Frequency and Pathogenicity of *Fusarium* spp. Isolated from First-Year Asparagus Grown from Transplants

JOHN P. DAMICONE, Graduate Assistant, and WILLIAM J. MANNING, Professor, Department of Plant Pathology, University of Massachusetts, Amherst 01003

ABSTRACT

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Isolations were made from internal crown tissues of 288 Mary Washington and 288 Jersey Centennial first-year asparagus plants grown from transplants. One or more species of *Fusarium* were isolated from every crown. All isolates were identified and assayed for pathogenicity by inoculating Mary Washington seedlings grown aseptically on Hoagland solution agar. *F. moniliforme* isolates made up 57.6% of the *Fusarium* population and had a mean disease index of 2.9 on a scale of 0–5. *F. oxysporum* isolates (30.4%) had a mean disease index of 1.5, mixed isolates of both *F. moniliforme* and *F. oxysporum* (10.6%) had a mean disease index of 2.7, and *F. solani* (1.4%) had a mean disease index of 1.3. The frequency of species isolated was not affected by cultivar, soil fumigation, or insecticide and herbicide applications.

Additional key words: *Fusarium moniliforme* var. *subglutinans*, integrated pest management

Fusarium oxysporum (Schlecht.) f. sp. *asparagi* (Cohen) and *F. moniliforme* (Sheld.) are both involved in asparagus decline (1,6,12). *F. oxysporum* f. sp. *asparagi* is the causal agent of wilt and root rot (1,10) and seedling blight (9). *F. moniliforme* causes stem and crown rot and is the most frequent and virulent species in older declining fields (6,12). Both species are seedborne (2,6,11) and frequently infect 1-yr-old crowns commonly used to establish plantings (7). *F. moniliforme* is also airborne in asparagus fields (8).

We began an integrated pest management program to determine the effects of cultivar, soil fumigation, and insecticide and herbicide applications on the establishment of a new asparagus planting. The planting was started with *Fusarium*-free transplants grown in a greenhouse rather than starting with preinfected field-grown crowns (2,17). This report pertains to the pathogenicity and frequency of *Fusarium* spp. isolated from these plants after one growing season. We also characterized the effects of cultural and pest management treatments, particularly cultivar type, on crown invasion by *Fusarium*.

MATERIALS AND METHODS

Plot design and cultural practices. Jersey Centennial (formerly Rutgers-Michigan hybrid 202), a high-yielding F-1 hybrid in *Fusarium*-infested soils (5), and Mary Washington, an open-pollinated *Fusarium*-susceptible cultivar, were used in this study. *Fusarium*-free transplants of each cultivar were grown from seed treated with benomyl in acetone (2). Treated seeds were germinated on 2% water agar for 7–10 days at 24 C in the dark, and uniform aseptic germlings were transplanted to compartmentalized flats containing steam-pasteurized, soilless growing medium (bark-peat-vermiculite, 1:1:1, v/v). Seedlings were grown in the greenhouse for 10 wk at 24–27 C and watered with half-strength Hoagland solution (19).

A sandy loam soil site (0.186 ha) typical of Connecticut River Valley asparagus culture was selected. Two applications of high-calcium lime were incorporated (one in the fall, one in the spring) at 4,876 kg/ha each to adjust soil pH to about 6.7 by planting. Granular fertilizer (10-10-10) was incorporated at 1,829 kg/ha before planting. The field was established by manually planting transplants in May 1980 in W-shaped planting furrows 30 cm deep with center planting beds 15 cm deep formed by tractor-mounted bed shaper (17).

The experimental design was a split-split plot with four replicates. The field was divided into two main plots, one fumigated with Vorlex (80% chlorinated C_3 hydrocarbons, 20% methylisothiocyanate) in the fall of 1979 and the other not fumigated (13,14) (whole-plot treatment). Each main plot was divided into two subplots, and the insecticide diazinon was applied to one subplot of each main plot. Diazinon was sprayed over entire plants at 0.63 kg a.i./ha on six dates during the 1980 growing season (split-plot treatment). Cultivar and tillage treatments were randomized within each subplot (split-split-plot treatments). Transplants of Mary Washington or Jersey Centennial were planted. Weeds were controlled either by manual cultivation or by simazine applied at 6.2 kg a.i./ha in June 1980. Two levels of each of the four treatments gave 16 treatment combinations that were replicated four times. Nine plants per replicate (36 per treatment combination, 576 total) were sampled at the end of the 1980 growing season by manually excavating the crown and root system and washing them free of soil under running tap water.

