3]); FO19 = *raphani* (ATCC 58110 [3]); FO25, FO26 = *vasinfectum*; FO28 = *niveum*; FO29 = *cucurmerinum*; and FO30 = *batatas*.

was isolated from asparagus were nonpathogenic on asparagus seedlings. Root lesion development was usually detectable after 1 wk (41), but seedlings inoculated with MA49 had no distinct lesions on the roots after 3 wk.

Recovery and characterization of *nit* mutants. All strains produced sectors on chlorate media after 5–11 days. Although data on sectoring frequency per strain were not recorded, there were differences in the number of attempts required to recover a nitM phenotype from the different strains of *F. o. asparagi*. Sectoring frequency on chlorate has been shown in *F. moniliforme* to be heritable and to vary with the strain (26). The nitM phenotype was recovered from 57 of 97 (59%) strains, whereas the *nit*1 phenotype was always recovered (Table 1). From the remaining 40 strains from which nitM phenotypes were not recovered, *nit*1 plus *nit*1 or *nit*1 plus *nit*3 complementary *nit* mutant pairs were recovered; all strains were self-compatible (5,6,24).

When complementary *nit* mutant pairs from a parental strain were composed of phenotypes *nit*1 plus *nit*1, the resulting heterokaryon would frequently be less robust than between nitM plus *nit*1 mutants (Table 1). No heterokaryons developed from *nit*3 plus *nit*3 phenotypes in intrastrain pairings.

Vegetative compatibility groups. Twenty-seven strains of *F. o. asparagi* and one nonpathogenic strain (MA49) were placed into eight VCGs (1001WE–1008WE) based on their ability to form heterokaryons with other strains in that VCG (Table 2). Complementary *nit* mutants from the other 58 strains of *F. o. asparagi* and 11 strains from other formae speciales did not fall into any of these VCGs. Because nitM phenotypes were recovered from 34 strains that were virulent on asparagus, these strains appear to each belong to a unique VCG. VCG 1001WE was the largest, with seven members from very diverse geographical areas including Taiwan, Washington state, and three counties in Michigan. All other VCGs (1002WE–1008WE) contained two to six strains from four counties in Michigan.

No clear relationship was observed in *F. o. asparagi* between VCG and locality (Table 2). For example, in one asparagus planting in Ingham County, MI, 23 strains of *F. o. asparagi* were isolated of which 13 strains were placed into VCGs 1001WE–1005WE; the other 10 strains of *F. o. asparagi* did not fall into these VCGs. From this same field strain, MA49 was isolated and rated as nonpathogenic in three seedling tests, but was vegetatively compatible with other strains of *F. o. asparagi* and placed in VCG 1002WE.

Of nine strains of *F. o. asparagi* isolated from infected asparagus roots in a field in Van Buren County, MI, four strains were placed into two VCGs (VCG 1006WE and 1007WE), whereas the other five strains of *F. o. asparagi* were not vegetatively compatible with one another or with other strains.

Fifteen isolates of *F. o. asparagi* were recovered from Oceana County, but only six were placed into VCGs 1001WE–1003WE, 1008WE; the other nine strains each represented a unique VCG. However, of three isolates recovered from a single plant in Clinton County, none was vegetatively compatible. Likewise, at least three distinct VCGs were identified among four strains of *F. o. asparagi* that were isolated from one asparagus crown from Bridgeport, NJ. With the exception of strain T-143 from Taiwan and strain FO3 from Washington state, all strains of *F. o. asparagi* collected outside Michigan represented unique VCGs.

The intensity of heterokaryotic growth in interstrain pairings within a VCG would range from a weak line of mycelial growth at the hyphal contact zone to robust mycelial growth (Fig. 1). Most heterokaryons between *nit* mutants from different strains were weak and would become macroscopic after 10 days at 25 C. Placing autoclaved cellophane strips between *nit* mutants on MM prevented the weak wild-type growth from developing, but not in areas where the hyphae were allowed to make contact.

