

## Relationship of Harvest Date and Host Genotype to Infection of Maize Kernels by *Fusarium moniliforme*

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### ABSTRACT

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Kernels of nine field-grown maize hybrids were assayed for fungi, ergosterol content, and germination 8, 11, and 14 wk after the mid-silk stage. *Fusarium moniliforme* and *Acremonium* sp. were the predominant fungi isolated from kernels. Frequencies of *F. moniliforme* differed significantly ( $P = 0.05$ ) among maize hybrids between harvests. Consistently low or high kernel colonization by *F. moniliforme* occurred in certain hybrids. Ergosterol content was greater in field samples with a high frequency of *F. moniliforme*, but there were no significant differences in ergosterol content among hybrids. *F. moniliforme* kernel infection had little influence on germinability of these hybrids.

Maize (*Zea mays* L.) is often left in the field well beyond physiological maturity

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before harvest. When harvest is delayed, maize kernels may be subject to infection by fungi. High frequencies of *Fusarium moniliforme* Sheldon have been implicated in severe maize epidemics (1,3,8,9) and mycotoxicosis of livestock (4,17,18) and humans (13,15).

King and Scott (10) reported high levels of asymptomatic kernel infection by *F. moniliforme* in commercially grown maize in Mississippi. They found differences in inbreds for asymptomatic kernel infection expressed in hybrids. Crosses where both parents were resistant had 11% infection compared with 55% where both parents were susceptible and 33% where one parent was resistant and one was susceptible.

Melchers and Johnston (16) found no

relationship between seed infected with *F. moniliforme* and maize diseases incited by this fungus. Kucharek and Kommedahl (12) demonstrated that plants grown from seed lots differing in percent kernel infection had no appreciable differences in field infection of crown roots. However, Futrell and Kilgore (6) reported poor maize stands in fields where the seed source was infected by *F. moniliforme*.

The purpose of this study was to determine the effect of delayed harvest on natural infection of maize hybrids by *F. moniliforme* in the field. Frequency of kernel infection, fungal biomass expressed as ergosterol content, and seed viability of maize hybrids, previously identified as susceptible or resistant to *F. moniliforme* by King and Scott (10), were evaluated at three harvest dates.

### MATERIALS AND METHODS

Nine maize hybrids were planted in single-row plots 5 m long and 1 m apart at Starkville, MS, in 1982 and 1983. Plots were later thinned to 20 plants per row. The uppermost ears from 10 plants in a plot were harvested 8 (physiological maturity), 11, or 14 wk after mid-silk stage and were dried to a constant weight in a forced-air drier at 42 C for 7 days.

Experimental design was a randomized block with three replicates.

Estimated damage inflicted on each ear by insects was determined on a scale of 1–6, where 1 = no damage and 6 = damage at the tip and sides of the ear. Ears harvested from each plot were machine-shelled, and randomly selected samples of about 200 kernels per ear were stored at 6 C and 45% relative humidity. Stored samples were later assayed for fungal infection frequency, ergosterol content, and germinability.

Maize kernels from stored samples were surface-sterilized by submersion in 70% ethanol for 5 sec, soaked for 3 min in 1.6% NaOCl, and rinsed twice in sterile distilled water. One hundred thirty surface-sterilized kernels from each plot were plated on Czapek solution agar. After 4 days of incubation in the dark at 28 C, plated kernels were examined for the presence of *F. moniliforme* and other fungi.

Ergosterol content of kernels was assayed according to the procedure described by Seitz et al (20,21). Thirty-gram samples of maize grain chosen randomly from each plot were ground in a grinding mill to pass a 16-mesh (1-mm) screen. A 10-g milled sample was then blended with 30 ml of methanol. The blended sample and a 20-ml methanol washing of the blender jar were combined and centrifuged for 5 min at 3,020 g. The resulting supernatant was decanted and the residue resuspended with 20 ml of methanol, shaken for 30 sec, then centrifuged again. Both supernatant samples were combined, mixed with 5 g of KOH and 10 ml of ethanol, and refluxed for 30 min at 65 C. The cooled, saponified mixture was diluted with 10 ml of distilled water and extracted three times with 20 ml of hexane; the hexane extracts were combined and evaporated to dryness under nitrogen with heating. The resultant residue was dissolved in 10 ml of 95% methanol and left at room temperature overnight in the dark, then

the suspension was filtered through a double glass fiber filter. Five milliliters of final extract was quantified by high-pressure liquid chromatography.

Germination tests were conducted according to the standard technique described by the Association of Official Seed Analysts (AOSA) rules for testing seed (2). Four random samples of 50 seeds per plot were rolled in moist paper towels and placed in a germinator at alternating temperatures of 20 and 30 C for 12-hr periods. Germination counts were made at 4 and 7 days. Data on percentage of kernels infected with *F. moniliforme*, ergosterol content, and percent germinability were subjected to analysis of variance and mean separation.

