Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues

J. A. LaMondia and W. H. Elmer[1]

Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504, U.S.A.

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Isolates of *Fusarium moniliforme* (Sheld.) emend. Snyd. & Hans., *F. oxysporum* (Schlecht) emend. Snyd. & Hans., and *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. were recovered from three 5-year-old field grown asparagus (*Asparagus officinalis* L. cv. Mary Washington) by isolating from symptomatic and asymptomatic feeder roots, storage roots, crown and basal stem segments. *Fusarium moniliforme* was more virulent than *F. oxysporum* on asparagus seedlings and *F. solani* was considered nonpathogenic. Isolates of *F. moniliforme* and *F. oxysporum* were placed into vegetative compatibility groups (VCGs) by demonstrating heterokaryosis with complementation tests using nitrate-nonutilizing (*nit*) mutants (pairing nitM and *nit1* mutants). Ninety-seven of 135 isolates of *F. moniliforme* were placed in 13 vegetative compatibility groups. The remaining 38 isolates were not classified by vegetative compatibility because of poor *nit* mutant recovery. Eight of 18 isolates of *F. oxysporum* were unique and classed as single members of eight different VCGs. The other 10 isolates were not placed in VCGs. All isolates of *F. moniliforme* were virulent, but mean disease ratings differed among the isolates in different VCGs. There was no correlation between vegetative compatibility group and tissue substrate or symptom expression on the tissue substrate. It appears that virulence on asparagus is a common trait with few exceptions among genetically distinct populations of *F. moniliforme* and *F. oxysporum* colonizing asparagus.

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Les auteurs ont obtenu plusieurs isolats du *Fusarium moniliforme* (Sheld.) emend. Snyd. & Hans., du *F. oxysporum* (Schlecht) emend. Snyd. & Hans. et du *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., à partir de trois champs cultivés en asperges (*Asparagus officinalis* L. cv. Mary Washington) au cours des 5 dernières années; les mises en cultures ont été tentées à partir de plantes montrant ou non des symptômes, au niveau des radicelles, des racines de réserve, du collet et de segments prélevés à la base de la tige. Sur plantules d'asperge, le *F. moniliforme* s'avère plus virulent que le *F. oxysporum* alors que le *F. solani* apparaît non pathogène. Les auteurs ont placé les isolats du *F. moniliforme* et du *F. oxysporum* dans des groupes de compatibilité végétative (VCG) en démontrant l'hétérocaryose avec des essais complémentaires à l'aide d'un mutant (*nit*) incapable d'utiliser les nitrates (couplage des mutants nitM et *nit1*). Parmi les 135 isolats du *F. moniliforme* 97 ont pu être placés dans 13 VCG. Les 38 isolats restants n'ont pu être classifiés par compatibilité végétative dû à une faible obtention de mutants *nit*. Des 18 isolats du *F. oxysporum*, 8 sont uniques et ont été classés dans huit VCG différents. Les autres groupes n'ont pas été placés dans des VCG. Tous les isolats du *F. moniliforme* sont virulents, mais les index de pathogénicité diffèrent entre les isolats des différents VCG. Il n'y a pas de corrélation entre le VCG et le tissu utilisé ou l'expression des symptômes sur le tissu utilisé. Sauf quelque exceptions, il semble que la virulence sur l'asperge soit un caractère commun parmi des populations génétiquemet distinctes du *F. moniliforme* et du *F. oxysporum* qui colonisent l'asperge.

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Introduction

Fusarium oxysporum (Schlecht) emend. Snyd. & Hans. (*F. oxysporum* f.sp. *asparagi* Cohen) and *F. moniliforme* (Sheld.) emend. Snyd. & Hans. are both incitants of Fusarium crown and root rot of asparagus (*Asparagus officinalis* L.) (Cohen and Heald 1941; Graham 1955; Grogan and Kimble 1959; Johnston *et al.* 1979). Both *Fusarium* species are soilborne (Nelson *et al.* 1983*b*), seedborne (Inglis 1980; Damicone *et al.* 1981), and competitive saprophytes of asparagus residues (W. H. Elmer, unpublished observations). In addition, they may symptomlessly colonize roots, crowns, and stems of asparagus plants. This behavior has made them ubiquitous inhabitants of asparagus plantings.

