Elimination of *Fusarium moniliforme* from Corn Seed

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ABSTRACT

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Fusarium moniliforme was eliminated from naturally infested seeds of several corn (*Zea mays*) hybrids when seeds were pretreated in distilled water for 4 hr at 18–22 C and then placed in water at 60 C for 5 min. The seeds remained viable, and neither seeds nor aseptically germinated seedlings yielded *F. moniliforme* when plated on Komada agar medium. For seeds that were treated in distilled water at 18–22 C for 5 hr and then 55 C for 10 min, water with benomyl at 2,000 ppm for 24 hr, or acetone with benomyl at 6,250 or 25,000 ppm for 24 hr, *F. moniliforme* was not isolated from seed but was isolated often from aseptically germinated seedlings.

Additional key words: stalk rot

Fusarium moniliforme Sheldon is a widespread plant pathogen causing mostly severe root, stem, or crown rots on a wide range of host crops (2), including root, stalk, or ear rots in maize (*Zea mays* L.) (3). Although *F. moniliforme* is commonly isolated from most corn seed (8,16,19; E. B. Lawrence, *unpublished*), the relative importance of this source of inoculum in field infection has been widely debated (6,9,12,13,18). Kucharek and Kommedahl (11) planted seed lots with high and low incidences of *F. moniliforme* but observed no differences in field infection of corn roots. They suggested that airborne inoculum may be a major source of field inoculum. However, Anderegg and Guthrie (1) demonstrated that partial seed disinfestation reduced *F. moniliforme* infection in field soil low in soilborne inoculum of *F. moniliforme*. Thus, the level of seed contamination by *F. moniliforme* may be of greater importance than was previously realized.

The importance of seed infestation in the development of field infections cannot be properly evaluated if seed free of *F. moniliforme* is not available. Therefore, many surface-sterilization techniques have been used with varied success. Kaiser (7) found that corn soaked 10 min in 0.525% NaOCl reduced, but did not eliminate, *F. moniliforme* infestation and that 10 min in 5.25% NaOCl was also unsuccessful. Anderegg and Guthrie (1) treated seeds for 5 min in 0.525% NaOCl and plated them on Nash's medium (15) and observed that infestation was reduced from 73 to 3%. Hot water treatments have also been suggested to disinfest corn seed. Moore (14) found that incidence of *F. moniliforme* in kernels was reduced by presoaking kernels in KSO_3 or by heating them in water at 54 C for 20 min. Salama and Mishricky (17) suggested that corn seed could be disinfested by soaking kernels in tap water for 5 hr at room temperature and then for 10 min at 53–56 C. This technique, however, eliminated *F. moniliforme* from seed only if the seed was subsequently treated with $HgCl_2$ (0.3%) for 15 min. El-Meleigi et al (5) reported that seed free of *F. moniliforme* could be obtained by a combined ethanol and hot water treatment, but in our laboratory this technique proved erratic; low germination (3–40%) and reduced growth rate resulted if *F. moniliforme* was eliminated (*unpublished*). Recently, benomyl in acetone was suggested to eliminate *F. moniliforme* from asparagus seed (4). This technique had never been tested for disinfestation of corn seed, however.

In comparing various surface-sterilization techniques, we observed that *F. moniliforme* was detected in aseptically germinated corn seedlings, even when less or no infestation had been evident in seed plated directly on a selective medium. Foley (6) also noted a similar phenomenon.

This paper reports a hot water treatment that eliminates *F. moniliforme* from corn seed. This technique is compared with other surface-sterilization techniques using a very sensitive germinated-plant assay. In addition, the efficacy of the benomyl in acetone treatment on corn was tested.

MATERIALS AND METHODS

Corn seed (O's Gold hybrid SX5500A) that had not been subjected to a commercial captan treatment remained untreated or a 15-g lot (about 50 seeds) was wrapped in a cheesecloth package and treated in one of the following ways: 1) 70% ETOH for 10 min, 2) 70% ETOH and 1.6% NaOCl (30% Clorox) for 3 min, 3) 0.525% NaOCl (10% Clorox) for 10 min, 4) distilled water containing benomyl at 2,000 ppm for 24 hr on a rotary shaker, or 5) acetone containing benomyl at 6,250 or 6) 25,000 ppm for 24 hr on a rotary shaker, or 7) distilled water at room temperature for 5 hr, distilled water at 53–56 C for 10 min, and then rinsing in sterile distilled water (18–22 C). Seed packages of SX5500A were also soaked for 4 hr in distilled water at room temperature, followed by 5-min treatments in distilled water at 55, 60, 65, or 75 C. Unless otherwise specified, seeds were treated at room temperature and treatments were followed by several rinses in sterile distilled water.