***Fusarium* isolation and identification.** Isolations were made from the internal crown tissue of each of the 576 plant samples. A 5-mm-diameter flame-sterilized cork borer was used to remove a core sample of vascular tissue from each crown. Four sections of vascular tissue were removed from the center of each core sample. The four sections from each crown were then surface-sterilized in aqueous 10% chlorine bleach for 7 min and placed on one potato-carrot agar plate (pH 5.5) amended with 100 mg/L of chlortetracycline (19). Plates were incubated at 24 C for 10 days. Resulting *Fusarium* colonies were transferred to carnation leaf agar plates that were incubated at 24 C under fluorescent lighting for 12 hr (18). Each isolate was identified according to Toussoun and Nelson's classification scheme (18). The species isolated from plants from each pest management treatment were tabulated to determine the effects of the treatments on *Fusarium* incidence. Treatment totals were compared with population totals, so a chi-square analysis was performed to decide if observed frequencies (treatment totals) differed from expected frequencies (population totals) at $P = 0.05$.

Pathogenicity of isolates. A seedling assay (4) was adapted to test the pathogenicity of each isolate, using susceptible aseptic Mary Washington seedlings. Seeds were surface-sterilized in aqueous 10% chlorine bleach for 10 min and germinated as described previously. Aseptic germlings were transferred to Hoagland solution agar slants (19) in 20-mm test tubes and placed in a growth chamber with a day length of 14 hr and temperatures of 28 C during the day and 25 C at night. When the seedling shoots were 4 cm long, the crowns were individually inoculated with the *Fusarium*

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isolates. Four seedlings were inoculated with each isolate by placing a 3-mm-diameter disk of mycelium, taken from the colony periphery of the carnation leaf agar plates, adjacent to each crown. Inoculated seedlings and uninoculated control seedlings were returned to the growth chamber and grown for 3 wk more, then evaluated. Seedlings were rated for crown, lower stem, and root rots using the following disease index: 0 = healthy plant; 1 = lesions on ≤25% of stem, crown, and root; 2 = lesions on >25 to ≤50% of stem, crown, and root; 3 = lesions on >50 to ≤75% of stem, crown, and root; 4 = lesions on >75 to ≤100% of stem, crown, and root; and 5 = dead plant. The mean disease index for each isolate and species was calculated. Mean indices for each species were compared using an analysis of variance and Duncan's multiple range test at $P = 0.01$.

RESULTS

***Fusarium* isolation and identification.** *Fusarium* spp. were isolated from each of the 576 crowns sampled after one growing season. *F. moniliforme* was isolated from 57.6% of the crowns, *F. oxysporum* from 30.4%, both *F. moniliforme* and *F. oxysporum* from 10.6%, and *F. solani* from 1.4% (Table 1). *F. moniliforme* was found in 68.2% of crowns sampled, making it the most prevalent *Fusarium* sp. Toussoun and Nelson (19) described a variant of *F. moniliforme* termed "var. *subglutinans*," which produces microconidia in false heads rather than chains. We observed this variant among our isolates growing on carnation leaf agar. Thirty-two of the 332 (9.6%) *F. moniliforme* isolates were of this type.

None of the pest management treatments applied for disease, insect, or weed management affected the frequency of *Fusarium* spp. isolated. Species isolated from whole-plot, split-plot, and split-split-plot treatments were tabulated and compared with the population totals. The species of *Fusarium* that colonized the crowns of the two cultivars tested were the same as those found in the population totals as indicated by a nonsignificant

Table 1. Frequency of *Fusarium* spp. isolated from two asparagus cultivars[a]

	F. oxysporum		*F. moniliforme*		*F. oxysporum* and *F. moniliforme*		*F. solani*		Total	
Cultivar	No.[b]	%[c]	No.	%	No.	%	No.	%	No.	%
Mary Washington	96	16.7	159	27.6	27	4.7	6	1.0	288	50
Jersey Centennial	79	13.7	173	30.0	34	5.9	2	0.3	288	50
Total	175	30.4	332	57.6	61	10.6	8	1.3	576	100

[a] Chi-square (3 df) = 5.04 (NS).
[b] Number of crowns from which isolation was made.
[c] Percentage of crowns from which designated species was isolated.

Table 2. Influence of soil fumigation (Vorlex) on frequency of *Fusarium* spp. isolated from first-year asparagus[a]

	F. oxysporum		*F. moniliforme*		*F. oxysporum* and *F. moniliforme*		*F. solani*		Total	
Vorlex (L/ha)	No.[b]	%[c]	No.	%	No.	%	No.	%	No.	%
0	81	14.1	164	28.5	38	6.6	5	0.9	288	50
363	94	16.3	168	29.2	23	4.0	3	0.5	288	50
Total	175	30.4	332	57.7	61	10.6	8	1.4	576	100

[a] Chi-square (3 df) = 5.18 (NS).
[b] Number of crowns from which isolation was made.
[c] Percentage of sampled crowns from which designated species was isolated.

