Importance of Different Pathways for Maize Kernel Infection by *Fusarium moniliforme*

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ABSTRACT

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The relative importance of several infection pathways (silks, stalks, and seed) leading to kernel infection of maize hybrids by *Fusarium moniliforme* was investigated in field experiments in 1993 and 1994. Systemic movement of specific fungal strains within plants was detected by using vegetative compatibility as a marker. Transmission of *F. moniliforme* from inoculated seed to stalks and developing kernels was detected in two of three field experiments; the seed-inoculated strain was detected in kernels on approximately 10% of ears. The percentage of kernels infected with the seed-inoculated strain ranged from 0 to 70%, with a mean of 0 to 2.5% (0 to 8.3% of *F. moniliforme*-infected kernels). Other pathways to kernel infection were more effective than seed transmission and systemic infection. *F. moniliforme* strains inoculated into the

crowns and stalks of plants were found throughout the stalks and in up to 95% of the kernels in individual plants. Infection through the silks was clearly the most effective pathway to kernel infection. This was the only inoculation method that significantly increased overall incidence of *F. moniliforme* infection in kernels; the silk-inoculated strain infected up to 100% of the kernels in individual ears, with a treatment mean as high as 83.7% of kernels. When plants were silk-inoculated, the percentage of kernels infected by other *F. moniliforme* strains from the seed or stalk was reduced, apparently due to competition among strains. This study provides evidence that systemic development of *F. moniliforme* from maize seed and stalk infections can contribute to kernel infection, but silk infection is a more important pathway for this fungus to reach the kernels.

Additional keywords: corn, *Gibberella fujikuroi*.

Fusarium moniliforme J. Sheld. is a widely distributed pathogen of maize (*Zea mays* L.), causing seedling disease, root rot, stalk rot, and ear or kernel rot. It is the most commonly reported fungal species infecting maize (18,32,33). At the time of harvest, *F. moniliforme* can be found in plants or crop residue in virtually every field of maize in the United States. It is common in maize seed, where it has variable effects on seed germination and seedling health. Some seed lots with a high incidence of *F. moniliforme* infection experience little or no reduction in germination or seedling growth, while others may be seriously affected by the fungus (28).

In addition to its effects on yield and seed quality, *F. moniliforme* infection can be detrimental to grain quality. *F. moniliforme* can produce a number of toxic compounds including fusaric acid, fusarins, gibberellins, moniliformin, and fumonisins (27,32,33). Recently, fumonisins have received the most attention, because fumonisin B1 is the most common toxin found in maize infected by *F. moniliforme* (26,33). Fumonisin B1 has been shown to cause a number of health problems in livestock and laboratory animals (7,33,37,38,40,46) and has been associated with an increased incidence of human esophageal cancer in some parts of the world (33). Fumonisin B1 can be found in moldy and symptomless infected kernels (1,3,31,32,33).

Systemic infection of maize plants by *F. moniliforme* was first proposed by Foley in 1962 (6). The fungus was present in seedlings, leaf sheaths, and stalk tissue without causing noticeable symptoms, and the frequency of isolation increased during the course of the season. Foley concluded that stalk infection occurred

through leaf sheaths, but that symptomless infection of seed led to systemic infection of the stalk. The relationship of systemic infection to subsequent stalk rot was not determined. Other research has supported the occurrence of *F. moniliforme* throughout symptomless maize plants (2,20). However, some authors investigating seed-to-stalk movement or kernel infection by *F. moniliforme* have not found evidence for systemic infection (5,16,17, 19). Because *F. moniliforme* is ubiquitous, natural infection by endemic strains can mask detection of movement of an inoculated strain from seed to stalk. In field experiments, noninoculated plants inevitably become infected with endemic strains; this can occur even in greenhouse experiments (44). Confirmation of the ability of *F. moniliforme* to move systemically in maize plants was provided when Kedera et al. (11) used vegetative self-compatibility as a marker to track specific strains from seed to kernel. Seeds were inoculated in the laboratory by soaking them in a suspension of microconidia. In plants grown from inoculated seed, the seed-inoculated strain was recovered from approximately 10% of *F. moniliforme*-infected kernels.

F. moniliforme is known to infect maize kernels through silks and through kernel damage caused by insects or other animals (8,9,18,32). Insects also have been shown to act as vectors of *F. moniliforme* (43). These infections may result in visible decay of the kernels, but many kernels are infected without showing symptoms (1,2,3,6,8,9,10,19,20,31,32,44,45). The relative importance of silk infection, insect-assisted infection, and systemic infection is likely influenced by the availability of inoculum for each type of infection. Many maize seedlots are heavily contaminated by *F. moniliforme*, and most seedlots contain some infected kernels (18,32,33). *F. moniliforme* also is known to survive in crop residue (18,35,36); however, this pathogen may be a minor component of the *Fusarium* populations surviving in maize residue (25). Airborne spores of the fungus are usually abundant in maize fields during the reproductive growth stages (36).

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Although various pathways to kernel infection have been established, the relative importance of each pathway is unclear. The objective of this research was to evaluate the relative importance of several pathways for infection of maize kernels by *F. moniliforme*. These pathways included seed transmission followed by systemic infection, stalk infection followed by systemic infection, and infection through silks. Preliminary results have been presented (29,30).

MATERIALS AND METHODS

Characterization of fungal strains. Five *F. moniliforme* strains, designated EA-1, EA-2, EA-3, EA-4, and 115, were obtained from maize seed or grain grown in Iowa by culturing whole kernels on Nash-Snyder medium (NSM). The strains were identified as *F. moniliforme* on carnation leaf agar (CLA) (34), according to the description by Nelson et al. (34). These strains were deposited with the Pennsylvania State University *Fusarium* Research Center and given the designations M-8113, M-8114, M-8115, M-8116, and M-8117, respectively. The strains were determined to belong to mating population A of *Gibberella fujikuroi* (21) by mating with known tester strains (M-3120 and M-3125) obtained from the Pennsylvania State University *Fusarium* Research Center. Sexual crosses were performed on carrot juice agar as described by Klittich and Leslie (14).