Individual isolates of *F. oxysporum* and *F. moniliforme* can vary in their virulence on asparagus seedlings in pathogenicity tests (Damicone and Manning 1985; Stephens and Elmer 1988). In fact, some isolates are morphologically identical to pathogenic isolates, but lack the ability to cause disease on asparagus seedlings. It is not uncommon for nonpathogenic *Fusarium* spp. to colonize roots (Correll *et al.* 1986*b*; Damicone and Manning 1985). The role of nonpathogenic isolates of *Fusarium* in disease development remains unclear; however, their potential in biological control against pathogenic species of *Fusarium* has been realized in asparagus and in other crops (Alabouvette *et al.* 1979; Damicone and Manning 1982; Schneider 1984).

In the past no differentiating characteristic, other than virulence on asparagus seedlings, has been used to study these important species. Recently, researchers have used vegetative compatibility, the ability of related isolates to form heterokaryons to subdivide *F. oxysporum* (Puhalla 1985) and *F. moniliforme* (Puhalla and Spieth 1985) into vegetative compatibility groups (VCGs). VCGs in *F. oxysporum* have been correlated with virulence on a specific host cultivar (Puhalla 1985; Correll *et al.* 1986*a*), colony size on selective media (Correll *et al.* 1986*a*), and isozyme patterns (Bosland and Williams 1987). Isolates belonging to other VCGs of *F. oxysporum* that are pathogenic on melon (Jacobson and Gordon 1988), banana (Ploetz and Correll 1988), and tomato (Elias and Schneider 1986) were restricted to specific localities. Conversely, Elmer and Stephens (1989) found no correlation

[1]Author to whom correspondence should be addressed.

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between VCG and location for isolates of *F. oxysporum* from Michigan and from global collections that were pathogenic on asparagus. At least 43 VCGs of *F. oxysporum* contain strains pathogenic to asparagus (Elmer and Stephens 1989). Likewise, isolates of *F. moniliforme* that were recovered from samples of corn and sorghum from different counties in Nebraska, U.S.A., were placed in 13 VCGs with no apparent pattern between VCGs and locality (Sidhu 1986).

Elmer and Stephens (1989) examined isolates from many diverse areas, but intense sampling and characterization of isolates recovered from a small habitat is lacking. Our objectives were to determine (*i*) what *Fusarium* species could be found colonizing different symptomatic and asymptomatic tissue from three asparagus plants, (*ii*) the pathogenicity of these isolates on asparagus seedlings, and (*iii*) if a preferential colonization of specific asparagus tissues exist among the VCGs within each species.

Materials and methods

Fungal isolation and pathogenicity tests

Three 5-year-old asparagus plants were removed from experimental plots at the Connecticut Agricultural Experiment Station, Valley Laboratory in Windsor, CT, in September 1987. Isolation of *Fusarium* species from asparagus tissue was approached factorially: 3 plants × 4 tissue types (basal stem segments, crown, storage roots, feeder roots) × 2 symptoms (symptomatic, asymptomatic) × 2 replicates; thus, 48 pieces of asparagus tissue were sampled. Tissues were washed with tap water, treated with 0.53% sodium hypochlorite (10% household bleach) for 1 min, and rinsed in distilled water. Each piece was serially sectioned into five segments to yield a total of 240 tissue sections, and placed onto Komada's medium that is selective for *Fusarium* spp. (Komada 1975). Symptoms included root lesions and crown and stem discoloration. Basal stem segments always contained vascular discoloration so they were sectioned into epidermal or vascular segments. Petri dishes were incubated at 20–25°C on laboratory benches for 5–7 days whereupon hyphal tips from each resulting colony were subcultured onto potato carrot agar (PCA) (Dhringa and Sinclair 1985). Tentative identification of the *Fusarium* spp. was made based on conidial morphology (Nelson *et al.* 1983*a*) and colony characteristics (Komada 1975). One single spore from each colony was subcultured again onto PCA (Dhringa and Sinclair 1985), KCl medium, or carnation-leaf agar (CLA) (Nelson *et al.* 1983*a*), and identified according to the criteria of Nelson *et al.* (1983*a*). Isolates were stored on agar slants of PCA for 3 months whereupon representative strains were placed in long term storage on silica gel (Windels *et al.* 1988).

Each isolate was tested for pathogenicity on asparagus seedlings (cv. Mary Washington) as described by Stephens and Elmer (1988). Disease severity ratings were recorded after 5 weeks using a scale of 1–5, where 1 represents no disease; 2, lesions on 0–25% of the root surface; 3, lesions on 25–50% of the root surface; 4, lesions on 50–75% of the root surface; and 5, lesions on 75–100% of the root surface or dead.

