Vegetative Compatibility Groups of *Fusarium proliferatum* from Asparagus and Comparisons of Virulence, Growth Rates, and Colonization of Asparagus Residues Among Groups

Wade H. Elmer

Assistant plant pathologist, Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, 06504.

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ABSTRACT

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Nitrogen metabolism mutants were selected from 110 isolates of *Fusarium proliferatum* isolated from four asparagus plantings during 1985 and 1987–1989. These nitrogen metabolism mutants were used in complementation tests for vegetative compatibility. Twenty vegetative compatibility groups (VCGs) were identified, but most (88) isolates fell into six VCGs that were found at more than one location. Isolates in the three most common VCGs (VCGs 5, 7, and 8) contained 39, 15, and 10% of the isolates, respectively, and were found at all locations. VCGs 13, 1, and 4 were found less frequently and comprised 5, 4, and 4% of isolates, respectively. Isolates from the six VCGs were compared for virulence on asparagus, for rates of radial growth on a minimal medium at 7, 12, 17, and 22 C, and for the rate that they colonized asparagus residues at 10 C and at 20 C. VCG was not correlated with virulence, or with radial growth rates at any temperature. Isolates in VCG 5 colonized asparagus residues at a higher rate than isolates in other VCGs, which may contribute to their frequent recovery in asparagus fields. More variation was found among VCGs in their rates of colonizing asparagus residues than within a VCG, which may indicate that these VCGs are genetically isolated and asexually propagated. There was no strong correlation between VCG and field. The frequent recovery of VCGs 5, 7, and 8 in many different plantings suggests that these VCGs are selectively maintained within and among asparagus fields.

Additional keywords: Asparagus officinalis, Fusarium crown and root rot, heterokaryosis.

Fusarium proliferatum (T. Matsushima) Nirenberg is one of several *Fusarium* spp. that causes a disease of asparagus (*Asparagus officinalis* L.) known as Fusarium crown and root rot (4,8,11,19) in the northeast United States. Although, until recently, *F. proliferatum* was taxonomically synonymous with *F. moniliforme* J. Sheld (27) but was separated by the presence of polyphialides in *F. proliferatum*, *F. moniliforme* only contains monophialides.

Symptoms of Fusarium crown and root rot commonly appear in mid to late season and include chlorosis and wilt of ferns, accompanied by crown rot and eventual plant death. Because asparagus plantings are perennial, the number of systemically infected crowns and ferns increases as the plantings age. *F. proliferatum* colonizes both vascular and epidermal tissue (24) and is highly pathogenic to asparagus transplants (11).

Although the teleomorph of *F. proliferatum* has not been described (27), isolates of *F. proliferatum* can sexually cross and produce perithecia of *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura (23,25). The perithecia of this fungus, however, have never been found in nature. The principle means of propagation is asexual, but it is conceivable that ascospores infrequently provide inoculum.

Vegetative incompatibility is a genetic trait controlled by *vic* loci (1,20,31); the hyphae of two vegetatively incompatible isolates are unable to fuse and form heterokaryons. At least 10 *vic* loci are known to control this trait in *F. moniliforme* (31). Vegetatively compatible isolates form heterokaryons when both isolates contain identical alleles at each of the *vic* loci (31). Compatible types form vegetative compatibility groups (VCGs). Given 10 *vic* loci with two alleles at each locus, sexual recombinations could produce 2^{10} or 1,024 different arrangements that could theoretically produce the same number of VCGs. Genetic exchange by parasexual recombination can only occur between vegetatively compatible isolates; therefore, isolates in each VCG are genetically separated from isolates in other VCGs. In addition, the evolutionary consequences of restricting gene flow to isolates within a VCG would give an advantage to VCGs sharing traits for competing in a particular niche. With these assumptions, one may expect less variation for a particular trait among isolates of *F. proliferatum* within a VCG than between VCGs, as has been demonstrated in other asexual fungi (3,7).