Complementation testing among nitrate nonutilizing (*nit*) mutants (14) of the five strains was conducted and determined that the strains were vegetatively incompatible with each other. Vegetative compatibility tests were conducted according to the methods of Klittich and Leslie (14). Strains were transferred from CLA to minimal medium amended with 2.5% potassium chlorate (MMC) and to potato dextrose agar (Difco Laboratories, Detroit) amended with 2.5% chlorate (PDC). Sectors growing rapidly on these media were usually *nit* mutants; wild-type strains do not grow on chlorate-amended media. Some mutants growing on MMC were able to grow on minimal medium (MM), which has nitrate as the sole nitrogen source. These were most likely *crn* mutants (15) and were discarded. The phenotypes of putative mutants growing on MMC were determined by transferring to both hypoxanthine medium (hx) and MM. Mutants growing on hx and not on MM were either *nit1* or *nit3* mutants. Mutants that grew on neither medium were classified as NitM mutants. Most mutants recovered from MMC were NitM, while most recovered from PDC were *nit1* or *nit3*. To test for vegetative compatibility, NitM mutants were paired on MM with *nit1* or *nit3* mutants. The *nit* mutants grew very sparsely across the medium, but complementation of the auxotrophic mutants was indicated by a line of vigorous growth where the mutants interacted. All five strains were self-compatible, but incompatible with the other strains.

The relative ability of the five strains to colonize maize stalk tissue was compared in greenhouse experiments in 1993. Maize seed (ICI hybrid 20051) was planted in 15-cm pots in a greenhouse soil mix. Plants were grown until three full internodes had developed above the soil line, and then the middle internode of each plant was injected with 1 ml of a suspension of microconidia (10^5 spores/ml). Ten plants were inoculated with each strain. Ten control plants were injected with sterile distilled water only. The experiment was arranged in a completely randomized design. After 4 weeks, the plants were removed from the pots, and the stalks were split longitudinally. The extent of stalk discoloration was measured, and stalk tissue samples were removed aseptically at 5-cm intervals and cultured on NSM to reisolate the fungi. Recovered *F. moniliforme* strains were tested for vegetative compatibility with the appropriate inoculated strains to confirm their identity. Based on the results of the reisolation and vegetative compatibility tests, the extent of stalk colonization was recorded for each stalk. The experiment was conducted twice.

Seed inoculation. A preliminary greenhouse experiment was conducted to evaluate the effectiveness of several seed-inoculation

methods. Three seed-inoculation methods were compared in a factorial experiment with wounding treatment as one factor and inoculation with *F. moniliforme* as the other factor. Seeds of maize hybrid ICI 20051 were wounded by abrasion with sandpaper or by puncturing the pericarp, or not wounded. Abrasion was performed by placing the seeds inside a glass jar lined with fine sandpaper (100 grit) and rotating for 2 h. Puncturing was done by hand with a sterile dissecting needle. Wounded and nonwounded seeds were soaked in a suspension of *F. moniliforme* strain EA-2 microconidia (10^6 /ml) for 24 h, air-dried, and hand-planted in 15-cm pots in the greenhouse. The noninoculated treatments were soaked in sterile water only. From each treatment, 25 inoculated seeds were checked for infection by surface-disinfecting and culturing on NSM. The experiment was in a completely randomized design with two replications of 10 plants for each treatment; plants were grown to stage V10. Tissue samples from the crown, growing point, and first three nodes of each plant were cultured on NSM. *F. moniliforme* isolates from these samples were tested for vegetative compatibility with the original strain (EA-2).

Field experiments. In 1993, a field experiment was conducted at the Iowa State University (ISU) Curtiss Research Farm near Ames. Seeds of a precommercial maize hybrid, ICI 20051, were inoculated without wounding, as described by Kedera et al. (11) with some modification. To reduce existing fungal contamination, seeds were soaked in tap water for 4 h, followed by a hot water treatment of 55°C for 15 min. Germination of seed was tested, and this duration of hot water treatment did not affect germination. The seeds were removed from the hot water treatment and placed in a spore suspension of *F. moniliforme* strain EA-2 macroconidia and microconidia (10^6 spores/ml) for 14 h. Seeds were then air-dried and planted with a commercial four-row planter. Noninoculated seed was planted first to avoid contamination of the planter boxes with strain EA-2. Small samples of seed were retained and cultured on NSM. Recovered *F. moniliforme* isolates were tested for vegetative compatibility with EA-2.

The field plot, planted on 1 June 1993 in a field that was cropped to soybeans the previous year, had a planting density of 64,246 plants/ha. The experiment was a split-plot design with five replicate blocks. Seed-inoculation treatments (inoculated or non-inoculated) were the main plots, and additional plant inoculation treatments were the subplots. The subplot treatments were no inoculation, crown inoculation (strain EA-3, growth stage V6), stalk inoculation (strain EA-4, growth stage V18, in the internode below the ear shank), silk inoculation (strain EA-1, stage R1-R2), or a combination of all three inoculation methods. Crown and stalk inoculations were conducted by injecting approximately 1 ml of a conidial suspension (10^5 to 10^6 spores/ml) into the plant tissue with a repeating syringe. For the silk inoculation, silks were sprayed with a spore suspension (10^5 spores/ml) once at stage R1 and once at stage R2. After the second spray, ears were loosely covered with plastic bags to retain moisture on the silks. The bags were left in place for 48 h. Subplots were four rows wide (0.76 m spacing) and 6.1 m long; 120 seeds were planted per subplot. There were 3-m-wide borders, planted with the same hybrid, between subplots.