Several strains of *F. oxysporum* that were pathogenic on asparagus, but that had previously been designated as pathogenic on onion (FO17, F110A), gladiolus (FO16, Gl, G2), and self-blanching celery cultivars (A8) were each vegetatively incompatible with all other strains except strains G1 and G2 from Italy. Strains G1 and G2 were vegetatively compatible with each other. Furthermore, strains from all other formae speciales that were nonpathogenic on asparagus were not vegetatively compatible with any strains of *F. o. asparagi*.

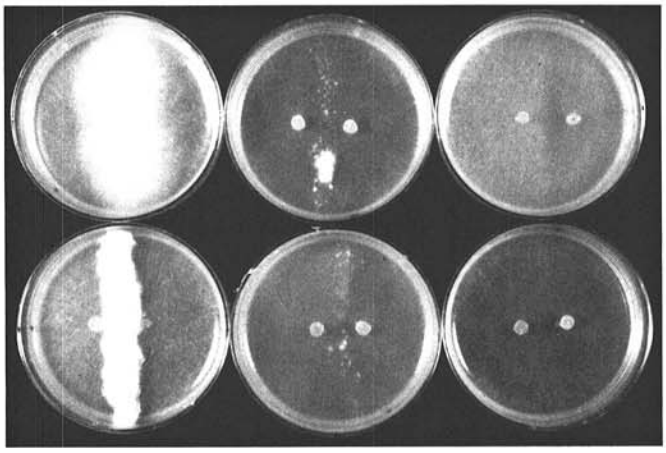

Fig. 1. Types of complementary growth observed between vegetatively compatible *nit* mutants of *Fusarium oxysporum* f. sp. *asparagi*. The column of plates on the left demonstrate strong heterokaryons formed between strains MA16 and MA50 (top) and between nitM and *nit*1 of F-11 (below); plates in the middle show weak heterokaryons formed between strains MA50 and MA34 (top) and between strains MA50 and MA49 (bottom); and plates on the right show no complementation between strains MA50 and FO3 (top) and between strains MA25 and FO16 (bottom).

DISCUSSION

Eight VCGs were identified among 28 strains with no specific pattern being observed between geographical origin and VCG. Thirty-four strains of *F. o. asparagi*, from which a nitM phenotype was recovered, each belonged to a unique VCG, which suggests that at least 43 VCGs of *F. o. asparagi* exist. The largest VCG contained strains from Taiwan, Washington state, and three counties in Michigan. It is not immediately apparent why strains in this VCG (1001WE) were detected in areas that were proximal to

TABLE 2. Vegetative compatibility groups, representative strains, and their respective localities of *Fusarium oxysporum* f. sp. *asparagi*

VCC[w]	Tester strain[x]	Localities	Strains
1001WE	MA25	Taiwan	T-143
		Washington	FO3
		Oceana, MI	MA25
		Lapeer, MI	MA30, MA31
		Ingham, MI	MA36, MA48
1002WE	MA16	Oceana, MI	F-10[y], MA16
		Ingham, MI	MA34, MA39, MA49[z], MA50
1003WE	MA24	Ingham, MI	MA33, MA47
		Oceana, MI	MA24
1004WE	MA43	Ingham, MI	MA43, MA44, MA45
1005WE	MA54	Ingham, MI	MA32, MA54, MA55
1006WE	MA9	Van Buren, MI	MA1, MA9
1007WE	MA6	Van Buren, MI	MA3, MA6
1008WE	MA23	Oceana, MI	MA22, MA23

[w] VCG = Vegetative compatibility groups; numbers represent a temporary VCG code followed by author's initials.

[x] These eight strains were chosen to represent each respective VCG; wild-type strains and nitM mutants have been deposited at the Fungal Genetics Stock Center, Kansas City Medical Center, Kansas City, KS, under the following accession numbers: MA25 = 6607; MA25 nitM = 6608, MA16 = 6609, MA16 nitM = 6610, MA24 = 6611, MA24 nitM = 6612, MA43 = 6613, MA43 nitM = 6614, MA54 = 6615, MA54 nitM = 6616, MA9 = 6617, MA9 = 6617, MA9 nitM = 6618, MA6 = 6619, MA6 nitM = 6620, MA23 = 6621, MA25 nitM = 6622.

[y] Strain F-10 = ATCC 38818.

