Influence of Inoculation Method on Systemic *Fusarium moniliforme* Infection of Maize Plants Grown from Infected Seeds

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ABSTRACT

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Two greenhouse and two field experiments were conducted in 1994 and 1995 to quantify the incidence of maize kernel infection resulting from systemic infection of maize plants by *Fusarium moniliforme*. Seeds were infected by two methods: (i) spray-inoculation of maize silks during seed development (field-infected), and (ii) soak-inoculation of mature seeds in a spore suspension (laboratory-infected). Plants were grown from infected seeds and assayed for systemic infection by the seed-inoculated strain, determined by vegetative compatibility of recovered isolates with the original strain. The seed-inoculated strain was recovered from stalks and kernels of plants grown from both types of infected seed. Mature plants grown from field-infected seeds had a higher percentage of their kernels infected with the seed-inoculated strain compared with plants from laboratory-infected seed. Mean incidence of infection by the seed-inoculated strain was 9.9 to 29.4% of all kernels (33.0 to 55.9% of *F. moniliforme*-infected kernels) in the plants grown from field-infected seed. Some plants from infected seed were subsequently silk-inoculated, and the silk-inoculated strain was recovered from a much higher percentage of kernels (26.5 to 37.5% of all kernels or 77.9 to 78.4% of *F. moniliforme*-infected kernels) than was the seed-inoculated strain; furthermore, silk inoculation significantly reduced incidence of kernel infection by the seed-inoculated strain. Seed infection by *F. moniliforme* resulted in systemic infection of plants and kernels. However, local infection (via silks) was a more important pathway to kernels than was systemic infection, and strains infecting the silks competed successfully against those entering the kernels through systemic development.

Additional keywords: *Gibberella fujikuroi*

Fusarium moniliforme J. Sheld. is a seed-transmitted pathogen of maize that is found throughout much of the world (14,27). Seedborne infection can result in poor germination and seedling decay (22), but the significance of seed transmission of *F. moniliforme* can extend well beyond the seedling stage. Seed-transmitted infections may contribute to stalk rot, ear rot, and symptomless, systemic infection of maize plants (2,5,16,22,24,25). Reductions in yield and grain quality can result from stalk and ear rot. Another detrimental effect of *F. moniliforme* infection of grain is the accumulation of mycotoxins. Strains of *F. moniliforme* isolated from maize are generally members of mating population A

of *Gibberella fujikuroi* (Sawada) Wollenw. (17). Mating population A includes strains that can produce high levels of fumonisins (18,20), which can cause fatal diseases in livestock and have been linked to cancer (6,28,33). Fumonisins can be found in visibly moldy and symptomless infected kernels (1–3,26,28). The discovery of fumonisins has therefore placed an added importance on reducing *F. moniliforme* infection of maize.

The inoculum for kernel infection has traditionally been assumed to be airborne spores infecting via silks and wounds caused by insects (4,7,12,14). *F. moniliforme* produces abundant windborne microconidia (31). The source of these spores is presumably maize residue, but there are contradictory reports regarding the survival of *F. moniliforme* in maize residue in the field (19,30). One report indicates that microconidia from tassels may be a source of conidia for silk infection (21).

Seed-transmitted, systemic infection of maize plants can also contribute to kernel infection (5,16,24,25). In 1962, Foley (5) concluded that stalk infection occurred through leaf sheaths, and that symptomless kernel infection led to symptomless systemic infection of the stalk and ultimately the kernels. Endophytic colonization of

maize plants by *F. moniliforme* has been observed by other researchers (2,16). Further evidence for the ability of *F. moniliforme* to cause systemic infection of maize was provided by Kedera et al. (8,9), who used vegetative self-compatibility as a marker to track the movement of specific strains from seeds, through the plants and into the kernels. However, other authors have not found evidence for systemic infection (5,12,13,15,34), and the overall significance of seed transmission of *F. moniliforme* has been debated in relation to its effects on seedlings, stalk rot, systemic infection, and kernel infection. The fungus is very common in corn seed, and in some cases, heavily infected seed shows no detrimental effects (22). Some researchers have reported that there is no relationship between incidence of seed infection and incidence of seedling infection (15,34).

Because *F. moniliforme* is ubiquitous, movement of a single fungal strain from seed to stalk has been difficult to monitor. In field experiments, noninoculated plants inevitably become infected with endemic strains; this can occur even in the greenhouse (34). Infection by endemic strains can mask the detection of movement of an inoculated strain from seed to stalk.

