Cross-Pathogenicity of *Fusarium moniliforme* Isolates from Corn and Asparagus

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ABSTRACT

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Fusarium moniliforme was isolated from overwintered corn stalks and dormant asparagus crowns. Single-conidial isolates were assayed for pathogenicity and virulence on potted corn seedlings and gnotobiotically grown asparagus seedlings. Isolates differed in pathogenicity and virulence on both hosts. However, some were cross-pathogenic and virulent to both crops. One cross-pathogenic isolate from each host, FmA4 from asparagus and FmC7 from corn, were selected for further evaluation. FmA4 and FmC7 reduced field emergence of sweet and dent corn and greenhouse emergence of asparagus. FmA4 and FmC7 also incited stalk rot of sweet and dent corn in the field and stem and crown rot of asparagus seedlings in the greenhouse. Corn and asparagus can serve as sources of inoculum of *F. moniliforme* pathogenic to both crops.

Fusarium moniliforme Sheldon is the causal agent of stem and crown rot of asparagus (*Asparagus officinalis* L.) and a contributing factor to early bed decline (4,5,7,10,13). *Fusarium moniliforme* also causes stalk and ear rots in corn (*Zea mays* L.) (1,14,16). The fungus reduces seedling stands in both crops through seed decay, damping-off, and seedling blight (11,15).

Stem and crown rot is presently the most serious limiting factor to asparagus production in western Massachusetts (4,5,8). Reports implicate *F. moniliforme* as the predominant and most virulent *Fusarium* species invading crowns of first-year (4) and older declining plants (13). Inoculum sources of *F. moniliforme* pathogenic to asparagus include seed (11,12), dormant crowns used for planting stock (8), and infested plant debris (8). *Fusarium moniliforme* is also airborne in asparagus fields (9). We have consistently observed sporodocia of *F. moniliforme* on overwintered corn and asparagus stubble and have suspected these to be a source of stem and crown rot inoculum. A preliminary report implicated corn stubble as a source of *F. moniliforme* var. *subglutinans* pathogenic to asparagus seedlings (3). Corn and asparagus are frequently grown in close proximity and often follow one another at a particular site. The objective of this research was to evaluate the cross-pathogenicity of isolates of *F. moniliforme* from both hosts. The effect of these isolates on seedling emergence, host colonization, and disease expression in both hosts was studied.

MATERIALS AND METHODS

Fusarium moniliforme was isolated from overwintered corn stalks and from dormant asparagus crowns. Corn stalks were split longitudinally and segments of pith tissue were excised and incubated on Nash medium (19) at 24 C. Dormant asparagus crowns were uprooted and a 5-mm core sample of crown tissue was taken using a flame-sterilized cork borer. Each core sample was trimmed free of external tissue and sliced transversely

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into 3-mm disks. Disks were surface-treated for 10 min in 0.5% aqueous NaOCl and incubated on Nash medium at 24 C. Single microconidial cultures were produced from nine corn isolates and five asparagus isolates. Cultures were transferred simultaneously to carnation leaf agar (CLA) for identification and to soil tubes for storage (19).

Isolates of *F. moniliforme* were evaluated for pathogenicity and virulence to asparagus using a modified seedling assay (5,6). Asparagus seeds of cultivar Mary Washington were treated with benomyl in acetone (2) and germinated on Hoagland solution agar (20) slants in a growth chamber (25 C day, 21 C night, 14-hr photoperiod). Four seedlings were inoculated with each isolate by placing a 3-mm plug of the fungus from CLA cultures adjacent to the crown. Inoculated seedlings and noninoculated control plants were returned to the growth chamber for 21 days. Each seedling was evaluated for stem, crown, and root rot using the following disease index: 0 = healthy; 1 = rot on <25% of basal stem, crown, and root area; 2 = rot on ≥25% to <50% of basal stem, crown, and root area; 3 = rot on ≥50% to <75% of basal stem, crown, and root area; 4 = rot on ≥75% to <100% of basal stem, crown, and root area; 5 = rot on 100% of basal stem, crown, and root area.