Plant stands were counted at stage V4 (39) to assess the effect of seed inoculation on emergence. Plants were sampled twice, at stage R4 and after physiological maturity, to assess infection by *F. moniliforme*. Five plants were selected arbitrarily from each subplot at each sampling date; whole plants were dug and transported to the laboratory. For the first sampling date, both stalks and kernels were cultured for *F. moniliforme* recovery; stalks were split longitudinally, and tissue samples were removed aseptically from the crown, lower stalk, ear node, and cob (a single sample of each tissue per plant). For the second sampling, ears were rated for the percentage of kernels showing symptoms of *Fusarium* infection, and 20 arbitrarily selected, symptomless kernels were cultured from each plant. All tissues were surface-disinfested in 0.5% so-

dium hypochlorite for 3 min, blotted dry, and cultured on NSM. After approximately 7 days, culture dishes were examined for the presence of *F. moniliforme*, and the proportion of samples infected was recorded. Each of the recovered isolates was transferred to PDC for generation of *nit1* or *nit3* mutants, which were then tested on MM for vegetative compatibility with NitM mutants of strains EA-1, EA-2, EA-3, and EA-4. The percentage of isolates that were compatible with each of the original strains was recorded.

In 1994, two field experiments were conducted in locations near Ames, IA, at the ISU Hinds and Woodruff Research Farms. Two commercial hybrids were used in each location; Pioneer hybrids 3417 and 3563 were planted on 12 May 1994 at the Hinds farm, and ICI hybrids 8708 and 8599 were planted on 5 May 1994 at the Woodruff farm. Both experiments were planted in a split-split plot design with four replications. Hybrid was the main plot, seed-inoculation treatment (inoculated or noninoculated) was the subplot, and additional inoculation treatment was the sub-subplot. Sub-subplots were four rows wide (0.76 m spacing) and 4.3 m long; 80 seeds were planted per sub-subplot. There were 3 × 2-m borders, planted to the same hybrid, between sub-subplots. In the Woodruff experiment, the inoculation treatments were identical to the 1993 experiment, with the addition of an inoculation of the ear shank at stage R4. Strains EA-1, EA-4, EA-2, EA-3, and 115 were used for inoculation of the seed, crowns, stalks, silks, and shanks, respectively. In the Hinds experiment, inoculation treatments were identical to the Woodruff experiment, with the addition of another multiple-inoculation treatment. Plants receiving this treatment were inoculated by injection in the crown, stalk, and shank, as described above. The silks also were inoculated, but they were sprayed only once with a suspension of *F. moniliforme* spores at the R2 stage, and the ears were not covered. In the Hinds experiment, strains EA-4, EA-3, EA-1, 115, and EA-2 were used for inoculation of the seed, crowns, stalks, silks, and shanks, respectively. At both locations, plants were sampled twice and tissues were assessed for infection by the original strains, as described for the 1993 experiment. In addition to the 20 symptomless kernels, up to five kernels per ear with Fusarium kernel rot were cultured for *F. moniliforme* recovery.

Data analysis. For all field experiments, analyses of variance were conducted on the percentage of stalk tissue samples, ears (arcsine-square-root transformed), or kernels (nontransformed) infected by *F. moniliforme*; a separate analysis was conducted for each *F. moniliforme* strain on the incidence of infection among the different treatments. The objective of these analyses was to test whether the incidence of infection by inoculated strains was greater in their respective inoculation treatments than in the control, and to compare the single-inoculation treatments with the multiple-inoculation treatment. Additional analyses were conducted to compare infection by the inoculated strains in each of their respective treatments. The objective of these analyses was to provide a direct comparison of the relative importance of each pathway to kernel infection. Recovered isolates that did not form *nit* mutants after three attempts were not included in the calculations or analyses.

The General Linear Models procedure of SAS (SAS Institute, Cary, NC), the frequency analysis procedure of SAS, or the analysis of variance procedures of SigmaStat Statistical Software (Jandel Scientific Software, San Rafael, CA) were used for the analyses. The Student-Newman-Keuls test (SigmaStat) was used for mean separation analyses, and frequency data were analyzed using the χ^2 test (SAS).

RESULTS

In the greenhouse stalk-inoculation experiments, all strains caused brown decayed lesions in maize stalks near the inoculation point. Mean lesion length was 25 mm after 4 weeks of incubation, and there were no significant differences in lesion length among the strains ($P > 0.05$). Each of the strains could be isolated from lesions and from stalk tissue up to 30 cm above the point of inoculation. Mean incidence of infection in tissue samples 30 cm from the inoculation point was 61%, and there were no significant differences in the frequency of isolation among the strains (χ^2 test, $\alpha = 0.05$).

Seed inoculation. All laboratory seed-inoculation techniques that were tested resulted in infection of a high percentage of the seed. Seed infection ranged from 88 to 100%, and there were no significant differences among the methods (χ^2 test, $\alpha = 0.05$). Infection of noninoculated seed averaged 4%. At stage V10, nearly all the plants were infected with *F. moniliforme* in some part of the plant. Seed inoculation did not significantly increase infection of crown tissue at this growth stage. For the node tissues sampled, there were no increases in infection due to inoculation, except for the abrasion treatment in which plants from noninoculated seed had little infection. For samples from meristematic tissue, there were some differences among treatments, but inoculation did not appear to increase infection.