Tests for vegetative compatibility

Isolates of *F. moniliforme* and *F. oxysporum* were placed in VCGs using complementation tests with nitrate-nonutilizing (*nit*) mutants to verify heterokaryon formation (Puhalla 1985; Puhalla and Spieth 1985). Methods for selecting and characterizing *nit* mutants in different classes (*nit1*, *nit3*, and nitM) have been described (Correll *et al.* 1986; Klittich and Leslie 1988). We attempted to recover a nitM mutant from every strain, but this was not always possible. Complementation tests were conducted by placing five agar plugs (3 × 3 mm) each colonized by a *nit1* mutant from each of five different strains on a nitrate minimal medium (Correll *et al.* 1987) equidistantly around a nitM mutant from another strain of that species. All nitM mutants were paired with a *nit1* mutant in every possible combination.

TABLE 1. Identification of *Fusarium* spp. isolated from different asparagus tissues

Asparagus tissue	Symptomatic[a]	No. of isolates recovered			
		Fo	Fm	Fs	Nf
Feeder roots	+	2	11	2	15
	—	3	4	5	18
Storage roots	+	7	18	0	5
	—	1	11	0	18
Crown	+	0	21	8	1
	—	2	19	4	5
Stem	Subepidermal	0	29	0	1
	Epidermal	3	22	2	0
Total		18	135	21	66

NOTE: χ^2 analysis: Fo, *F. oxysporum*, not associated with tissue type; Fm, *F. moniliforme*, most common in crown and stem segments ($P = 0.005$); Fs, *F. solani*, most common in the crown ($P = 0.005$); symptomatic tissue vs. asymptomatic tissue was not significant for all *Fusarium* spp. Nf, not identified as Fusarium.

[a]Symptomatic tissue was evidenced by lesions on roots or vascular discoloration in crowns and stems; vascular discoloration was present in all stems.

Plates were incubated on laboratory benches at 20–25°C and examined weekly for the dense mycelial growth characteristic of heterokaryon development. Two isolates of the same species were scored vegetatively incompatible if dense aerial mycelial growth indicative of a heterokaryon did not develop between the *nit1* mutant and nitM mutant after 2 weeks. Compatible isolates were assigned a two-digit identification number. Representative wild-type and nitM mutants of *F. moniliforme* from each VCG were stored on silica gel (Windel *et al.* 1988).

Results

Of the 240 asparagus tissue sections placed on Komada's medium, 174 pieces (73%) yielded *Fusarium* spp.; other unidentified fungal genera grew from the remaining 66 pieces of asparagus tissue (Table 1). A tentative species identification was made of each *Fusarium* culture based on microphialide morphology and presence or absence of chlamydospores (Nelson *et al.* 1983*a*). The 174 *Fusarium* cultures were subcultured by removing hyphal tips from the colonies. Although 28% (46 of 174) contained mixed species of *Fusarium*, 39, 43, and 18% of these cultures were tenatively identified as being predominantly *F. moniliforme*, *F. oxysporum*, and *F. solani*, respectively. After single conidial cultures were established from each of the 174 cultures, isolates were confirmed to species (Nelson *et al.* 1983*a*) and found to contain 72, 14, and 14% isolates of *F. moniliforme*, *F. oxysporum*, and *F. solani*, respectively. *Fusarium moniliforme* was often recovered from mixed cultures believed to also contain *F. oxysporum* and (or) *F. solani*.

Fusarium moniliforme was most commonly isolated from basal stem segments and from the crown (Table 1). *Fusarium oxysporum* was recovered in low frequencies (8%) from basal stem segments, crown tissue, storage roots, and feeder roots and *F. solani* was recovered in low frequency (12%) from crown sections. *Fusarium* spp. were isolated in approximately equal numbers from asymptomatic and symptomatic tissues. Twenty-eight percent of the sampled tissue yielded no *Fusarium* spp.

Ninety-seven of the 135 isolates of *F. moniliforme* could be classed into 13 VCGs (Table 2). No correlation was observed

TABLE 2. Frequency of vegetative compatibility groups (VCGs) of *Fusarium moniliforme* colonizing asparagus tissue

Plant part	Symptomatic[a]	VCGs present
Feeder roots	+	04, 05, 06, 07, 13
	—	01, 03
Storage roots	+	02, 03, 04, 05, 06, 07
	—	04, 05, 06, 10, 11, 13
Crown	+	01, 02, 03, 04, 05, 06, 07, 09
	—	01, 04, 05, 06, 08, 09, 13
Basal stems	Subepidermal	03, 04, 06, 07, 09, 13
	Epidermal	04, 05, 07, 08, 09, 12, 13

NOTE: For χ^2 analysis, VCG was not associated with tissue type or symptomatic vs. asymptomatic tissue.
[a]Symptomatic tissue was evidenced by lesions on roots or vascular discoloration in crowns and stems; vascular discoloration was present in all stems.