Isolates were assayed for pathogenicity and virulence on corn by toothpick inoculation (21) of greenhouse-grown seedlings. Seedlings of hybrid sweet corn (Stewart's Butter and Sugar) were grown in 10-cm-diameter pots containing steam-pasteurized potting mix (1 peat:1 soil:1 sand, v/v) for 14 days at 21–27 C. Sterile toothpicks were soaked in 7-day-old cultures of each isolate growing in potato-carrot broth (PCB), pH 5.5 (20), at room temerature or in sterile PCB (control) for 2 days. Four seedlings were inoculated per isolate by inserting toothpicks through stems 1 cm above the soil line. Seedlings were grown for 14 days and were evaluated for stalk rot. Stalks were split longitudinally and the length of discolored tissue was measured. Discoloration lengths were converted to the following disease index: 0 = rot <5 mm; 1 = rot ≥5 mm to <10 mm; 2 = rot ≥10 mm to <15 mm; 3 = rot ≥15 mm to <20 mm. Isolations were then made from stalks at 2, 4, and 6 cm above inoculation points on potato-carrot agar (PCA), pH 4.5 (20).

Mean disease indices were calculated for each isolate per host. One isolate from each host with the highest total disease index (asparagus assay + corn assay) was selected for further evaluation. Oat inoculum of isolates FmA4 from asparagus and FmC7 from corn was produced by inoculating moistened, sterile oat grain with 2 ml of 7-day-old cultures grown on PCB. Inoculum was incubated at room temperature for 21 days, air-dried, and used immediately.

The influence of isolates FmA4 and FmC7 on corn emergence and stalk rot development was assessed in a field containing Hinkly loamy sand (Typic Udorthents). The field received preplant incorporations of 1,200 kg/ha ground limestone and 39-39-39 kg/ha NPK granular fertilizer. Experimental designs were randomized complete blocks with four replicates for emergence tests and three replicates for the stalk rot test. Emergence tests consisted of single row plots 1.2 m long and 4.5 m apart planted with 100 seeds of sweet corn (Stewart's Butter and Sugar) or dent corn (Stewart's No. 290). Oat inoculum of FmA4, FmC7, or sterile oats (control) were added to each seed furrow at 250 ml per row. The experiment was done once with non-treated seed, and again with thiram-treated seed (Arasan, 60% thiram, E. I. DuPont de Nemours & Co., Wilmington, DE). Emergence was determined by counting surviving seedlings after 14 days. The stalk rot test contained single-row plots 30 m long and 1.2 m apart, planted with sweet or dent corn seed. Plants were spaced 23 cm apart and were inoculated at the pretassel stage (42 days after planting) by inserting toothpicks through stalks 20 cm above the soil line. Ten plants per plot were inoculated with FmA4, FmC7, or control toothpicks. At maturity, stalks were split longitudinally, the length of rot was measured, and isolations were made from the periphery of rotted tissue on Nash medium.

The effects of FmA4 and FmC7 on asparagus emergence and stem and crown rot development was tested in the greenhouse using soil from the field previously described. Soil was amended with FmA4, FmC7, or control oat inoculum at 75 ml/L of soil and placed in 26-cm-diameter pots. Four pots per isolate and control were planted with 100 seeds of the cultivar Mary Washington. Pots were randomized on a greenhouse bench and maintained at 21–24 C for 6 wk preceding stand counts. Twelve weeks after planting, 75 seedlings per

Table 1. Pathogenicity of *Fusarium moniliforme* isolates to corn and asparagus seedlings

Isolate	Source	Corn disease index[x]	Asparagus disease index[y]
FmA1	Asparagus	2.7	3.7
FmA2	Asparagus	2.0	3.2
FmA3	Asparagus	2.7	3.5
FmA4	Asparagus	2.7	4.0
FmA5	Asparagus	1.0	3.5
FmC1	Corn	2.5	3.2
FmC2	Corn	1.0	2.0
FmC3	Corn	1.0	2.0
FmC4 *[z]	Corn	1.0	1.7
FmC5 *	Corn	2.0	0.5
FmC6 *	Corn	2.0	0.5
FmC7	Corn	3.0	3.0
FmC8	Corn	2.2	2.2
FmC9 *	Corn	1.0	0

[x] Corn stem rot disease index (0–3) based on length of stalk rot. Mean values of four seedlings 14 days after inoculation.
[y] Asparagus stem, crown, and root rot index (0–5). Mean values of four seedlings 21 days after inoculation.
[z] * = Colonies with orange pigment on potato-carrot agar.