After seed had dried 24 hr in an ethanol-disinfested laminar flow hood, 20 seeds were selected, halved, and placed on Komada medium (10) prepared by combining 1.0 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Fe-Na-EDTA; 2.0 g of L-asparagine; 20.0 g of D-galactose; 1,000 ml of distilled water; and 10.0 g of agar with 1.0 g of pentachloronitrobenzene (75% WP), 0.5 g of oxgall, 1.0 g of $Na_2B_4O_7 \cdot 10H_2O$, 0.3 g of streptomycin sulfate, 0.05 g of tetracycline, and 0.05 g of cloramphenicol added to the cooled basal medium after autoclaving. After 5–8 days at 20–22 C, the developing colonies were examined microscopically. If chains of microconidia were observed, the colony was identified as *F. moniliforme* and percentage of infestation was recorded.

To further test the efficacy of the various disinfestation techniques, glass test tubes (25 × 200 mm) were filled to one-fourth with vermiculite, capped, and autoclaved. A corn seed was added to each tube and the vermiculite was moistened with sterile distilled water. Twenty seeds disinfested by each treatment were tested. The tubes were incubated for 2–3 wk in continuous fluorescent light (3,228 lux) at 24 C. After incubation, percentage of germination was assessed; the plants were removed under a sterile hood, shaken free of adhering vermiculite, and cut into 1–2 cm root and stem pieces and the seeds were halved. These pieces and halved seeds

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Table 1. Comparison of Komada medium[x] with potato-dextrose agar (PDA) for detection of colonies of *Fusarium moniliforme*

Suspension[y] (microconidia/ml)	Colonies recovered (mean no.)	
	Komada	PDA
64	49.6 a[z]	41.6 a
32	28.3 b	24.3 b
16	14.3 c	11.0 c
8	8.0 d	8.3 d

[x]Containing 1.0 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Fe-Na-EDTA; 2.0 g of L-asparagine; 20.0 g of D-galactose; 1,000 ml of distilled water; and 10.0 g of agar with 1.0 g of pentachloronitrobenzene (75% WP), 0.5 g of oxgall, 1.0 g of $Na_2B_4O_7 \cdot 10H_2$, 0.3 g of streptomycin sulfate, 0.5 g of tetracycline, and 0.05 g of cloramphenicol added to the cooled basal medium after autoclaving.

[y]One milliliter of each conidial suspension was used to seed petri plates containing 20 ml of solidified medium.

[z]Numbers followed by the same letters in both columns are not significantly different ($P = 0.05$) as determined by Duncan's multiple test.

from the germinated seedlings were plated on Komada medium and incubated for 5–8 days at 20–22 C. *F. moniliforme* was identified from the developing colonies as described previously.

The superiority of the 4-hr soak, 5 min at 59–60 C hot water disinfestation treatment as compared with the more standard surface sterilization technique (0.525% NaOCl, 10 min) was further tested on nine Funk's seed hybrids (G4444, G4143, G4574, G4082, G4628, G4646, G4321, G4449, and G4606) that had not been treated with a commercial seed treatment. The infestation level of untreated seed was compared with that of seed treated with hot water or NaOCl by directly placing seed on Komada medium and by aseptically germinating seed in tubes and plating the plant parts on Komada medium. All of these experiments were repeated twice using 20 seeds for each treatment of each hybrid.

Komada medium (10) developed to isolate *Fusarium* spp. from soil was used in these experiments because attempts to use potato-dextrose agar (PDA) failed when plates became overridden with *Trichoderma* and *Rhizopus* spp. The efficacy of Komada medium was compared with that of PDA to ensure that Komada medium, because of its selectivity, does not significantly reduce the *F. moniliforme* population that can be isolated. A plate of PDA on which *F. moniliforme* had been cultured was scraped and washed with sterile distilled water into a 50-ml beaker. The suspension was filtered through two layers of cheesecloth to remove mycelial fragments. A hemacytometer count indicated that the filtrate contained 64 microconidia per milliliter. From this, three twofold dilutions (50-50) were prepared by adding 1, 3, and 7 ml of sterile distilled water to each 1.0 ml of the original suspension. Each dilution was delivered and spread on three replicate petri plates containing Komada or PDA, and the colonies that developed were counted and compared after 1 wk.