Sidhu (32) used nitrate nonutilizing (*nit*) mutants (5,20) from 38 corn and sorghum isolates of *F. moniliforme* in complementation tests for heterokaryosis and found 13 VCGs. He postulated that genes for pathogenicity influence which VCG types will be promoted or demoted in a population. LaMondia and Elmer (24) identified 13 VCGs from 97 isolates of *F. moniliforme* recovered from three asparagus plants in Windsor, CT. The 13 VCGs identified by LaMondia and Elmer were not equally represented, and, in pathogenicity tests on asparagus seedlings, the largest VCGs could not be characterized by higher virulence. It was later determined that isolates in these VCGs contained polyphialides and were therefore redesignated as VCGs in *F. proliferatum*. Inasmuch as this survey was confined to three plants, it was not known if these findings were typical of the VCG structure in other asparagus fields.

The objectives of this study were to examine populations of *F. proliferatum* from asparagus fields in different areas for their VCG composition; to compare representative isolates assigned to VCGs recovered from different sites for competitive traits, such as virulence, growth rates on agar media, and colonization of asparagus residues; and to compare the relative distribution of variance among and within VCGs for each of the aforementioned traits.

MATERIALS AND METHODS

Collection of isolates. A total of 110 isolates of *F. proliferatum* were isolated from the base of chlorotic asparagus ferns from fields of varying ages (Table 1). Two asparagus fields in Connecticut were approximately 35 miles apart: a young vigorous

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planting, and an abandoned 30- to 35-yr-old asparagus field. The Massachusetts field was a typical 7- to 10-yr-old asparagus planting. The four sampled fields in Hart Co., Michigan were commercial plantings between 4 and 10 yr old that were in close proximity to each other, and were therefore grouped as representative of that area.

Four or five wedge-shaped pieces of tissue no larger than 0.5 × 0.5 cm were removed from the base of a chlorotic stem, surface-treated with 0.53% Na hypochlorite (10% household bleach) for 1 min, rinsed with distilled water, and placed on Komada's medium, which is selective for *Fusarium* spp. (22). Plates were incubated in plastic bags on laboratory benches (20–24 C) for 5–7 days. The dominant colonist was *F. proliferatum*, which grew profusely from the tissue. Isolates were established from single conidia that were subcultured onto potato-carrot agar (PCA) (10). Each isolate came from a different plant.

Vegetative compatibility tests. Isolates were placed in VCGs by complementation tests for heterokaryon formation using *nit* mutants (5,20). Although the techniques for selecting *nit* mutants and phenotypically characterizing them as *nit*1, *nit*3, and nitM types have been reported for *F. oxysporum* Schlectend.:Fr. (5) and for *F. moniliforme* (20), the procedure will be briefly described. Agar plugs of minimal medium (MM) (31) colonized by each isolate were transferred to potato-dextrose agar containing 1.5% potassium chlorate and incubated for 14 days at 18–25 C. After 5–14 days, rapidly expanding sectors that grew away from the restricted growth were transferred to MM, where they grew as thin expanding colonies without aerial mycelium. *Nit* mutants were classified into one of three classes by culturing each *nit* mutant on nitrite agar (MM modified by replacing sodium nitrate with sodium nitrite, 0.5 g/l) or on hypoxanthine agar (MM modified by replacing sodium nitrate with hypoxanthine, 0.2 g/l). *Nit*1 mutants grew as wild types on all media except MM, and nitM mutants only grew as wild types on nitrite agar, whereas *nit*3 mutants only grew as wild types on hypoxanthine agar (5,20). When *nit*1 and nitM mutants from the same isolate were paired on MM, robust heterokaryotic growth would develop at the point of anastomosis. Complementation tests were conducted as described by Klittich and Leslie (21). Multiwell plates (Falcon No. 3047; Becton Dickinson Co., Lincoln Park, NJ) containing 24 wells of MM were uniformly spread with a spore suspension (10^5–10^6 conidia per milliliter) of a nitM mutant from one isolate that had been cultured on MM. Each well then received a spore suspension of a *nit*1 mutant from another isolate. Positive and negative controls were always included. Plates were incubated for 7–10 days on laboratory benches. When paired *nit* mutants gave rise to wild type growth, the two *nit* mutants were placed 2–3 cm apart on petri plates filled with MM. The presence of dense wild type growth at the point of hyphal contact was evidence of heterokaryosis and vegetative compatibility.