Vegetative compatibility tests revealed that the inoculated strain EA-2 was present in tissues of some plants from seed that received each inoculation treatment. EA-2 was not recovered from any plants from noninoculated seed. There were significant differences among inoculation treatments ($P < 0.05$), with the non-wounded and puncture treatments having higher infection levels than the abrasion treatment for all tissues. Based on these results, nonwounded inoculated seed was used for the field experiments.

Field experiments. All seeds inoculated with *F. moniliforme* for field experiments were infected with the appropriate strain. Non-inoculated seed had a low level of *F. moniliforme* infection following the heat treatment, but strains recovered from noninoculated seed were not vegetatively compatible with the strains used for seed inoculation. Seed inoculation did not significantly affect emergence ($P > 0.05$) in any of the field experiments. Mean emergence ranged from 80 to 90% for all treatments in the field experiments.

In 1994, seedlings sampled from both the Hinds and Woodruff field experiments were infected with *F. moniliforme*; there were no significant effects of hybrid on seedling or stalk infection in either experiment, so data were combined for the two hybrids at each location. In both experiments, infection of seedling tissues was

TABLE 1. Incidence of infection by *Fusarium moniliforme* in 3-week-old maize seedlings (growth stage V4) in field experiments near Ames, IA, in 1994^a

	All <i>F. moniliforme</i> strains (% of seedlings)				Seed-inoculated strains (% of seedlings) ^b			
	Crown	Mesocotyl	Root	Plant	Crown	Mesocotyl	Root	Plant
Hinds farm								
Inoculated seed	70.0 ^c	67.5 ^c	77.5 ^c	87.5 ^c	57.5 ^c	57.5 ^c	72.5 ^c	80.0 ^c
Noninoculated seed	27.5	27.5	27.5	50.0	10.0	2.5	2.5	10.0
Woodruff farm								
Inoculated seed	82.5	97.5 ^c	82.5 ^c	97.5	38.8 ^c	67.3 ^c	62.5 ^c	97.5 ^c
Noninoculated seed	57.5	65.0	42.5	77.5	2.5	3.1	0.0	6.6

^a Data are means of eight replications, five seedlings per replication (combined for two hybrids).

^b Seeds were inoculated with *F. moniliforme* strains EA-4 and EA-1 in the Hinds and Woodruff experiments, respectively. Their presence in sampled tissues was determined by vegetative compatibility with the original strains.

^c Seed inoculation significantly increased infection (F test, $P < 0.05$, analysis of variance of arcsine-square root-transformed proportions).

Fig. 1. Mean incidence of maize stalk and cob tissue infection by *Fusarium moniliforme* in plants from a field experiment at Iowa State University Curtiss Farm, near Ames, IA, in 1993. Within each tissue type, asterisks indicate values that were significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of 10 replications of five plants each. Data are combined for plants from inoculated and noninoculated seed. Crowns and stalks were inoculated by injection of a *F. moniliforme* spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all three inoculation treatments. Tissues were sampled after physiological maturity.

Fig. 2. Mean incidence of maize stalk and shank tissue infection by *Fusarium moniliforme* in plants from 1994 field experiments. **A**, Hinds farm and **B**, Woodruff farm. Within each tissue type, asterisks indicate values that were significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of 16 replications of five plants each. Data are combined for plants from inoculated and noninoculated seed, two hybrids per location. Crowns, stalks, and shanks were inoculated by injection of a *F. moniliforme* spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all four inoculation treatments. Plant tissues were sampled after physiological maturity.

significantly increased ($P \leq 0.05$) by seed inoculation (Table 1). Considering all three tissue samples (root, mesocotyl, and crown) taken from each plant, overall plant infection was significantly increased by seed inoculation in the Hinds, but not the Woodruff, experiment. Seed-inoculated strains were recovered from all three tissue types from seedlings in both experiments (Table 1). Neither total infection nor infection by inoculated strains differed among the tissue types.

In all three field experiments, seed inoculation had no significant effect on the percentage of crown, stalk, or cob samples or kernels that were infected with *F. moniliforme* at growth stage R4 ($P > 0.05$). Other inoculation treatments, however, did significantly increase infection of these tissues (Figs. 1 and 2). Crown, stalk, and shank inoculation significantly increased infection of the inoculated tissues, but usually did not affect the incidence of infection in tissues adjacent to the inoculation site (Student-Newman-Keuls test, $\alpha = 0.05$). Silk inoculation also significantly increased shank infection in the Hinds experiment (Fig. 2). Cob infection was not affected by the inoculation treatments ($P > 0.05$). The multiple inoculation (this treatment included crown, stalk, and silk inoculation) significantly increased both crown and stalk infection (Student-Newman-Keuls test, $\alpha = 0.05$). Incidence of *F. moniliforme* infection did not differ between hybrids in either 1994 experiment.

In 1993, the strain used for seed inoculation was not detected in any plant tissues, but, in 1994, it was detected in 18.1% (Hinds) and 9.8% (Woodruff) of crowns and a lower percentage of lower stalk, ear node, and shank tissues. Strains used for crown, stalk, shank, and silk inoculations were recovered from most inoculated plants (Tables 2 and 3). Each of the strains was recovered from a high percentage of the inoculated tissues, and movement of the strains within plants also was apparent by their recovery from adjacent tissues. For example, in the Hinds experiment, the stalk-inoculated strain was recovered from 100% of lower stalks, 89% of infected crowns, and 33% of infected ear nodes and shanks. In the multiple-inoculation treatments, all inoculated strains were recovered, with the highest incidence of infection by each strain occurring near its respective inoculation site. In general, recovery of a particular strain from the multiple-inoculation treatment was more restricted within the plant compared with the single-inoculation treatments. Strains vegetatively compatible with each of the inoculated strains were detected in a few samples from noninoculated plants.