RESULTS AND DISCUSSION

When the sensitivity of Komada medium was compared with PDA, it was observed that colonies of *F. moniliforme* grew slower on Komada medium; however, the numbers that eventually appeared were not significantly different ($P = 0.05$) from the number on PDA (Table 1), justifying the use of Komada medium in these experiments.

All untreated corn seeds plated directly onto Komada medium were infested with *F. moniliforme* (Table 2), as were germinated seedlings of this control group. Several of the seeds treated with 70% ETOH, hot water, or benomyl in water or acetone were not infested when plated on agar. However, *F. moniliforme* was recovered from 10–75% of the seedlings from treated seeds. Thus, the germinated seedling assay for the presence of *F. moniliforme* in corn seed was proven to be more sensitive than direct plating of seed.

The hot water treatment suggested by Salama and Michricky (17) did not eliminate *F. moniliforme* from seed in this experiment (Table 2) and was only effective on most hybrids in their own experiments when coupled with an $HgCl_2$ treatment. Water treatments of 60, 65, and 75 C were effective in eliminating *F. moniliforme* from corn seed, but at 65 and 75 C, germination was inhibited (Table 3). Although germination of seed treated at 60 C was slightly reduced, this treatment successfully eliminated *F. moniliforme*. This is the only technique known to eradicate *F. moniliforme* from corn seed without affecting seed viability. This technique, which eliminates seed-borne *F. moniliforme* rather than delaying its appearance (as may occur with fungistatic compounds such as benomyl), has certain benefits for research. However, to maintain reasonably

Table 2. Influence of corn seed disinfestation treatments on seed germination and the recovery of *Fusarium moniliforme* from seed and seedlings

Treatment	Germination (%)	Infestation (%)[x]	
		Seed	Seedling[y]
Untreated (control)	100 a[z]	100 a	100 a
70% ETOH (10 min)	100 a	0 d	35 c
70% ETOH dip, 1.6% NaOCl (10 min)	100 a	25 c	40 c
0.525% NaOCl (10 min)	100 a	25 c	35 c
Water, 18–22 C (5 hr); water, 55 C (10 min)	95 a	0 d	10 cd
Benomyl at 2,000 ppm in H_2O (24-hr soak)	100 a	0 d	80 b
Benomyl at 6,250 ppm in acetone (24-hr soak)	70 b	0 d	75 b
Benomyl at 25,000 ppm in acetone (24-hr soak)	65 b	0 d	15 cd

[x]Determined by plating 20 halved seeds or seedling pieces on Komada medium containing 1.0 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Fe-Na-EDTA; 2.0 g of L-asparagine; 20.0 g of D-galactose; 1,000 ml of distilled water; and 10.0 g of agar with 1.0 g of pentachloronitrobenzene (75% WP), 0.5 g of oxgall, 1.0 g of $Na_2B_4O_7 \cdot 1OH_2$, 0.3 g of streptomycin sulfate, 0.5 g of stage R7 (50% of leaves yellow, physiologic maturity) for Florestal-1, Viçsa-1, and Urbana-1; at tetracycline, and 0.05 g of cloramphenicol added to the cooled basal medium after autoclaving.

[y]Treated seeds were germinated aseptically in glass tubes filled to one-fourth with vermiculite. After 2–3 wk, the 20 seedlings or ungerminated seeds were removed, shaken free of vermiculite, and plated on Komada medium.

[z]Percentages followed by different letters in any column are significantly different ($P = 0.05$) as determined by the chi-square test.