Complementary pairs of *nit*1 and nitM mutants from isolates of *F. proliferatum* used to represent VCGs in past studies (24) were included to add to the preexisting VCG framework. Compatible isolates were assigned to known VCGs when they complemented the *nit* mutants of the isolate representing that VCG or were assigned to new VCGs when two or more isolates were compatible with each other. All isolates and *nit* mutants were stored in a refrigerator on silica gel (35) or were placed in 15% glycerol and stored at −40 C (36). Representative isolates from VCGs 1, 4, 5, 7, 8, and 13 were confirmed to be *F. proliferatum* by P. E. Nelson, Pennsylvania State University, and deposited at the Fusarium Research Center, Pennsylvania State University, University Park, under accession numbers: M-6372, M-6371, M-6374, M6375, M-6376, and M-6373, respectively.

Virulence tests. Conidial suspensions of the selected isolates were prepared from young cultures growing on petri dishes filled with MM. Each plate was seeded with an agar plug colonized by one of the isolates, incubated at 22 C in the dark for 10 days, and washed twice with 20 ml of sterile distilled water. Suspensions were filtered through four layers of sterile cheesecloth and diluted to 1×10^7 conidia per milliliter after counting with a hemacytometer.

Fusarium-free asparagus seeds (cv. Mary Washington) were germinated under axenic conditions described elsewhere (13). Seedlings were grown in potting mix (ProMix BX, Premier Brand, New Rochelle, NY) for 10 wk. Transplants were washed and culled to uniform individuals and then placed into 10-cm plastic pots filled with washed sand (one plant per pot). One week after transplanting, 100 ml of the conidial suspension was poured around the base of each plant. The process was repeated 1 wk later. Three pots were treated with each isolate and the noninoculated controls were treated with distilled water. Each pot received an application of 100 ml of Hoagland's solution (16) every 10–14 days. Three months later, plants were removed from the pots, washed in tap water, and weighed. Disease severity, expressed as the percentage of discolored or rotted roots, was determined on each plant by estimating the total root length on a 2- × 2-cm grid using the modified line intersect method (34). The length of discolored and rotten roots was estimated using the same

TABLE 1. Number and percentage of isolates of *Fusarium proliferatum* from different asparagus fields assigned to vegetative compatibility groups (VCGs)

	Hamden, CT		Southington, CT		Whately, MA		Hart, MI		Total	
VCG[z]	No.	%	No.	%	No.	%	No.	%	No.	%
1	2	4	0	0	0	0	3	23	5	4
4	4	7	0	0	0	0	0	0	4	4
5	23	39	5	29	11	50	6	46	45	39
7	5	9	6	35	4	18	2	15	17	15
8	4	7	4	23	1	5	2	15	11	10
12	2	3	0	0	0	0	0	0	2	2
13	5	9	1	6	0	0	0	0	6	5
14	2	4	0	0	0	0	0	0	2	2
15	1	2	0	0	0	0	0	0	1	1
16	1	2	0	0	0	0	0	0	1	1
17	1	2	0	0	0	0	0	0	1	1
18	1	2	0	0	0	0	0	0	1	1
19	0	0	1	6	0	0	0	0	1	1
20	1	1	0	0	0	0	0	0	1	1
21	1	1	0	0	0	0	0	0	1	1
22	1	1	0	0	0	0	0	0	1	1
23	2	3	0	0	0	0	0	0	2	2
24	0	0	0	0	4	18	0	0	4	5
25	0	0	0	0	2	9	0	0	2	2
26	2	4	0	0	0	0	0	0	2	2
total	58	100	17	100	22	100	13	100	110	100

[z] VCGs identified by complementation tests using nitrate nonutilizing mutants (5,21); VCGs 2, 3, 6, 9, 10, and 11 were not found in these surveys.

technique. The experiment was repeated once with similar results.

Residue colonization studies. Clear plastic cups (4×4 cm in diameter) were filled with approximately 28 g of sterile dry sand. Conidia from the selected isolates were prepared as described above and diluted in distilled water, and 8 ml of the resulting suspension was incorporated into the sand to yield approximately 5×10^4 conidia per gram of sand. Cups were capped and placed in the incubator in the dark at 10 or 20 C for 4–6 days. Small amounts of infested sand were then transferred with a spatula to PCA (10) to confirm the presence of the fungus.