Table 2. Influence of *Fusarium moniliforme* isolates from corn and asparagus on field emergence of sweet and dent corn

Host	Isolate[w]	Emergence[x]	
		Thiram-treated seed[y]	Nontreated seed
Sweet corn	FmA4	72.7 ab[z]	35.5 cd
	FmC7	74.0 ab	40.7 d
	Control	75.7 ab	65.0 ab
Dent corn	FmA4	67.8 ab	44.0 d
	FmC7	63.2 b	57.0 bc
	Control	79.7 a	77.0 a

[w] Soil amended with 250 ml/4.5 m of row of oat inoculum of *F. moniliforme* isolates FmA4, FmC7, or noninoculated oats.
[x] Mean values of four replicates of 100 seeds each.
[y] Seed treated with 60% thiram.
[z] Values in a column followed by the same letter do not differ significantly at $P < 0.05$, according to Duncan's multiple range test.

isolate and control were randomly chosen, uprooted, washed, and blotted dry. Stem and crown rot was evaluated using the disease index (0–5) described above and plant fresh weights were recorded.

Table 3. Influence of *Fusarium moniliforme* isolates from corn and asparagus on stalk rot development of dent and sweet corn in the field

Isolate[x]	Length of stalk rotted (cm)[y]	
	Dent corn	Sweet corn
FmA4	9.7 a[z]	9.0 a
FmC7	10.2 a	9.5 a
Control	1.9 b	2.2 b

[x] Toothpick inoculation of *F. moniliforme* isolates FmA4, FmC7, or sterile toothpicks inserted through stalks 20–30 cm above soil line 40 days after planting.
[y] Mean values for three replicates of ten stalks.
[z] Values followed by the same letter do not differ significantly at $P < 0.05$, according to Duncan's multiple range test.

RESULTS

All single-conidial isolates of *Fusarium* were *F. moniliforme*. However, some differed in cultural appearance growing on PCA. When viewed through the bottom of culture dishes, all asparagus isolates and most from corn had purple pigmentation. Some from corn had orange pigmentation.

Isolates differed in disease development on corn and asparagus (Table 1). Some isolates were considered to be cross-pathogenic, having disease indices ≥2 in both assays. Orange isolates were nonpathogenic or low in virulence on asparagus. All isolates were recovered from corn stalks at 2 cm and 4 cm above inoculation points.

FmA4 and FmC7 reduced emergence of dent and sweet corn when nontreated seeds were planted in infested soil in the field (Table 2). FmA4 and FmC7 reduced emergence of dent corn 43 and 26%, respectively, and sweet corn emergence by 45 and 37%, respectively, compared with the control. Seed treatment with thiram significantly improved emergence of all treatments, except FmC7 on dent corn. Observed stand reductions were attributed to seed rot and preemergence damping-off, because no seedling blight was observed. Both isolates incited equal amounts of stalk rot in sweet and dent corn (Fig 1, Table 3). Plants inoculated with sterile toothpicks exhibited only minor discoloration, typically 1 cm above and below inoculation points. *Fusarium moniliforme* was recovered from 100% of stalks inoculated with FmA4 and FmC7 and from 35% of control plants.

FmA4 and FmC7 significantly reduced emergence of asparagus seeds in the greenhouse (Table 4). Soil infestation with FmA4 and FmC7 resulted in 38 and 31% stand loss compared with the control. Seedling blight, as well as preemergence damping-off, contributed to observed stand loss. Infestation of soil with FmA4 and FmC7 resulted in greater stem and crown rot development than in noninfested soil (Table 4). Plant fresh weight was not affected by either isolate. *Fusarium moniliforme* was recovered from 100% of crowns grown with FmA4 and FmC7, and from 16% of control crowns.