Table 3. Influence of insecticide (diazinon) application on frequency of *Fusarium* spp. isolated from first-year asparagus[a]

	F. oxysporum		*F. moniliforme*		*F. oxysporum* and *F. moniliforme*		*F. solani*		Total	
Diazinon (kg a.i./ha)	No.[b]	%[c]	No.	%	No.	%	No.	%	No.	%
0.00	90	15.6	157	27.3	34	5.9	7	1.2	288	50
0.63	85	14.8	175	30.4	27	4.7	1	0.2	288	50
Total	175	30.4	332	57.7	61	10.6	8	1.4	576	100

[a] Chi-square = 6.42 (NS).
[b] Number of crowns from which isolation was made.
[c] Percentage of crowns from which designated species was isolated.

Table 4. Influence of herbicide (simazine) application on frequency of *Fusarium* spp. isolated from first-year asparagus[a]

	F. oxysporum		*F. moniliforme*		*F. oxysporum* and *F. moniliforme*		*F. solani*		Total	
Simazine (kg a.i./ha)	No.[b]	%[c]	No.	%	No.	%	No.	%	No.	%
6.2	98	17.0	154	26.7	34	5.9	2	0.3	288	50
0.0	77	13.4	178	30.9	27	4.7	6	1.0	288	50
Total	175	30.4	332	57.6	61	10.6	8	1.3	576	100

[a] Chi-square = 7.06 (NS).
[b] Number of crowns from which isolation was made.
[c] Percentage of sampled crowns from which designated species was isolated.

chi-square (Table 1). Fall preplant fumigation (Table 2), diazinon insecticide applications (Table 3), and simazine weed management (Table 4) also did not alter *Fusarium* incidence.

Pathogenicity of *Fusarium* isolates. The seedling assay detected variability in pathogenicity and virulence of pathogenic isolates among and within species. *F. moniliforme* was significantly more virulent than *F. oxysporum* or *F. solani*; *F. moniliforme* isolates had a mean disease index of 2.9 compared with 1.5 and 1.3, respectively, for the others (Table 5). Mixed isolates of *F. oxysporum* and *F. moniliforme* were also significantly more virulent than isolates of *F. oxysporum* or *F. solani*.

Distribution frequencies are given for the isolate types as the percentage of the total for each type in each disease index category (0–5) (Fig. 1). Seven and one-half percent of *F. moniliforme* isolates and 5% of mixed isolates killed seedlings after 3 wk, whereas none of the *F. oxysporum* isolates killed seedlings. Twenty-seven percent of *F. oxysporum* isolates were nonpathogenic (0 rating), whereas only 3.3% of *F. moniliforme* isolates assayed were nonpathogenic. Mixed isolates were similar in distribution to *F. moniliforme* isolates although slightly lower in virulence. All *F. solani* assayed fell in the 0, 1, and 2 disease index categories.

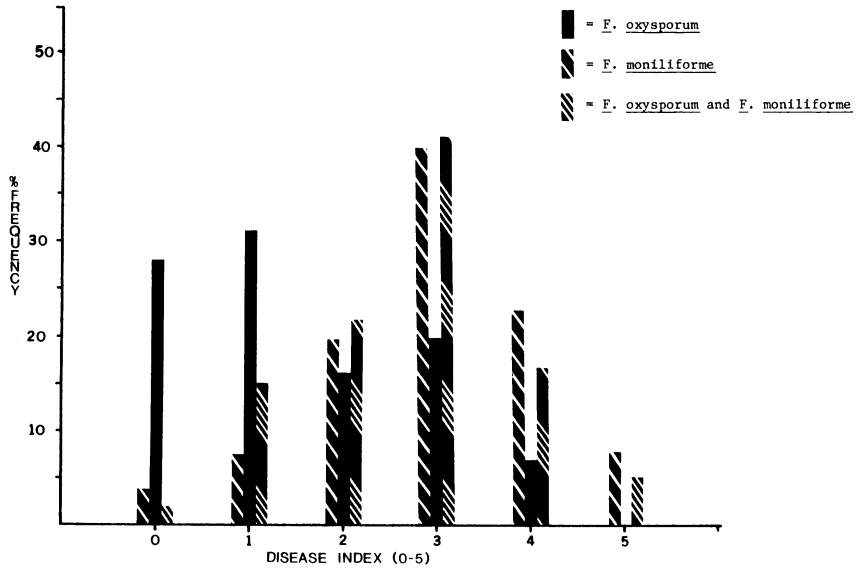

Fig. 1. Frequencies of *Fusarium* spp. isolated from 576 asparagus crowns (1 yr old) for each disease index category (0–5) by seedling assay.