TABLE 2. Origin of *Fusarium proliferatum* isolates in different vegetative compatibility groups (VCGs), their effect on mean disease ratings and fresh weights of asparagus plants, and comparisons of variance components for isolates within a VCG and among VCGs

Isolate	Origin	Fresh plant weights	Disease[x] rating
VCG 5			
87-9	Hamden, CT	2.60 a[z]	40 a
NF27	Whately, MA	2.37 a	56 a
SA38	Southington, CT	2.53 a	55 a
88-15-1	Hamden, CT	1.90 a	59 a
93WT	Windsor, CT[y]	1.97 a	65 a
Mean		2.27 (a)	55 (a)
VCG 7			
87-28	Hamden, CT	2.33 a	45 a
153WT	Windsor, CT[y]	2.63 a	58 a
88-3-3	Hamden, CT	1.80 a	51 a
SA23	Southington, CT	2.33 a	42 a
NF21	Whately, MA	1.70 a	60 a
Mean		2.10 (a)	51 (a)
VCG 8			
172WT	Windsor, CT[y]	2.10 a	37 a
87-5	Hamden, CT	2.10 a	55 a
NF8	Whately, MA	1.67 a	52 a
SA25	Southington, CT	1.60 a	38 a
SA45	Southington, CT	1.73 a	48 a
Mean		1.80 (a)	46 (a)
VCG 13			
87-46	Hamden, CT	2.10 a	44 a
87-37	Hamden, CT	2.10 a	48 a
88-34	Hamden, CT	2.07 a	33 a
78WT	Windsor, CT[y]	1.97 a	41 a
88-37	Hamden, CT[x]	2.03 a	49 a
Mean		2.10 (a)	43 (a)
VCG 1			
88-14-2	Hamden, CT	2.17 a	63 a
88-39	Hamden, CT	1.90 a	60 a
24WT	Windsor, CT[y]	2.43 a	39 a
Mean		2.20 (a)	54 (a)
VCG 4			
87-31	Hamden, CT	1.67 a	55 a
87-38	Hamden, CT	2.23 a	37 a
WT11	Windsor, CT[y]	2.43 a	39 a
Mean		2.18 (a)	51 (a)
Noninoculated Control		3.73 b	8 b

Source	df	ms	F-value	Variance component
Nested analysis of variance for fresh weights				
Between VCGs	5	0.33	1.15^{ns}	0.0
Between isolates within a VCG	20	0.29	0.86^{ns}	0.0
Error	52	0.32		
Nested analysis of variance for percent disease				
Between VCGs	5	0.03	1.0^{ns}	0.0
Between isolates within a VCG	20	0.03	1.0^{ns}	0.0
Error	52	0.03		

[x] Disease ratings based on the percentage of discolored roots determined by modified line intersect method.

[y] Isolates 11WT, 24WT, 78WT, 93WT, 153WT, and 172WT were recovered in a previous study, and deposited at Fusarium Research Center under accession numbers: M-6371, M-6372, M-6373, M-6374, M-6375, and M-6376, respectively.

[z] Values followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$; letters in parentheses pertain to statistical tests on VCG means.

Asparagus (cv. Mary Washington) stalk residues were recovered from a field in February 1990 and air-dried at 75 C for 24 h. Pieces of the outer epidermal stem tissue were separated from the inner pith tissue, and the outer stem pieces were cut to 0.2–0.4 $\times$ 3 cm and autoclaved for 1 h. (Later experiments used stem pieces that were not autoclaved. Samples from these stem pieces were incubated for 1 wk on Komada's selective media (22) and were observed to be free of *Fusarium* spp.) Ten stem pieces were vertically inserted approximately 1.0 cm into the sand in each cup. Stem pieces were also placed into cups containing sand and distilled water to serve as controls. The cups were capped and returned to the incubators.