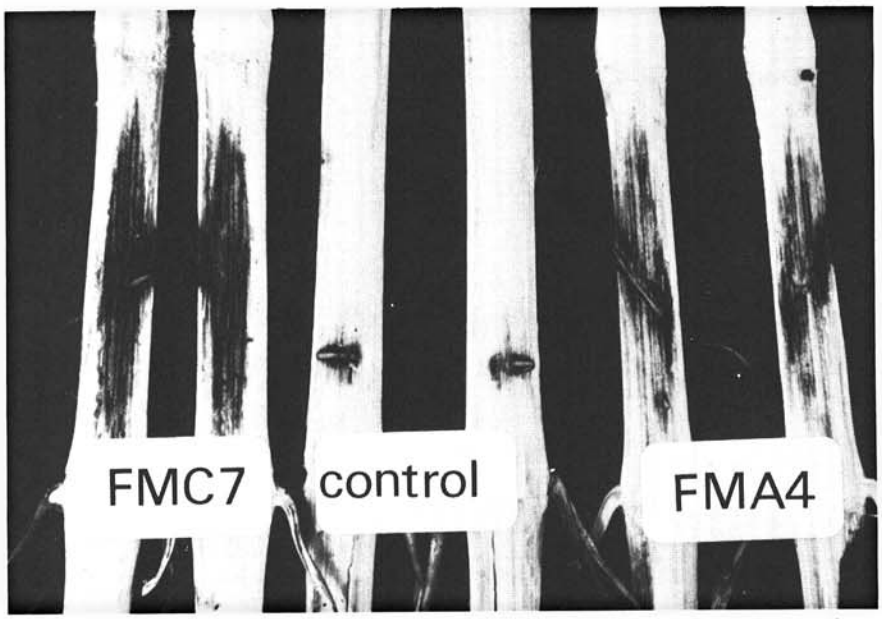

Fig. 1. Stalk rot development in dent corn inoculated with *Fusarium moniliforme* isolates from corn (FmC7), asparagus (FmA4), or sterile toothpick (control).

Table 4. Influence of *Fusarium moniliforme* isolates from corn and asparagus on growth and disease development of cv. Mary Washington asparagus seedlings in a greenhouse

Isolate[w]	Emergence[x] (%)	Plant fresh weight[y] (g)	Disease index[z] (0–5)
FmA4	36.8 a	0.23 ± 0.06	1.93 ± 0.57
FmC7	41.0 a	0.25 ± 0.05	1.72 ± 0.51
Control	59.5 b	0.25 ± 0.06	0.72 ± 0.47

[w] Steam-pasteurized soil amended at 75 ml/L soil with oat inoculum of *F. moniliforme* isolates FmA4, FmC7, or sterile oats.
[x] Mean values of four replicates of 100 seeds each determined 6 wk after planting. Values followed by the same letter do not differ significantly at $P < 0.05$, according to Duncan's multiple range test.
[y] Mean values (± standard deviations) of 75 plants randomly chosen 12 wk after planting.
[z] Mean values (± standard deviations) of 75 plants, stem and crown rot disease index.

DISCUSSION

This study demonstrated that *F. moniliforme* isolates cross-pathogenic to corn and asparagus can survive, colonize, and incite typical disease symptoms in both hosts. *Fusarium moniliforme* produces no chlamydospores and requires plant debris or a living host for survival (16,17). The pathogen can colonize and persist in corn stalks and residues as a saprophyte and can sporulate aboveground on these substrates (16,17). *Fusarium moniliforme* can also survive in lesions on dormant asparagus crowns, and can survive and sporulate on overwintered asparagus stubble, insect damaged stalks, or on dead and dying stalks during the growing season (8,10). Airborne inoculum of *F. moniliforme* arising from such sources may explain the 70.2% infection of first-year asparagus grown in fumigated soil planted with *Fusarium*-free transplants (4). It is concluded that corn stalks are an additional source of inoculum of *F. moniliforme* capable of causing stem and crown rot of asparagus.

Results also suggest that colony color may be a useful criterion for differentiation of isolates from corn pathogenic to asparagus because only a portion of these isolates were cross-pathogenic. Colony color is not generally thought to be a useful taxonomic character in *Fusarium* spp. (19). However, color-specific mutants have been used to study the ecology of *F. oxysporum* f. sp. *apii* race 2 (18). A more extensive survey is needed to determine the reliability and usefulness of this trait and the relative importance of corn stubble as a source of inoculum for asparagus stem and crown rot.

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