TABLE 2. Incidence of infection of maize plant tissues by *Fusarium moniliforme* strains used for plant inoculations in a field experiment conducted at the Curtiss farm near Ames, IA, 1993^a

Inoculation site ^b	Inoculated strain	Plant tissue			
		Crown	Lower stalk	Stalk (ear node)	Cob
Single inoculations ^c					
Crown	EA-3	79	50	37	19
Stalk	EA-4	44	92	33	38
Silk	EA-1	53	67	20	80
Multiple inoculation ^d					
Crown	EA-3	53	0	0	0
Stalk	EA-4	11	94	8	11
Silk	EA-1	14	0	23	61
Noninoculated control					
	EA-3	10	0	0	10
	EA-4	0	0	0	0
	EA-1	0	0	0	0

^a Entries are percentage of *F. moniliforme*-infected plants from which isolates vegetatively compatible with inoculated strains were recovered, based on means of 10 replications and five plants per replication.

^b Plants were inoculated by injection of a spore suspension into crowns at growth stage V6, injection into stalks at growth stage V18, or spraying silks with a spore suspension at growth stage R1-R2.

^c In the single-inoculation treatments, each plant was inoculated once, at one of the three inoculation sites.

^d In the multiple-inoculation treatment, each plant was inoculated three times, once at each inoculation site.

In each field experiment, only the silk-inoculation and multiple-inoculation treatments significantly increased (Student-Newman-Keuls test, $\alpha = 0.05$) the incidence of symptomless kernel infection by *F. moniliforme* compared with the control (Figs. 3 to 6). Incidence of kernel infection differed significantly ($P < 0.05$) between the two kernel sampling dates in 1993, with greater incidence in the later sampling (Fig. 3). Maize hybrids did not differ significantly ($P = 0.0943$) in the Hinds experiment, and there were no differences between the multiple-inoculation treatments with or without postinoculation plastic bags on the ears. Incidence of symptomless infection was lower in the Woodruff experiment than in the Hinds experiment, and it differed significantly between the two hybrids at Woodruff farm ($P = 0.0007$).

Each of the inoculated strains was recovered from symptomless kernels in its respective treatment, except for the seed-inoculated strain in 1993 (Figs. 3 to 6; Table 4). The silk-inoculated strains were always recovered from a high percentage of ears (86 to 100%)

in the silk-inoculated treatments. Incidence of the crown-, stalk-, and shank-inoculated strains was significantly lower, ranging from 1.2 to 64% of ears (Table 4). In both 1994 experiments, the seed-inoculated strain was recovered from 10% of ears receiving the seed inoculation. In the multiple-inoculation treatments, the silk-inoculated strain infected a greater percentage of kernels than any other strain (Figs. 3 to 6). Incidence of kernel infection by the crown- and stalk-inoculated strains was significantly lower (Student-Newman-Keuls test, $\alpha = 0.05$) in the multiple-inoculation treatments than in their respective single-inoculation treatments. A low percentage of isolates recovered from noninoculated plants were vegetatively compatible with strains used in inoculations.

Within individual ears in both 1994 experiments, silk-inoculated strains were recovered from 0 to 100% of symptomless kernels. In those ears infected with the silk strains, mean incidence of infection by the strains was 33.0 to 72.5% of all kernels, or 60.0 to 90.1% of symptomless *F. moniliforme*-infected kernels. Similarly,

TABLE 3. Incidence of infection of maize plant tissues by *Fusarium moniliforme* strains used for plant inoculations in field experiments conducted at two locations near Ames, IA, in 1994^a

		Hinds farm					Woodruff farm				
			Plant tissue					Plant tissue			
Inoculation site ^b	Seed also inoculated	Inoculated strain	Crown	Lower stalk	Ear node	Shank	Inoculated strain	Crown	Lower stalk	Ear node	Shank
Single inoculations ^c											
Crown	Yes	EA-3	81.0	40.0	18.2	0	EA-4	91.9	47.4	18.2	17.8
		EA-4	10.8	13.3	18.2	0	EA-1	2.7	5.3	18.2	0
Stalk	Yes	EA-1	62.5	97.5	46.7	20.0	EA-2	52.6	100	32.7	15.8
		EA-4	12.5	0	0	0	EA-1	5.3	6.9	8.2	26.3
Silk	Yes	115	0	5.9	0	83.3	EA-3	0	0	16.7	45.8
		EA-4	18.1	0	0	0	EA-1	27.8	9.1	0	0
Shank	Yes	EA-2	2.5	20.0	14.3	90.0	115	0	9.1	33.3	97.0
		EA-4	37.5	10.0	7.1	0	EA-1	16.7	0	0	0
Multiple inoculation ^d											
Crown	Yes										
	Yes	EA-3	56.8	0	8.3	0	EA-4	74.3	5.3	14.3	3.7
Stalk	Yes	EA-1	21.6	97.5	41.7	2.9	EA-2	11.4	92.1	28.6	3.7
	Yes	115	0	0	8.3	25.7	EA-3	0	0	7.1	11.1
Shank	Yes	EA-2	0	0	8.3	65.7	115	0	0	28.6	62.9
	Yes	EA-4	16.2	0	0	0	EA-1	2.9	0	0	0
Noninoculated control											
	Yes										
	Yes	EA-3	0	0	0	0	EA-4	0	10.0	0	0
	Yes	EA-1	0	0	0	0	EA-2	0	0	0	0
	Yes	115	0	0	0	0	EA-3	0	0	0	0
	Yes	EA-2	0	0	0	0	115	0	0	0	0
Seed	Yes	EA-4	44.4	0	0	0	EA-1	25.0	0	11.1	6.5
Mean ^e	Yes	EA-4	18.1	2.4	4.3	0	EA-1	9.8	3.5	5.4	4.5
Single inoculations ^f											
Crown	No										
	No	EA-3	83.8	37.5	33.3	0	EA-4	100	60.0	33.3	34.8
Stalk	No	EA-1	88.9	100	33.3	33.3	EA-2	100	97.4	20.0	11.8
	No	115	18.1	6.7	6.7	64.3	EA-3	0	9.1	27.8	34.8
Shank	No	EA-2	0	25.0	8.3	71.4	115	0	25.0	30.6	86.2
Multiple inoculation ^g											
Crown	No										
	No	EA-3	68.6	0	0	0	EA-4	80.0	8.1	10.5	9.4
Stalk	No	EA-1	20.0	100	25.0	9.1	EA-2	17.1	91.9	10.5	0
	No	115	0	0	6.3	30.3	EA-3	0	0	10.5	3.1
Shank	No	EA-2	0	0	6.3	51.5	115	0	0	15.8	71.9
Noninoculated control											
	No										
	No	EA-3	0	9.1	0	6.2	EA-4	0	0	0	0
	No	EA-1	0	9.1	0	0	EA-2	9.1	0	0	0
	No	115	0	0	0	0	EA-3	0	0	0	0
	No	EA-2	0	0	0	0	115	0	0	0	0
Mean ^e	No	EA-4	0	0	0	0	EA-1	0	0	0	0