Stem pieces were removed after 1, 2, 3, and 4 days of incubation at 10 and 20 C. The stem portion in contact with the sand plus the adjacent 0.5 cm was aseptically removed and discarded. The upper portion was treated with 0.5% sodium hypochlorite (10% household bleach) for 1 min and rinsed with distilled water. Pieces were blotted dry, placed onto PCA (10), and incubated in plastic bags on laboratory benches (18–25 C). After 5 days, the number of pieces colonized by *F. proliferatum* were counted and expressed as percentage of residue colonized. Three replicate cups per isolate were used, and the experiment was repeated using 16 g of autoclaved 1:1 sand-potting mix instead of sand. Before analysis, homogeneity of variance was established by transformation to the arcsine of the square root. Both repetitions of the experiment were included in the analysis.

Radial growth studies. Radial fungal growth was measured on plates of MM incubated at different temperatures. A colonized agar plug (4 mm in diameter) was removed with a no. 1 cork borer from the actively growing margins of each isolate, and transferred to the centers of petri plates (1.5×10 cm) filled with MM (31). Plates were inverted and incubated in the dark at 7, 12, 17, and 22 C. The largest and smallest colony diameters were measured to the nearest mm on three replicate plates, every 3 days for plates held at 7 C, every 2 days for plates held at 12 and 17 C, and daily for plates held at 22 C. Measurements continued until the hyphae reached the edge of the plate (measurements included the 4-mm agar plug). Strong correlations ($R^2 =$ .99–1.000) existed between mean radial growth and time. Slopes of each growth curve were calculated for each replicated isolate at each temperature and were expressed as the mean growth (mm) per day. The standard error of each slope was negligible.

Statistical procedures. Virulence experiments were analyzed as one-way nested designs, whereas data on radial growth and colonization of asparagus residues were analyzed as factorial nested designs (33), with means compared by Duncan's multiple range test. Temperature, sampling time, and VCGs were assumed to be fixed variables (meaning they do not represent all temperatures, all times from which a sample could be taken, or all VCGs), whereas isolates within VCGs were considered random and accepted as representative of the population in that VCG. Variance components (33) were computed to assess whether isolates within a VCG had less variation than among VCGs. In a mixed model, it is not valid to directly compare variance components from random and fixed variables or to assume that variance components are proportional to the total variance of the experiment. They are presented with caution, however, to demonstrate the relative distribution of variation in these experiments.

RESULTS

Vegetative compatibility tests and VCG distribution. *Nit* mutants emerged from restricted growth on chlorate media after 5–11 days, with an average of 2 sectors per colony. Most *nit* mutants recovered were *nit*1 type, whereas *nit*3 and nitM mutants were rare. All *nit*1 and nitM mutants from the same isolate were self compatible and would form dense heterokaryotic growth when paired on MM.

Out of 110 isolates of *F. proliferatum* collected and used in complementation tests, 88 isolates fell into one of six previously described VCGs (25) (Table 1). The other 22 isolates fell into 14 new VCGs. All new VCGs contained less than 4 isolates recovered from the same planting at the same time.

The percentage of isolates belonging to each VCG was computed for each location (Table 1). Isolates in VCGs 5, 7, and 8 were common and were recovered in every sampled site. They comprised 39, 15, and 10%, respectively, of the total isolates assigned to VCGs. Most of the isolates recovered from asparagus ferns in Hamden, CT, belonged to the VCG 5 type. VCG 13 was represented in all sites (except Whately, MA), and VCG 1 was found only in Hamden and Hart Co., MI. Isolates in VCG 4 were found only in Hamden. No strong correlation was observed between VCG and field.

Virulence studies. Isolates were selected from VCGs that were found at at least two different sites (Table 2). Isolates from VCG 4 were also included because of their relatively high occurrence in a past survey (24). Five isolates were selected from the more common VCGs (VCGs 5, 7, 8). Five isolates were also selected from VCG 13, whereas only three isolates were chosen from VCG 1 and VCG 4, because fewer isolates were recovered from these VCGs. Isolates were chosen to represent different areas and included those isolates that were recovered previously in Windsor, CT (24).

All isolates of *F. proliferatum* incited 33–65% root rot on asparagus transplants (Table 2). Similarly, all isolates reduced the final plant weights by the same relative amount when they were compared to noninoculated plants. The variance components for fresh weights and the percentage of diseased roots derived for both sources (isolates within a VCG and among VCGs) were 0.