TABLE 3. Mean virulence ratings of vegetatively compatible groups (VCGs) of *Fusarium moniliforme* isolated from asparagus

VCG[a]	No. of isolates[b]	Mean virulence rating[c]
01	5	3.0*bc*
02	4	2.2*a*
03	9	2.4*ab*
04	17	3.1*bc*
05	13	3.5*c*
06	14	3.3*c*
07	15	3.5*c*
08	3	2.8*abc*
09	4	3.1*bc*
10	1	3.5*c*
11	1	3.3*c*
12	2	3.2*c*
13	11	3.5*c*

[a]VCG determined from heterokaryosis tests with complementary nitrate-nonutilizing mutants.
[b]These values represent the number of isolates of *F. moniliforme* belonging to that VCG.
[c]Virulence ratings of asparagus seedlings (cv. Mary Washington) were based on a disease severity scale of 1–5 where 1 represents no disease; 2, lesions on 0–25% of the roots; 3, lesions on 25–50% of the roots; 4, lesions on 50–75% of the roots; and 5, lesions on 75–100% of the roots or dead. Values followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$.

for isolates in any VCG towards any tissue type. In fact, there were several examples where asymptomatic tissue was colonized by isolates belonging to some of the VCGs that contained members colonizing diseased tissue. VCG 04 contained the greatest number of isolates and isolates in this group were found in every tissue type sampled. VCGs 09 and 10 were each composed of a single isolate that was recovered from crown and stem tissue, respectively. The remaining 38 isolates of *F. moniliforme* were not placed into VCGs because, despite repeated attempts, chlorate-resistant sectors did not develop (Klittich and Leslie 1988), or because nitM mutants were not recovered and those *nit1* mutants that were recovered did not complement the nitM mutants from other isolates.

Of the 18 isolates of *F. oxysporum* subcultured from colonies isolated from asparagus tissues, only 8 yielded the nitM mutant necessary for complementation tests. These 8 isolates of *F. oxysporum* were not vegetatively compatible with each other or with *nit* mutants from the other 10 isolates, and each appeared to represent a unique VCG.

Mean virulence ratings for *F. solani*, *F. oxysporum*, and *F. moniliforme* were 2.1, 2.6, and 3.2, respectively. Isolates of *F. moniliforme* were significantly more virulent on asparagus seedlings than the other *Fusarium* spp., which did not give statistically different ratings in these tests. The low virulence reaction associated with *F. solani* indicates that it is nonpathogenic to asparagus.

Differences were noted between the mean virulence ratings of isolates belonging to each VCG of *F. moniliforme* (Table 3). Disease ratings ranged from 2.2 to 3.5; however, isolates comprising 11 VCGs were not significantly different in virulence on asparagus seedlings. Although isolates in VCG 02 were rated significantly less virulent than other VCGs, each one was recovered from symptomatic tissue. Conversely, VCG 10 contained a single isolate that was recovered from asymptomatic tissue, but was highly virulent on asparagus seedlings.

Discussion

Previous studies have demonstrated that asparagus roots, crowns, and stems are reservoirs for many different species of *Fusarium* (Graham 1955; Damicone and Manning 1985). However, in the eastern regions of North America only isolates of *F. oxysporum* and *F. moniliforme* have been virulent in seedling tests (Graham 1955; Johnston *et al.* 1979; Damicone and Manning 1985). Damicone and Manning (1985) recovered *F. moniliforme* and *F. oxysporum* from the crown tissue of 1-year-old asparagus transplants 57 and 30% of the time, respectively, with a 10% recovery of mixed cultures of *F. moniliforme* and *F. oxysporum*. Inasmuch as mixed species of *Fusarium* were noted in 28% of our cultures, our data would reflect similar findings. It appears that the technique of retrieving one single spore from a colony of mixed species of *Fusarium* favors the isolation of *F. moniliforme*. This is probably the result of the large ratio of *F. moniliforme* conidia to *F. oxysporum* conidia that occur on PCA, a medium that promotes sporulation in fungi (Dhringa and Sinclair 1985). Perhaps avoiding the first subculture would have reduced the nonrandom retrieval of *F. moniliforme*. Nevertheless, we conclude, as have others (Gilbertson and Manning 1980; Damicone and Manning 1985; Stephens and Elmer 1988), that as a species, *F. moniliforme* is a more aggressive colonizer of asparagus tissue and more virulent on asparagus seedlings than *F. oxysporum*.