In most studies on seed transmission of *F. moniliforme*, either seeds were inoculated in the laboratory after maturity, or naturally infected seeds were used (8,9,15,16,34). In the former case, there is a potential for artifactual results because the mode of seed infection is quite unnatural. In the latter case, it is generally not possible to identify specific strains moving from seed to plant because naturally infected seed may contain a diversity of indistinguishable strains. More accurate results should be obtained when seeds are infected during their development in the field with an *F. moniliforme* strain that can be distinguished from endemic strains.

The significance of seed transmission and systemic infection by *F. moniliforme* requires further investigation because of the widespread occurrence of symptomless infection of kernels and the potential for systemic infection to lead to fumonisin contamination. The objective of this research was to determine and compare levels of systemic infection of maize plants from seed inoculated in the laboratory and in the field. Preliminary results have been presented (23).

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MATERIALS AND METHODS

Characterization of fungal strains.

Three *F. moniliforme* strains, designated EA-1, EA-2, and 115, were obtained from maize seed or grain grown in Iowa by culturing whole kernels on Nash-Snyder Medium (NSM) (29). After isolation, single spores of each strain were transferred to carnation leaf agar (CLA) (29) and stored on silica gel pellets at 4°C. All strains used in the study were grown on CLA and identified as *F. moniliforme* according to the description by Nelson et al. (29). These strains were deposited with the Pennsylvania State University *Fusarium* Research Center, where they were designated M-8113, M-8114, and M-8117. The strains were determined to belong to mating population A of *G. fujikuroi* (17) 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 (10).

Complementation testing among nitrate-nonutilizing (*nit*) mutants (10) of the three strains was used to test for vegetative compatibility among the strains. Vegetative compatibility tests were conducted according to the methods of Klittich and Leslie (10). Strains were transferred from CLA to minimal medium +2.5% potassium potassium chlorate (MMC) (10) and potato-dextrose agar (Difco) + 2.5% chlorate (PDC). Sectors growing rapidly on these media were usually *nit* mutants; wild-type isolates do not grow on chlorate-amended media. The phenotypes of putative mutants growing on MMC were determined by transferring colonies to both hypoxanthine medium (hx) and minimal medium (MM),

which has nitrate as the sole nitrogen source. Mutants growing on hx and not on MM were either *nit-1* or *nit-3* mutants. Mutants that grew on neither medium were classified as *nit-M* mutants. Most mutants recovered from MMC were *nit-M*, whereas most recovered from PDC were *nit-1* or *nit-3*. Some sectors that grew on MMC also grew well on MM. These were likely chlorate-resistant, nitrate-utilizing (*crn*) mutants (11) and were discarded. To test for vegetative compatibility, *nit-M* mutants were paired on MM with *nit-1* or *nit-3* 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 three strains were self-compatible but incompatible with the other strains.

Systemic infection. Two greenhouse experiments and two field experiments were conducted to compare the incidence of systemic infection in plants grown from field-infected seeds (harvested from silk-inoculated plants) and laboratory-infected seeds.

Seeds used for the experiments described here were generated in a 1993 field experiment (25). A precommercial corn hybrid, ICI 20051, was planted in a field near Ames, Iowa. At growth stage R1 (32), silks of some plants were inoculated with *F. moniliforme* strain EA-1 by spraying to runoff with a conidial suspension (10^6 spores per ml) in sterile water (approximately 2 ml per plant). One week later, silks were inoculated again and the ears were covered with plastic bags for 48 h. Control plants were not inoculated or covered with bags. Ears were harvested after maturity and kernels were removed.

The incidences of *F. moniliforme* infection and strain EA-1 infection in kernels were determined by surface-disinfesting kernels in 0.5% sodium hypochlorite for 3 min, placing whole kernels (500 each of inoculated and noninoculated kernels) on NSM, and testing recovered *F. moniliforme* isolates for vegetative compatibility with EA-1, as described previously.