^a Entries are percentage of *F. moniliforme*-infected plants from which isolates vegetatively compatible with inoculated strains were recovered. Percentages are based on combined data for two hybrids (eight replications of five plants each).

^b Plants were inoculated by injection of a spore suspension into crowns at growth stage V6, injection into stalks at growth stage V18, or spraying silks with a spore suspension at growth stage R1-R2.

^c Seeds were inoculated and each plant was inoculated once, at one of the three inoculation sites.

^d Seeds were inoculated and each plant was inoculated four times, once at each inoculation site.

^e Mean incidence of infection by the seed-inoculated strain.

^f Each plant was inoculated once, at one of the four inoculation sites.

^g Each plant was inoculated four times, once at each inoculation site.

the stalk-inoculated strains were recovered from 0 to 95% of kernels in individual ears; considering only the ears infected with these strains, mean incidence of infection was 13.5 to 32.5% of kernels (29.3 to 55.0% of symptomless *F. moniliforme*-infected kernels). Incidence of kernel infection by the crown-inoculated strains ranged from 0 to 75% of kernels within individual ears; mean incidence of the crown-inoculated strains in these ears was 5.0 to 20.5% of kernels (7.9 to 50.0% of infected kernels). Incidence of the shank-inoculated strains in individual ears was 0 to 60% of kernels; mean incidence of the shank-inoculated strains from these ears was 9.6% of kernels (20.3% of infected kernels). Incidence of the seed-inoculated strains in individual ears was 0 to 70% of kernels; mean incidence of the seed strains from these ears was 0 to 15.5% of kernels (0 to 48.2% of infected kernels).

The percentage of kernels with symptoms of Fusarium kernel rot at maturity was low in all experiments, ranging from 0.7 to 5.0% (Table 5). Silk-inoculated treatments had slightly greater severity than the others, but this difference was significant only for the 1993 experiment (Student-Newman-Keuls test, $\alpha = 0.05$). The presence of Fusarium kernel rot symptoms and the presence of insect damage were significantly related (χ^2 test, $P < 0.0001$) in all three experiments. Recovery of the inoculated strains from symptomatic kernels was greater in the Hinds experiment than the Woodruff experiment (Fig. 7). In the Hinds experiment, the silk-inoculated

strain caused kernel rot in a high percentage of symptomatic kernels. Other inoculated strains were detected, but not the seed-inoculated strain. In the Woodruff experiment, the seed-inoculated strain infected a small percentage of symptomatic kernels (Fig. 7).

DISCUSSION

The results of this study confirm earlier reports (2,5,11,20) that *F. moniliforme* can infect maize plants and grow systemically without causing symptoms, and that such infections can progress into the kernels. More importantly, this study has provided a measure of the relative contributions of various pathways to symptomless kernel infection when plants are inoculated at specific growth stages. Some kernel infection by *F. moniliforme* resulted from introduction of the fungus into the seed or the stalk, but infection through the silks was, by far, the most important pathway to kernel infection in these experiments.

Transmission of a fungus from maize seed to kernels can be divided into four steps: (i) transmission from seed to seedling, (ii) movement within the stalk, (iii) movement into the ear, and (iv) spread within the ear. In our experiments, transmission of *F. moniliforme* from seed to seedling occurred frequently (Table 1). Less

Fig. 3. Mean incidence of symptomless maize kernel infection by *Fusarium moniliforme* in plants from a field experiment at Iowa State University Curtiss Farm, near Ames, IA, in 1993. **A**, Immature kernels and **B**, mature kernels. Mult. = multiple-inoculation treatment in which crowns, stalks, and silks were inoculated. Data are combined for plants from inoculated and noninoculated seed. Patterns indicate the percentage of infection by each inoculated strain. EA-1 was silk-inoculated, EA-3 was crown-inoculated, and EA-4 was stalk-inoculated. Bars with the same letter are not significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of 10 replications of 100 kernels each. Seeds were inoculated by soaking in a *F. moniliforme* spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2.