DISCUSSION

All 576 crowns sampled after one growing season were invaded by one or more species of *Fusarium*. This occurred in spite of using *Fusarium*-free transplants as planting stock. Although half of the field was fumigated with Vorlex, which has fungicidal properties, plants were universally infected by *Fusarium*. The cultivar type or weed and insect management techniques also had no effect on *Fusarium* incidence.

Ellison et al (5) described Jersey Centennial as a high-yielding cultivar in *Fusarium*-infested soils in New Jersey. Our results show that internal crown tissues of this cultivar are infected with the same species of *Fusarium* as the standard Mary Washington cultivar. The mechanism resulting in higher yields could be a vigor-related tolerance and not an active resistance to *Fusarium*. This is not surprising because both Jersey Centennial parents are Mary Washington selections (5).

We are not sure why none of the pest management treatments tested in our study altered or reduced *Fusarium* infections. *Fusarium* spp. may have survived fumigation, rapidly recolonized the fumigated soil, or infections may have occurred during the growing season. Although *F. moniliforme* does not form chlamydospores (18), survival mechanisms have been reported that include the production of thickened, resistant hyphae (15) and the ability to survive as a saprophyte in corn stubble (16). We have reported that *F. moniliforme* var. *subglutinans* isolated from corn stubble can be pathogenic on asparagus (3). *F. moniliforme* can infect asparagus flowers and fruit via airborne inoculum, and this inoculum source is common in Massachusetts (7,8). We have consistently observed salmon-pink sporodochia on asparagus and corn stubble in the spring, particularly during wet conditions. Sporodochia also appear on lower stems of dead and dying asparagus stems during wet periods throughout the growing season. Such inoculum sources from nearby plots or farms could account for the observed infections.

Our results indicate *F. moniliforme* is the most prevalent and virulent species isolated from crowns of first-year asparagus. This follows results in New Jersey and California, where *F. moniliforme* predominated in older declining fields and was considered more virulent than *F. oxysporum* (6,12). This is the first report of *F. moniliforme* being the predominant crown-rotting pathogen in first-year asparagus. *F. moniliforme* has recently been proposed to cause a disease distinct from that caused by *F. oxysporum* (12). Our pathogenicity assay indicated that *F. moniliforme* attacked the stems and crown more severely than *F. oxysporum*, which primarily affected roots and caused some crown discoloration. The seedling death symptom caused by isolates of *F. moniliforme* in our study is similar to that reported in Ontario (9) and to what we occasionally observe in the field and greenhouse when asparagus is direct-seeded into infested soils. *F. moniliforme* was not a primary incitant of seedling blight in Ontario (9). Our results agree with those of others (8) in that *F. solani* does not appear to be an important pathogen of asparagus because of its low frequency of isolation and virulence.

Table 5. Disease indices of *Fusarium* spp. from 576 first-year asparagus crowns

Fusarium spp.	No. of isolates	Disease index[y]
F. oxysporum	175	1.5 a[z]
F. moniliforme	332	2.9 b
F. oxysporum and *F. moniliforme*	61	2.7 b
F. solani	8	1.3 a

[y] Mean crown, stem, and root rot index of four Mary Washington seedlings per isolate on a scale of 0–5, where 0 = healthy plant; 1 = lesions on ≤25% of stem, crown, and root; 2 = lesions on >50 to ≤75% of stem, crown, and root; 4 = lesions on >75 to ≤100% of stem, crown, and root; and 5 = dead plant.

[z] Mean values followed by the same letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

The nature of crown invasion of pathogen-free transplants by *Fusarium* spp., particularly *F. moniliforme*, reported in this paper warrants more research on the relation of these crown infections to asparagus decline. It is impossible to grow *Fusarium*-free asparagus. The slow progress of asparagus decline indicates that syndrome may be stress-related, so we are investigating the effects of integrated pest management on the yield and survival of this planting. Additional research is needed on the effects of fumigation on *F. moniliforme*, its survival in soil and crop residue, and its host range and transmission. Programs for developing asparagus cultivar resistance to *Fusarium* should focus on stem and crown rot caused by *F. moniliforme*.

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