Kernels harvested in 1993 were used for the greenhouse experiments. In experiment 1, two treatments (field-infected seed, harvested from inoculated plants, and control seed, harvested from noninoculated plants) were arranged in a completely randomized design with five replicates of 10 plants each. Incidence of infection by strain EA-1 was 72% for infected seeds and 0% for control seeds. Plants were grown in 15-cm pots to stage V10, at which time the plants were aseptically dissected and cultured on NSM for isolation of *F. moniliforme*; isolates recovered from the crown and the first four nodes were tested for vegetative compatibility with strain EA-1, as described previously. Isolates that failed to form stable *nit* mutants after three attempts were not included in calculations and analyses. The experiment was repeated (experiment 2) with an added treatment (laboratory-infected seed). For this treatment, seeds collected from noninoculated plants in the 1993 field experiment were inoculated in the laboratory according to the method of Kedera et al. (8), with some modifications. 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. This duration of hot water treatment did not affect germination. Seeds were removed from the hot water treatment, placed in a spore suspension of *F. moniliforme* strain EA-1 macroconidia and microconidia (10^6 spores per ml) for 14 h, and air-dried. A sample of laboratory-inoculated seeds was surface-disinfested and tested for infection by strain EA-1; 100% of the seeds were infected with the inoculated strain. Noninoculated seeds in this experiment also received the hot water treatment. The experimental design was completely randomized with five replicates of 10 plants each.

A field experiment was conducted in 1994 at the Iowa State University Curtiss Farm to assess transmission of *F. moniliforme* from field-infected seeds to kernels. There were three treatments consisting of seeds collected from the 1993 field experiment. Treatments 1 and 2 were field-infected seeds that differed in their incidence of infection by strain EA-1 (72 and 61%, respectively). The third treatment was a control; the seeds had no infection by strain EA-1. The treatments were planted in single rows approximately 30 m long. After maturity, 50 ears each were selected arbitrarily from each treatment. Ten arbitrarily selected kernels were re-

Table 1. *Fusarium moniliforme* infection (%) of maize plants grown in the greenhouse from field-infected seed, 1994 (experiment 1)^w

<i>F. moniliforme</i> infection	Plant tissue	Seed inoculation treatment	
		Field-infected ^x	Control ^y
All strains	Crown	70.0* ^z	44.0
	Node 1	42.8*	26.0
	Node 2	32.0*	20.0
	Node 3	16.7*	0.0
	Node 4	6.0	0.0
Strain EA-1 (% of all samples)	Crown	50.0*	0.0
	Node 1	30.0*	0.0
	Node 2	20.8*	0.0
	Node 3	8.7*	0.0
	Node 4	0.0	0.0
Strain EA-1 (% of <i>F. moniliforme</i> - infected samples)	Crown	71.4*	0.0
	Node 1	70.1*	0.0
	Node 2	65.0*	0.0
	Node 3	52.0*	0.0
	Node 4	0.0	0.0

^w Percentages are based on five replicates of 10 plants (some data were missing due to contamination).

^x Field-infected seeds were harvested from plants that were silk-inoculated with *F. moniliforme* in the field.

^y Control seeds were from noninoculated plants.

^z * means value was significantly higher than the control (*F* test, *P* < 0.05).

moved from each ear, surface-disinfested, and cultured on NSM; recovered isolates were tested for vegetative compatibility with strain EA-1, as described previously. The percentage of kernels infected with *F. moniliforme* and the percentage from which EA-1 was recovered were recorded. Isolates that failed to form stable *nit* mutants after three attempts were not included in calculations and analyses.

Another field experiment was conducted in 1995 to compare incidence of kernel infection originating from local (silk) infection with systemic plant infection. This experiment utilized a split-plot design, with seed-inoculation treatments (field-infected, laboratory-infected, and control, as in greenhouse experiment 2) as the main plots and silk inoculation (inoculated or noninoculated) as the subplots. There were five replicate blocks. Subplots were four rows wide (0.76-m spacing) and 4.3 m long, with 3 × 2 m borders, planted to the same hybrid, between subplots; 80 seeds were planted per subplot. All seeds planted were collected from a 1994 field experiment (Pioneer Brand hybrid 3563). Field-infected seed had been harvested from plants silk-inoculated with *F. moniliforme* strain 115 as described previously. This strain was detected in 80% of 500 seeds cultured on NSM. Laboratory-infected and control seeds were from noninoculated plants. Laboratory inoculation of kernels was conducted with strain 115 as described previously. After inoculation, strain 115 was recovered from 100% of a sample of the laboratory-infected seeds. In the 1995 experiment, when the plants reached stage R2, silks of plants in half of the subplots were spray-inoculated with strain EA-2 and covered with plastic bags for 48 h as described previously. Stalks were sampled twice during the season for isolation of *F. moniliforme*. At growth stage V12, five plants per subplot were selected arbitrarily, and tissue samples from the crowns, the third and sixth nodes above the soil line, and the ear shoot were aseptically removed, surface-disinfested in 0.5% NaOCl for 2 min, and cultured on NSM. At stage R4, another five plants per subplot were sampled, and the same tissues were cultured except that the ear shank was substituted for the ear shoot. The percentage of tissue samples that were infected with *F. moniliforme* was recorded, and the recovered isolates were tested for vegetative compatibility with the seed-inoculated strain (115). After the plants were mature, 10 ears were selected arbitrarily from each subplot, and 20 kernels were removed aseptically from each ear and cultured for *F. moniliforme* recovery as described previously. Recovered isolates were tested for vegetative compatibility with strains 115 and EA-2. Isolates that failed to form stable *nit* mutants after three attempts were not included in calculations and analyses.