Fig. 4. Mean incidence of symptomless maize kernel infection by *Fusarium moniliforme* in plants from a field experiment at Iowa State University Hinds Farm, near Ames, IA, in 1994. Plants from **A**, noninoculated seed and **B**, inoculated seed. Data are combined for two hybrids. Seeds were inoculated by soaking in a *F. moniliforme* spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all five inoculation treatments. Silk-inoculated ears were covered with a plastic bag for 48 h after inoculation. The treatment designated Multiple/n did not have plastic bags placed over the ears. Patterns indicate the percentage of infection by each inoculated strain. EA-1 was stalk-inoculated, EA-2 was shank-inoculated, EA-3 was crown-inoculated, EA-4 was seed-inoculated, and 115 was silk-inoculated. Bars with the same letter are not significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of eight replications of 100 kernels each.

frequent was the movement of the fungus from crown tissue of seedlings to stalk tissue of growing plants. In the 1993 experiment, this step did not occur or was not detected; in the 1994 experiments, this step occurred in a low percentage of plants (Table 4). In our experiments, movement of the fungus from seedling crown tissue to stalk tissue appeared to be the limiting factor in the transmission of seed-inoculated strains to kernels. Previous reports (11) have shown a higher incidence of stalk infection by seed-inoculated strains. In our work, stalk sampling was performed earlier in the season, and this may have reduced detection of the seed strains. It is possible, as suggested by Lawrence et al. (20) and Nelson (32), that seed-transmitted strains do not advance above the plant roots and crown until after pollination, and stalk colonization by these strains progresses more rapidly in senescing (or senescent) stalks. Another possible explanation for reduced recovery of seed-inoculated strains in our experiments is the use of hybrid maize genotypes, rather than inbreds (11). The next step in *F. moniliforme* transmission, movement from stalk to ear, apparently was not as limiting as movement from seedling to stalk. For example, incidence of seed-inoculated strains in ears was at least as high as in upper stalks and shanks in the 1994 experiments (Tables 3 and 4). Spread of the fungus within ears or among kernels also did not seem to be limiting; in ears infected with the seed-inoculated strains, up to 70% of kernels were infected with the strain. Spread within the ear occurred late in the season; when kernels

were sampled twice (1993), infection frequency nearly tripled between stage R4 and the final sampling (after physiological maturity).

F. moniliforme strains introduced into the crowns, stalks, or shanks of plants moved both up and down within the plants (Tables 2 and 3). In most stalk-inoculated plants, a decayed lesion developed within the stalk near the inoculation point. In some cases, discolored vascular strands extended several nodes up or down. However, most stalks remained symptomless beyond a few centimeters from the inoculation point. Results generally indicated that *F. moniliforme* strains entering the plant through stalk and shank wounds, as would be made by insects such as the European corn borer, can contribute significantly to kernel infection. Strains inoculated into shanks did not infect as many kernels as those inoculated into stalks, possibly due to the late timing of the shank inoculations.

Fungal vegetative compatibility was a useful marker for identifying inoculated strains after their recovery from the field. The method is effective for *F. moniliforme* because vegetative incompatibility is widespread in the population of this fungus (22,24,42); vegetative compatibility is controlled by multiple alleles at approximately 10 loci (22). This method has limitations, however, because it is labor-intensive and vegetative compatibility is not completely specific to a single strain (23). In most of the experiments, a few isolates from noninoculated plants were found to be vegetatively compatible with inoculated strains. This might have been the result of unintentional spread of the original strains or the existence of endemic strains that were vegetatively compatible with the original strains. Because recovery of vegetatively compatible strains from

Fig. 5. Mean incidence of symptomless maize kernel infection by *Fusarium moniliforme* in plants from a field experiment at Iowa State University Woodruff Farm, near Ames, IA, in 1994. ICI hybrid 8599 plants from **A**, noninoculated seed and **B**, inoculated seed. Seeds were inoculated by soaking in a *F. moniliforme* spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation (Mult.) included all five inoculation treatments. Patterns indicate the percentage of infection by each inoculated strain. EA-1 was seed-inoculated, EA-2 was stalk-inoculated, EA-3 was silk-inoculated, EA-4 was crown-inoculated, and 115 was shank-inoculated. Bars with the same letter are not significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of four replications of 100 kernels each.

Fig. 6. Mean incidence of symptomless maize kernel infection by *Fusarium moniliforme* in plants from Woodruff Farm, in 1994. ICI hybrid 8708 plants from **A**, noninoculated seed and **B**, inoculated seed. Seeds were inoculated by soaking in a *F. moniliforme* spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation (Mult.) included all five inoculation treatments. Patterns indicate the percentage of infection by each inoculated strain. EA-1 was seed-inoculated, EA-2 was stalk-inoculated, EA-3 was silk-inoculated, EA-4 was crown-inoculated, and 115 was shank-inoculated. Bars with the same letter are not significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Values are means of four replications of 100 kernels each.

TABLE 4. Incidence of infection of maize ears and kernels by *Fusarium moniliforme* strains inoculated into plant crowns, stalks, silks, or shanks in field experiments conducted near Ames, IA, in 1993 and 1994^a

Experiment	Tissue	Site of maize plant inoculation with <i>F. moniliforme</i> ^b						
		Seed	Crown	Stalk	Silk ^c	Shank	Multiple ^c	Multiple
Curtiss 1993 ^d	Ears (%)	0 c	56.0 b	64.0 b	100 a	NA	100 a	NA
	Kernels (%)	0 c	13.7 b	19.2 b	53.3 a	NA	57.4 a	NA
	<i>F. moniliforme</i> -infected kernels (%)	0 c	44.2 b	49.4 b	90.1 a	NA	91.9 a	NA
Hinds 1994 ^e	Ears (%)	10.0 c	1.2 d	30.0 b	100 a	16.2 bc	100 a	94.2 a
	Kernels (%)	0.1 b	0.1 b	6.3 b	83.7 a	1.4 b	79.8 a	69.1 a
	<i>F. moniliforme</i> -infected kernels (%)	0.7 c	0.6 c	30.4 b	94.1 a	7.3 c	94.1 a	93.4 a
Woodruff 1994 ^e	Ears (%)	10.0 c	57.5 b	35.0 bc	86.2 a	33.7 bc	98.0 a	NA
	Kernels (%)	2.5 c	12.5 c	5.2 c	29.8 b	4.8 c	44.7 a	NA
	<i>F. moniliforme</i> -infected kernels (%)	8.3 d	41.2 c	17.2 d	62.7 b	15.8 d	87.8 a	NA

^a Within a row, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test, $\alpha = 0.05$. Percentages are based on combined data for two hybrids (eight replications of five plants each).