The data reported here demonstrate that the virulence trait is present among a minimum of 14 distinct VCGs of *F. moniliforme*. No relationships were observed between isolates in a VCG and the substrate from which the isolates were recovered. All of the 135 isolates of *F. moniliforme* tested were virulent in the asparagus seedling assay. Therefore, virulence on asparagus may not be a useful criterion for subdividing *F. moniliforme*. Avirulent isolates of *F. moniliforme* may lack ability to compete with virulent strains in colonizing asparagus tissue. Other VCGs of *F. moniliforme* may still exist among the uncharacterized, but virulent, isolates we recovered that did not produce chlorate-resistant sectors. The phenomenon of nonsectoring is heritable (Klittich *et al.* 1988) and may reflect a common trait in *F. moniliforme*. Other procedures for generating complementary mutants may aid in classifying this

large and virulent population of *F. moniliforme* (Correll and Leslie 1987).

The 18 strains of *F. oxysporum* that were isolated from three plants in this study represent at least nine distinct VCGs. This finding is in contrast to other pathogenic groups (*formae speciales*) of *F. oxysporum* inhabitating a locale; these populations were usually represented by one or two VCGs (Correll *et al*. 1986*a*; Elias and Schneider 1986; Bosland and Williams 1987; Jacobson and Gordon 1988; Ploetz and Correll 1988). Elmer and Stephens (1989) reported that over 11 VCGs of *F. oxysporum* that contained strains pathogenic to asparagus seedlings were recovered from one asparagus field in Michigan and at least 43 VCGs were identified from global collections. It was suggested that isolates of *F. oxysporum* that were pathogenic on asparagus consist of genetically distinct populations including ones that contain members pathogenic on multiple hosts (Elmer and Stephens 1989). Virulence on asparagus may be a common trait in *F. oxysporum*, bringing into question the utility of the *formae speciales* concept (Snyder and Hansen 1940) in regard to asparagus.

Both pathogens are commonly isolated from seeds (Inglis 1980; Damicone *et al*. 1981), crowns (Cohen and Heald 1941; Grogan and Kimble 1959), and soil not previously planted to asparagus (Graham 1955). This may explain the wide distribution of VCGs encountered in our study of only three plants. This large number of genetically distinct isolates of *F. oxysporum* found parasitizing asparagus not only highlights the insidious nature of the disease caused by this fungus, but may also explain, in part, why asparagus cultivars that are resistant to such diverse populations of pathogens have been scarce (Stephens and Elmer 1988).

Puhalla (1985) described how VCGs in *F. oxysporum* could become genetically isolated after the teleomorph stage became nonfunctional, and asexual reproduction thereafter restricts combinations of genetic traits to individual VCGs. This situation would be different in *F. moniliforme* that can produce its teleomorph stage (*Gibberella fujikuroi* (Sarv.) Wr.) and thus undergo meiosis, which would permit redistribution of genetic traits among new VCGs (Puhalla and Spieth 1985; Sidhu 1986), including genes for pathogenicity on asparagus. However, the teleomorphic state is only produced under laboratory conditions (Kulhman 1982) and appears not to be involved in natural recombination (Puhalla and Spieth 1985; Sidhu 1986). If we assumed that meiotic recombination in *F. moniliforme* is not operative, then isolates in each VCG would be genetically isolated from isolates in other VCGs. It is not known if other isolates of *F. moniliforme* found colonizing other hosts, such as corn and sorghum, would belong to any of these 13 VCGs. Cross pathogenicity of isolates of *F. moniliforme* on corn and asparagus is known to exist (Damicone *et al*. 1988); however, no data presently indicate if certain VCGs of *F. moniliforme* possess a propensity for colonizing only one particular host. It is interesting that Sidhu (1986) also found 13 VCGs of *F. moniliforme* among isolates recovered from samples of corn and sorghum grain. Additional comparisons between members of these VCGs and others (Klittich and Leslie 1988) will provide a better understanding of the genetic diversity of this important species.

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