Data analysis. Analyses of variance were conducted on the percentage of tissue samples or kernels infected by *F. moniliforme*. Analyses of variance also were conducted for each strain on the arcsine-square-root transformed proportions of ears that had one or more kernels infected with the inoculated strains. The general linear models procedure of SAS (SAS Institute, Cary, NC), the frequency analysis procedure of SAS, or analysis of variance procedures of SigmaStat Statistical Software (Jandel Scientific, San Rafael, CA) was used for the analyses. Mean separation analyses were conducted using the Student-Newman-Keuls test, and chi-square tests were used to compare frequency data.

RESULTS

In plants grown from inoculated seed in greenhouse experiment 1, *F. moniliforme*

was isolated from 70% of the crowns; incidence of node infection decreased with height above the soil line. Incidence of infection was significantly greater for the inoculated treatment than for the non-inoculated treatment (*F* test, *P* < 0.05). Strain EA-1 was recovered from 50% of the crowns, and recovery of this strain decreased with respect to node height (Table 1). No strains vegetatively compatible with EA-1 were recovered from the plants from noninoculated seeds.

In greenhouse experiment 2, results were similar. Incidence of *F. moniliforme* infection differed significantly among the treatments (*P* < 0.05) and usually was greatest for the laboratory-infected seed (Table 2). When data from different plant tissues were analyzed separately, only crown infection differed significantly among treatments. When expressed as a

Table 2. *Fusarium moniliforme* infection (%) of maize plants grown in the greenhouse from field-infected, laboratory-infected, and noninoculated seed, 1994 (experiment 2)^y

<i>F. moniliforme</i> infection	Plant tissue	Seed inoculation treatment		
		Field-infected ^w	Laboratory-infected ^x	Control ^y
All strains	Crown	78.0 b ^z	100.0 a	22.0 c
	Node 1	22.0 a	16.0 a	20.0 a
	Node 2	12.0 a	24.0 a	8.0 a
	Node 3	0.0 a	8.0 a	0.0 a
	Node 4	0.0 a	8.0 a	0.0 a
Strain EA-1 (% of all samples)	Crown	42.0 a	44.0 a	2.0 b
	Node 1	12.0 a	8.0 a	2.0 b
	Node 2	12.0 a	0.0 b	0.0 b
	Node 3	0.0 a	0.0 a	0.0 a
	Node 4	0.0 a	0.0 a	0.0 a
Strain EA-1 (% of <i>F. moniliforme</i> - infected samples)	Crown	59.0 a	44.0 b	7.1 c
	Node 1	54.5 a	50.0 a	9.1 b
	Node 2	100.0 a	0.0 b	0.0 b
	Node 3	0.0 a	0.0 a	0.0 a
	Node 4	0.0 a	0.0 a	0.0 a

^y Percentages are based on five replicates of 10 plants.

^w Field-infected seeds were harvested from plants that were silk-inoculated with *F. moniliforme* in the field.

^x Laboratory-infected seeds were harvested from noninoculated plants and infected by soaking in a spore suspension of *F. moniliforme*.

^y Control seeds were from noninoculated plants.

^z Within a row, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test ($\alpha = 0.05$).

Table 3. *Fusarium* symptoms, total symptomless kernel infection by *Fusarium moniliforme*, and infection by *F. moniliforme* strain EA-1 in kernels on plants grown from field-infected seeds^x, Curtiss Farm, 1994

Treatment	Symptoms (% kernels)	Symptomless infection (% kernels)	EA-1 infection		
			Kernels (%)	Infected kernels (%)	Ears (%)
Treatment 1 ^y	2.3 a ^z	52.6 a	29.4 a	55.9 a	78.0
Treatment 2	3.2 a	42.2 b	14.2 b	33.7 b	60.0
Control	ND	34.4 b	0.0 c	0.0 c	0.0

^x Field-infected seeds were harvested from plants that were silk-inoculated with *F. moniliforme* in the field.