^b Seeds were inoculated by soaking in a spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all five inoculation treatments.

^c Ears were covered with plastic bags for 48 h after silk inoculation

^d Values are means of five replications of five ears or 100 kernels each.

^e Values are means of eight replications of five ears or 100 kernels each.

control plants rarely occurred, it did not interfere with interpretation of the results. Occurrence of vegetatively compatible strains in control plants was not reported in similar work on *F. moniliforme* by Kedera et al. (11), but was reported to be a more significant factor when vegetative compatibility was used to identify strains of *Aspergillus flavus* released in the field and recovered (4).

Incidence of infection of symptomatic kernels by inoculated strains was higher in the Hinds experiment than in the Woodruff experiment (Fig. 7). In the Hinds experiment, the pattern was similar to that observed for symptomless kernels; the silk-inoculated strain was dominant and the crown-, stalk-, and shank-inoculated strains infected lower percentages of the kernels. In some cases, symptomless kernels infected with an inoculated strain may have developed symptoms after being wounded by insects. However, the low incidence of the seed-, stalk-, and crown-inoculated strains in symptomatic kernels indicated that kernel rot was not as closely related to systemic plant infection as was symptomless kernel infection. In the Woodruff experiment, most infection in symptomatic kernels was apparently due to strains other than those used for inoculations; however, the seed-inoculated strain was recovered from some kernels. The high level of background infection in symptomatic kernels at Woodruff farm may have been due to the introduction of endemic strains by insects. Insects are an important factor in the infection of maize kernels by *F. moniliforme* (18,32,43), but their role was not examined in this study.

Competition among *F. moniliforme* strains within plants was evident in this study. For most of the inoculated strains, the percentage of kernels infected with a particular strain was lower in the multiple-inoculation treatments than in the single-inoculation treatments (Figs. 2 and 5; Tables 2 and 3). Strains inoculated onto silks appeared to have a competitive advantage. It is common for maize plants to be infected with more than one strain of *F. moniliforme* and other *Fusarium* species (12); consequently, multiple pathways are probably functioning in these plants and the strains are competing for colonization of the kernels and other tissues.

The predominance of silk infection as a pathway to the kernels supports the pursuit of resistance factors that are expressed in silks, kernel pericarps, and other kernel tissues (8,9,10,13,41). However, it is clear that some kernel infection can occur by other pathways. These other pathways may gain importance in genotypes that have reduced infection through the silks.

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TABLE 5. Mean percentage of maize kernels with symptoms of *Fusarium* kernel rot in field experiments conducted near Ames, IA, in 1993 to 1994^a

Site of inoculation ^b	Experiment		
	Curtiss 1993 ^c	Hinds 1994 ^d	Woodruff 1994 ^d
Control	3.1 b	0.7 b	2.0 ns
Crown	2.9 b	0.7 b	2.4
Stalk	2.9 b	0.8 b	2.1
Silk ^e	5.0 a	1.7 ab	3.2
Shank	NA	4.1 a	2.4
Multiple	NA	1.9 ab	NA
Multiple ^e	3.8 ab	1.4 ab	2.7

^a Values in a column followed by the same letter are not significantly different according to the Student-Newman-Keuls test, $\alpha = 0.05$.

^b Crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all five inoculation treatments.

^c Percentages are based on combined data for plants grown from inoculated and noninoculated seed (means of 10 replications of five ears each).

^d Percentages are based on combined data for two hybrids and for plants grown from inoculated and noninoculated seed (means of 16 replications of five ears each).

^e Ears were covered with plastic bags for 48 h after silk inoculation.

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Fig. 7. Mean incidence of infection by *Fusarium moniliforme* in maize kernels with *Fusarium* kernel rot symptoms in field experiments in 1994. **Top**, Hinds Farm; **Bottom**, Woodruff Farm. Seeds were inoculated by soaking in a *F. moniliforme* spore suspension; crowns, stalks, and shanks were inoculated by injection of a spore suspension at growth stages V6, V18, and R3-R4, respectively; silks were inoculated by spraying a spore suspension onto silks at stage R1-R2; multiple inoculation included all five inoculation treatments. Silk-inoculated ears were covered with a plastic bag for 48 h after inoculation. The treatment designated Multiple/n did not have plastic bags placed over the ears (this treatment was not included in the Woodruff experiment). Patterns indicate the percentage of infection by each inoculated strain. In the Hinds experiment, EA-1 was stalk-inoculated, EA-2 was shank-inoculated, EA-3 was crown-inoculated, EA-4 was seed-inoculated, and 115 was silk-inoculated. In the Woodruff experiment, EA-1 was seed-inoculated, EA-2 was stalk-inoculated, EA-3 was silk-inoculated, EA-4 was crown-inoculated, and 115 was shank-inoculated. Bars with the same letter are not significantly different from the control according to the Student-Newman-Keuls test ($\alpha = 0.05$). Data are combined for two hybrids at each location. Values are means of eight replications of 25 kernels each.

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