Colonization of asparagus residues. No isolate could be recovered from asparagus residues after 1 or 2 days at 10 C, but many were isolated after 3 days (Table 3). Colonization at 20 C was much faster than at 10 C and was detectable after 2 days. The average frequency of recovery after 3 days at 20 C was roughly twice that after 4 days at 10 C. All stem residues were colonized by *F. proliferatum* after 4 days at 20 C. At 10 C, isolates in VCG 5 were among the fastest in colonizing the tissue, but differences were slight. After 2 days at 20 C, isolates in VCG 5 invaded the asparagus stems significantly faster than isolates in other VCGs. On the third day, approximately 80% of all stem residues were colonized by the isolates.

Consideration of the nested analysis of variance for the colonization of asparagus residues revealed that the time of sampling accounted for the majority of the variance (Table 3). As expected, the temperature and the interaction between the time of sampling and the temperature also contributed considerable variation to the isolate recovery. When considering the genetic sources of variance, three times as much variation was detected among VCGs as within a VCG.

Radial growth studies. No significant differences in growth rates were detected between VCGs at any temperature (data not shown). The regressions for the radial expansion (mm) (Y) for all isolates over time (days) (X) at 7, 12, 17, and 22 C were: $Y = -0.18 + 1.56X$, ($R^2 = .98$); $Y = 1.17 + 3.42X$, ($R^2 = .98$); $Y = -1.58 + 7.26X$, ($R^2 = .98$); and $Y = 0.88 + 10.02X$, ($R^2 = .99$), respectively. The averaged growth rate increased 2.94 mm/day per degree C. Similarly, there was no appreciable difference between the variance components for isolates within a VCG (0.178 mm) and among VCGs (0.183 mm).

DISCUSSION

Using *nit* mutants of *F. proliferatum* in complementation tests, 20 VCGs were identified out of 110 isolates recovered from asparagus fields in Connecticut, Massachusetts, and Michigan; however, six VCGs comprised 79% of all isolates. Isolates assigned to VCGs 5, 7, and 8 were found at every site, whereas isolates belonging to VCGs 1, 4, and 13 were represented less frequently. These six VCGs also contained 65% of the isolates sampled from three asparagus plants in one field in Windsor, CT, but, in that survey, isolates in VCG 4 were the most frequent (24). These results suggest that these VCGs may be the dominant colonists in asparagus plantings.

The presence of the same VCGs (VCG 5, 7, and 8) in different asparagus fields indicates that these types are being selectively maintained. The less frequent VCGs (VCGs 1, 4, and 13) may lack competitive traits necessary to ensure their dispersal and survival with an asparagus planting. Sidhu (32) postulated that the host would ultimately select for VCGs in *F. moniliforme* with phenotypes adapted for surviving within a population; VCGs that contained highly virulent strains would quickly become extinct after destroying their host while less virulent types would survive. Although no differences in virulence were detected in these studies,

TABLE 3. Percentage of asparagus stem residues colonized by isolates of *Fusarium proliferatum* in different vegetative compatibility groups (VCGs) and comparisons of variance components for isolates within a VCG and among VCGs

	Percent fungal recovery							
	10 C days				20 C days			
VCG	1	2	3	4	1	2	3	4
1	0	0	3.3 b[x]	47.8 ab	0	20.0 b	76.7 a	100.0
4	0	0	13.2 ab	37.7 ab	0	6.7 b	81.6 a	100.0
5	0	0	15.3 a	56.3 a	0	52.3 a	86.3 a	100.0
7	0	0	6.7 b	30.6 b	0	14.7 b	83.1 a	100.0
8	0	0	1.5 b	36.7 ab	0	20.1 b	84.0 a	100.0
13	0	0	13.9 ab	25.4 b	0	16.7 b	84.7 a	100.0

Source	df	ms	F-value	Variance component
Nested analysis of variance				
Between VCGs	5	32.30	5.7**[y]	0.53
Between isolates within a VCG	20	5.62	1.6*	0.16
Sampling time[z]	1	1737.75	479.4***	11.49
Sampling time *VCG	5	5.88	1.6	0.09
Temperature	1	654.84	180.7***	4.31
Temperature*VCG	5	7.90	2.2	0.17
Sampling time*temperature	1	254.34	69.3***	3.28
Error	268	3.49		

[x] Values represent the mean of six replicates from two experiments; one replicate represents the percentage of ten asparagus residues; values were transformed to the arcsine of the square root before analysis; values in columns followed by differing letters are significantly different by Duncan's multiple range test at $P = 0.05$.