^y Treatments 1 and 2 were seed lots that differed in incidence of seed infection (72 and 61%, respectively).

^z Values within a column followed by the same letter are not significantly different according to the Student-Newman-Keuls test ($\alpha = 0.05$). ns = no significant effect of treatment. ND = no data were collected. Percentages are based on 500 kernels from 50 ears per treatment.

percentage of all plants, incidence of infection by strain EA-1 did not differ significantly between the two inoculated treatments (Student-Newman-Keuls test, $\alpha = 0.05$), except for the second node above the crown. When expressed as a percentage of the *F. moniliforme*-infected plants, incidence of infection by EA-1 in crowns and the second node was significantly greater for plants from field-infected seed. One control plant was infected with strain EA-1.

In the 1994 Curtiss Farm experiment, symptoms of *Fusarium* kernel infection occurred at a very low incidence, were closely associated with insect damage to kernels (χ^2 test, $P < 0.0001$, data not shown), and were not significantly affected by seed inoculation. Symptomless kernel infection by *F. moniliforme* differed significantly among the treatments (Student-

Newman-Keuls test, $\alpha = 0.05$), with plants in treatment 1 expressing the highest incidence of infection (52.6% of kernels, Table 3). Incidence of infection by strain EA-1 also differed significantly among the treatments; maximum incidence was 55.9% of *F. moniliforme*-infected kernels from plants in treatment 1. EA-1 was not detected in the control treatment.

In the 1995 Curtiss Farm experiment, plants grown from field-infected seed had significantly more (Student-Newman-Keuls test, $\alpha = 0.05$) crown infection than did plants from laboratory-infected or control seed at stages V12 and R4 (Fig. 1). Incidence of stalk and shank infection were significantly higher for the field-infected treatment at stage R4. Plants grown from laboratory-infected seed did not have consistently higher infection of these tissues than did the control plants. In plants grown

from inoculated seed, the incidence of *F. moniliforme* infection and strain 115 infection decreased with height of the tissue samples. This trend did not occur with the plants from control seed. Incidence of infection by the inoculated strain (115) from crowns was significantly greater for the plants from field-inoculated seed than for those from laboratory-inoculated seed.

Seed inoculation (laboratory or field infection) did not significantly affect incidence of *F. moniliforme* infection in kernels ($P = 0.0945$); however, silk inoculation did increase kernel infection ($P = 0.0207$) (Fig. 2). The seed-inoculated strain (115) was isolated from 9.6% of the kernels (33.0% of *F. moniliforme*-infected kernels) on plants from field-infected seeds if the plants were not silk-inoculated. This was significantly greater than the incidence of infection by strain 115 in plants grown from laboratory-infected seeds. The percentage of ears with one or more kernels infected by strain 115 (Fig. 2) also was significantly affected by seed-inoculation method ($P = 0.0106$). The silk-inoculated strain (EA-2) was isolated from a significantly greater (Student-Newman-Keuls test, $\alpha = 0.05$) percentage of kernels than was the seed-inoculated strain. Silk inoculation significantly reduced ($P = 0.0321$) incidence of kernel infection by the seed-inoculated strain and reduced the percentage of ears infected by the seed-inoculated strain ($P = 0.005$). Kernel infection by the silk-inoculated strain did not differ significantly ($P = 0.438$) among the seed inoculation treatments.

DISCUSSION

These results further confirmed the ability of *F. moniliforme* to move systemically from seed to stalk to kernel in maize plants grown in the field. Seed inoculation method was a significant factor in the incidence of systemic infection. In general, incidence of systemic infection was greater in plants grown from field-infected seeds than in plants grown from laboratory-infected seeds. The highest incidence of systemic infection occurred in the 1994 Curtiss Farm experiment, using field-infected seed, and incidence of systemic infection was significantly greater for field-infected than for laboratory-infected seed in the 1995 Curtiss Farm experiment. This difference is more notable when one considers that incidence of infection of laboratory-infected seed was 100%, whereas that of field-infected seed was $\leq 80\%$. Even with the reduced incidence of seed infection, systemic infection incidence was higher with the field-inoculated seed. Considering that seed infection was 80% for the field-infected treatment in the 1995 Curtiss Farm experiment, the seed-inoculated strain infected 42.5% of ears in plants from field-infected seed compared with 28% for laboratory-infected seed. Field-infection of seeds is a more natural