Table 3. Viability of seed and recovery of *Fusarium moniliforme* from seeds that were treated in distilled water for 4 hr at 18–22 C and then treated with hot water

Treatment	Germination (%)	Infestation (%)[x]	
		Seed	Seedling[y]
Untreated (control)	100 a[z]	100 a	100 a
55 C (5 min)	95 a	0 b	15 b
60 C (5 min)	90 a	0 b	0 b
65 C (5 min)	0 b	0 b	0 b
75 C (5 min)	0 b	0 b	0 b

[x]Determined by plating 20 halved seeds or seedling pieces on Komada medium containing 1.0 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Fe-Na-EDTA; 2.0 g of L-asparagine; 20.0 g of D-galactose; 1,000 ml of distilled water; and 10.0 g of agar with 1.0 g of pentachloronitrobenzene (75% WP), 0.5 g of oxgall, 1.0 g of $Na_2B_4O_7 \cdot 1OH_2$), 0.3 g of streptomycin sulfate, 0.5 g of tetracycline, and 0.5 g of cloramphenicol added to the cooled basal medium after autoclaving.

[y]Treated seeds were germinated aseptically in glass tubes filled to one-fourth with vermiculite. After 2–3 wk, the 20 seedlings or ungerminated seeds were removed, shaken free of vermiculite, and plated on Komada medium.

[z]Percentages followed by different letters in any column are significantly different ($P = 0.05$) as determined by the chi-square test.

Table 4. Percentage of *Fusarium moniliforme* isolated from hybrid corn seed before and after sodium hypochlorite (NaOCl) and hot water treatment at 60 C

	Isolated from seed (%)[x]			Isolated from seedling (%)[y]		
Hybrid	Control (untreated)	NaOCl	Hot water	Control (untreated)	NaOCl	Hot water
G4444	90 abc[z]	75 cdef	0 q	90 abc	85 abcd	0 q
G4143	90 abc	80 bcdef	0 q	95 ab	60 cdefgh	0 q
G4574	0 q	0 q	0 q	10 nopq	20 lmnopq	0 q
G4082	20 lmnopq	25 jklmnop	0 q	50 efghijk	65 defgh	0 q
G4628	80 bcde	25 jklmnop	0 q	80 bcde	90 abc	0 q
G4646	100 a	40 ghijklm	0 q	100 a	65 cdefgh	0 q
G4321	70 cefg	35 hijklmn	0 q	70 cdefg	95 ab	0 q
G4449	90 abc	15 mnopq	0 q	90 abc	60 defghi	0 q
G4606	80 bcde	75 cdef	0 q	90 abc	70 cdefg	0 q

[x] Both treated and untreated seeds or seedling pieces were plated on Komada medium containing 1.0 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Fe-Na-EDTA; 2.0 g of L-asparagine; 20.0 g of D-galactose; 1,000 ml of distilled water; and 10.0 g of agar with 1.0 g of pentachloronitrobenzene (75% WP), 0.5 g of oxgall, 1.0 g of $Na_2B_4 \cdot 1OH_2$, 0.3 g of streptomycin sulfate, 0.5 g of tetracycline, and 0.05 g of cloramphenicol added to the cooled basal medium after autoclaving.

[y] Treated and untreated seed were germinated aseptically in glass tubes filled to one-fourth with vermiculite. After 2–3 wk, the 20 seedlings or ungerminated seeds were removed, shaken free of vermiculite, and plated on Komada medium.

[z] Means (percentages) in any column followed by the same letters are not significantly different ($P = 0.05$) as determined by the chi-square test.

high germination (80–100%), the temperature should not exceed 60 C. This treatment was also effective on nine other hybrids (Table 4). For each hybrid, treatment of seeds with hot water eliminated *F. moniliforme* from both seeds and seedlings, but treatment with NaOCl resulted in a high recovery of the fungus from both seeds and seedlings. Regardless of hybrid, there were no statistical differences ($P = 0.05$) in seed germination (range 85–100%) whether seeds were treated with hot water, NaOCl, or not treated. The effectiveness of the hot water treatment as compared with the more standard NaOCl surface-sterilization technique suggests that *F. moniliforme* was eliminated from the internal portion of the seed.

The importance of seed infestation as a source of *F. moniliforme* inoculum has been widely debated. Infestation levels of seed lots have been compared by plating seed on agar media; seed with varying levels of infestation could then be compared in the field (9). Alternatively, highly infested seed was compared in the field with surface-disinfested seed that appeared largely disinfested after plating on agar (1). Based on results reported here, levels of seed infestation may be higher than are observed by assay of kernels directly on an agar medium. To explore further the importance of seed infestation, we recommend the hot water treatment of seed (4-hr soak, 5 min at 60 C); alternatively, infestation levels in seed lots should be determined by the more sensitive germinated-seedling assay.

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