[y] Values followed by *, **, or *** denote significance at $P = 0.05$, 0.01, or 0.001, respectively.

[z] Only data from two sampling times were included for each temperature.

these assays for measuring virulence could not reflect the natural course of disease progress over several years as it occurs in nature; therefore, these data may not prove or disprove the assumption that genes for pathogenicity on asparagus can affect the VCG composition of *F. proliferatum*.

F. proliferatum is also a colonist of corn (23,25). Inasmuch as isolates of *F. moniliforme* from corn and asparagus were virulent in cross-pathogenicity tests (9), similar traits may also exist in isolates of *F. proliferatum*. If *F. proliferatum* share hosts in nature, it may not be surprising to recover the same VCGs from different hosts. Many examples exist where isolates in a VCG infect different hosts. VCGs in *F. oxysporum* f. sp. *apii* (R. R. Nelson & Sherb.) W. C. Snyder & H. N. Hans. contain isolates that infect asparagus (12). Similarly, one VCG of *Verticillium dahliae* Kleb. contained isolates pathogenic on several different hosts (30). Strains that have successfully adapted to one host may also survive beyond the point of introduction in other host environments and be maintained at lower frequencies. The disproportionately large number of small VCGs of *F. proliferatum* found at single sites may represent recent introductions from other host environments. Surveys comparing the VCG structure on several hosts are needed to support this hypothesis.

Traits other than virulence may also influence the relative occurrence of a pathogen. Since these fungi survive as colonists of plant debris (14,26), isolates that more rapidly invade uncolonized residues may increase their probability of surviving, sporulating, and competitively colonizing new tissue. LaMondia and Elmer (24) reported that 28% of asparagus tissue sampled in late season was not colonized by *Fusarium* spp., suggesting that tissue is available if not colonized by other organisms. One explanation for isolates in VCG 5 being frequently recovered from several asparagus fields could be that they colonized asparagus residues faster than isolates in other VCGs. Moreover, *F. proliferatum*, like *F. moniliforme*, is a contaminant of asparagus seed (17), which suggests that isolates that could increase their rate of colonizing flowers and berries would increase their capacity for dispersal. This hypothesis, however, would not account for the prevalence of VCGs 7 and 8, which were slower in colonizing asparagus residues. Other unexamined traits, such as increased sporulation and greater survival in plant debris or in the guts of insect vectors (15), may possibly explain why these other VCGs are selectively maintained in asparagus plantings.

Radial growth rates have been correlated with VCGs in asexual fungi (3,29). The lack of significant differences observed in these studies suggests that this particular trait is relatively stable. This may indicate that radial growth rates on MM have no influence on the relative occurrence of a VCG in an asparagus field. Butcher et al (3) found differences in radial growth rates among VCGs of *Aspergillus nidulans* (Eidam) G. Wint., but did not attempt to relate their findings to the population structure. In addition, Ploetz and Shokes (29) found that different VCGs of *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. f. sp. *meridionalis* (G. Morgan-Jones) varied in growth rates in vitro, but rapid growth was not correlated with the more prevalent VCGs.

Asexual fungi usually have fewer VCGs within a locale than sexually reproducing fungi (1,2,6,18,28,30,37). If the perithecia of *F. proliferatum* (*Gibberella fujikuroi*) are functional in asparagus fields, and if these isolates contain all alleles on at least 10 *vic* loci, as in *F. moniliforme*, then the ascospores released could create a possible 1,024 VCGs (31). These VCGs would all differ in *vic* loci, whereas genes for other traits, such as competitive saprophytic ability, would be randomly distributed throughout isolates in all the VCGs. In this study, most of the isolates that were sampled were assigned to six VCGs, and isolates within a VCG had less variation in colonizing asparagus residues than among VCGs; therefore, it is likely that sexual reproduction in nature is limited and that VCGs of *F. proliferatum* are genetically isolated.

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