Fig. 1. Maize crown, stalk, and shank infection by *Fusarium moniliforme* in plants grown from laboratory- or field-infected seed, Curtiss Farm, 1995: (A) growth stage V12, (B) growth stage R4. Field-infected seeds (Field) were harvested from plants that were silk-inoculated with *F. moniliforme* in the field. Laboratory-infected seeds (Lab) were harvested from noninoculated plants and infected by soaking in a spore suspension of *F. moniliforme*. Control seeds were from noninoculated plants. Strain 115 data are the percentages of samples infected with the seed-inoculated strain (115). Within each tissue type and sampling period, bars with the same letter are not significantly different according to the Student-Newman-Keuls test ($\alpha = 0.05$).

infection process and may result in fungal colonization of the embryonic tissue, whereas soaking mature seeds is likely to cause a more superficial infection. The true importance of systemic infection of maize by *F. moniliforme* is probably better reflected by results from field-infected seed.

Transmission from seed to immature stalks was not significantly different between inoculation methods in the greenhouse experiments when incidence was expressed as a percentage of all plants. However, considering only the infected plants, a greater percentage was infected with the inoculated strain in the field-infected treatment than in the laboratory-infected treatment. This comparison is probably more meaningful than a comparison considering all the plants, because initial seed infection was less in field-infected seed (72%) than in laboratory-infected seed (100%). The inoculated strain

also was detected in tissues higher in the plant (second node) in the field-infected treatment than in the laboratory-infected treatment. We have observed much higher levels of seed transmission in plants grown from both field- and laboratory-infected seed in experiments where the plants were sampled during the seedling stage (25). Apparently, many seedling infections are not persistent into the later vegetative-growth stages of the plants. The movement of *F. moniliforme* from the crown into the stalk seems to be a limiting factor in the occurrence of systemic infection. Infections that do persist often are not detectable above the crown tissue until the late reproductive stages or even after maturity (8,9,16,27). Relatively low incidence of stalk infection by the seed-inoculated strain in the 1995 field experiment may have been due to the earliness of the stalk sampling (stage R4).

Fungal vegetative compatibility was a useful marker for identifying inoculated strains after their recovery from the field. Even when total *F. moniliforme* infection was not affected by seed inoculation, it was usually possible to observe differences in incidence of systemic infection based on results of vegetative compatibility tests. In two 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 inoculated strains or the existence of endemic strains that were vegetatively compatible with the inoculated strains. Because this occurred at a low frequency, it did not interfere with interpretation of the results.

While field infection of seed resulted in significant levels of subsequent kernel infection, infection through silks was the dominant pathway to kernel infection in the 1995 field experiment. Competition among *F. moniliforme* strains within plants was evident in this experiment; silk inoculation significantly reduced kernel infection by the seed-inoculated strain. When conditions favored silk infection, strains entering the kernels by systemic infection were at a competitive disadvantage. This suggests that systemic plant infections do not reach the kernels until after pollination, an observation supported by previous work (16,25,27). In the 1995 Curtiss Farm experiment, plastic bags over the ears probably favored infection through silks; however, we have observed similar results when silk-inoculated ears were not covered (25).

In studies on seed transmission and systemic infection by *F. moniliforme*, the use of seed from inoculated plants is desirable to mimic results from natural infection. However, there are two disadvantages of this method: an extra growing season is required to produce infected seed, and the level of seed infection is usually less than 100%. Laboratory inoculation may not be as effective in terms of producing systemically infected plants; its main advantage is reduced time and labor.

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Fig. 2. Infection by *Fusarium moniliforme* in maize plants grown from laboratory- or field-infected seed, Curtiss Farm, 1995. Field-infected seeds (Field) were harvested from plants that were silk-inoculated with *F. moniliforme* in the field. Laboratory-infected seeds (Lab) were harvested from noninoculated plants and infected by soaking in a spore suspension of *F. moniliforme*. Control seeds (Ctrl.) were from noninoculated plants. Shading patterns indicate the percent infection by each inoculated strain. EA-2 was a silk-inoculated strain, and 115 was a seed-inoculated strain. (A) Percentage of ears with one or more kernels infected. (B) Percentage of kernels infected.

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