[z] MA49 was rated as nonpathogenic on asparagus seedlings.

other vegetatively incompatible, but equally virulent, strains. However, it is known that *F. o. asparagi* is seedborne (13,22) and carried on the roots and crowns of transplants (4,21); these patterns of dissemination may have aided in long-distance transport of certain VCGs of *F. o. asparagi* into fields where other virulent strains existed. Because fields never planted to asparagus are known to harbor a resident population of *F. o. asparagi* (20; unpublished), it may explain how such genetic diversity among pathogenic strains of *F. o. asparagi* could exist within an asparagus planting.

Strains within a VCG are usually more similar genetically than strains in different VCGs (12). However, strain MA49 was rated as nonpathogenic in three different seedling tests, but formed heterokaryons with all other virulent members in that VCG (1002WE). This response was also observed with other asparagus cultivars (unpublished). It is not unreasonable to expect some genetic heterogeneity within a VCG because variation could arise in individual strains after the teleomorph was lost. It is interesting that this genetic variation is in virulence traits. Such heterogeneity in virulence has been reported for strains in other VCGs of *F. oxysporum* (3,9,16,24,36). We recognize that subculturing could have rendered strain MA49 avirulent; however, this explanation is questioned inasmuch as 23 other isolates retrieved from this field in the same manner each retained their virulence. It is possible that the virulence trait was lost in strain MA49's progenitors whereas the vegetative compatibility loci (*vic* or *het*) were retained. Additional studies are needed to confirm if other nonpathogenic strains can share VCGs with *F. o. asparagi*. Such nonpathogenic strains may be so closely related to virulent strains that they may be useful in biological control by competing with and excluding virulent strains from niches in the root and rhizosphere. Additionally, these strains would be valuable in studies on the molecular genetics of virulence on asparagus.

Our strain of *F. o. apii* race 2 (FA3) from Michigan was nonpathogenic on asparagus, whereas the *F. o. apii* race 1 (A8) isolate from France was pathogenic. Puhalla (37) doubted that these races of *F. o. apii* arose from the same progenitor. *F. o. apii* race 1 contains at least two VCGs (5), whereas *F. o. apii* race 2 belongs to a single VCG (7). If all VCGs of *F. o. apii* race 1 are pathogenic on asparagus, soils in Michigan that were heavily infested with *F. o. apii* race 1 during the 1910s–1950s (34) should be avoided for asparagus culture. Armstrong and Armstrong (2) first described the virulence of their isolate of *F. o. apii* race 1 on asparagus crowns and stated that common genes for pathogenicity probably exist in many formae speciales. Because the reciprocal pathogenicity tests of our *F. o. asparagi* strains on other crops were not done, it is not known if these strains infect multiple hosts. However, Graham (20) demonstrated that his strains of *F. o. asparagi* would incite lesions on gladiolus bulbs.

Although only a limited number of strains were examined, our findings suggest that the virulence trait in *F. o. asparagi* is not confined to only a few VCGs. Instead, virulence in *F. o. asparagi* on asparagus may be a less specialized trait that is common to many VCGs in *F. oxysporum*. These results are in contrast to those of other researchers working with VCGs of other formae speciales in which VCG has been correlated with formae speciales or race (3,7,9,16,24,25,36,38), or with formae speciales from a specific locality (16,24,36). Identifying field isolates of *F. o. asparagi* by vegetative compatibility would be impractical because the number of VCGs that could exist could be very large and unmanageable. Also, there is no assurance that vegetatively compatible isolates recovered are pathogenic. Pathogenicity tests on asparagus plants may continue to be the most reliable means of identifying this forma speciales. Nevertheless, because so many VCGs of *F. o. asparagi* might exist, strains from rare VCGs may be very useful in epidemiological studies.

The pattern noted in this study closely resembled that discovered by Correll et al (8) with nonpathogenic strains of *F. oxysporum* colonizing celery roots. Correll et al (8) placed 50 out of 110 strains of *F. oxysporum* into 14 VCGs, but also found many strains, each of which appeared to be a unique VCG. Additional pairings between representatives of other VCGs (5) and those reported here may provide a better understanding of the nature of virulence on asparagus.

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