## RESULTS

*F. moniliforme* and *Acremonium* sp. were the predominant fungal species isolated from maize kernels (30 and 17%, respectively), with only very low isolation frequencies (<10%) for *Alternaria*, *Trichoderma*, *Mucor*, *Drechslera*, *Curvularia*, *Penicillium*, *Nigrospora*, *Aspergillus*, *Epicoccum*, and other *Fusarium* spp. In both 1982 and 1983, there were significant differences ( $P = 0.05$ ) in the percentage of *F. moniliforme* isolated from hybrids harvested at different times after mid silk stage, and the data have been presented as the averages of three replicates (Table 1). The fungus was least often isolated from hybrids CI90C × SC170, DeKalb XL395, and Coker 77 and most often from Mp68:616 × Mp440, Trojan TXS114, Mp68:616 × Mp303, and Mp68:616 × Mp317. Frequencies of isolation increased progressively with harvest dates. In 1982, the frequencies of isolation of *F. moniliforme* from all hybrids harvested 8, 11, and 14 wk after mid silk stage were 18, 36, and 42%, and in 1983, 15, 36, and 33%, respectively.

Only one hybrid, Mp68:616 × Mp317, had significantly ( $P = 0.05$ ) higher ergosterol content than other hybrids

and that was at the final harvest. Mean ergosterol contents for all hybrids harvested at 8, 11, and 14 wk were 1.46, 2.09, and 3.12  $\mu\text{g}$  of ergosterol per gram of grain, respectively. Despite the trend toward increased ergosterol content from the first to the last harvest for most hybrids, the differences were not significant ( $P = 0.05$ ).

Significant ( $P = 0.05$ ) differences in maize kernel germination occurred among hybrids harvested 8 wk after mid silk but not at later dates. Northrup King PX675 had a significantly ( $P = 0.05$ ) lower germination percentage (93%) than eight other hybrids (mean = 96%) harvested at physiological maturity (8 wk after mid silk). The only hybrid with significantly ( $P = 0.05$ ) reduced germination at advanced harvest dates was Trojan TXS114 (89%). Maize kernels that did not germinate or develop into normal seedlings were overgrown by *F. moniliforme*.

## DISCUSSION

During this study, *F. moniliforme* and *Acremonium* sp. were the most abundant and frequently isolated fungi from field-grown maize. Many kernels that appeared asymptomatic and had been surface-sterilized yielded colonies of one or both of these fungi. Thus, we concluded that these fungi were borne internally. *F. moniliforme* has not been effectively eliminated by fungicides or hot water treatment in attempts to reduce the incidence of seedborne fungi in maize (1,5,9,19).

The progressive increase in maize kernel infection by *F. moniliforme* from 8 to 14 wk after mid silk suggests a continuing susceptibility to this fungus during kernel development or continued colonization after initial infection. The fact that a high percentage of kernels of certain hybrids contained *F. moniliforme*, even 8 wk after mid silk, means that the fungus may pose a potential health hazard when high moisture maize is stored hermetically (22). Consumption of maize infected by this fungus has been shown to be a contributing factor in the estrogenic syndrome in swine, and ingestion may coincide with a high incidence of human esophageal cancer (14,15,17).

Seitz et al (20) postulated that a lack of significant difference in ergosterol content of maize samples might be attributed to greater sampling variability compared with small grains. Harvested maize may contain many fungi-free or lightly invaded kernels, and only a few kernels that decay are heavily invaded. Ergosterol levels of maize hybrids in this study were consistently low except for the last harvest of Mp68:616 × Mp317 (3.12  $\mu\text{g}/\text{g}$ ). The bulk of kernels from this hybrid were in an advanced stage of decomposition by the last harvest, and this probably accounts for the high

**Table 1.** Infection of kernels of nine maize hybrids with *Fusarium moniliforme* in field plots at Starkville, MS, over two growing seasons (1982 and 1983)

Hybrid	Percent infection at harvest <sup>2</sup> (period after mid silk stage [wk])			
	8	11	14	Mean
CI90C × SC170	6	16	16	13 d
DeKalb XL395	5	21	22	16 cd
Coker 77	6	35	21	21 cd
CI90C × Mp303	10	35	28	24 bc
Northrup King PX675	16	33	38	29 b
Mp68:616 × Mp317	27	51	35	38 a
Mp68:616 × Mp303	31	34	65	43 a
Trojan TXS114	21	54	56	44 a
Mp68:616 × Mp440	29	48	56	44 a
Mean	17	36	37	

<sup>2</sup>Infection determined after 4 days of incubation in the dark of surface-sterilized kernels on Czapek solution agar at 28 C. Each datum represents the mean percentage infection of 12 replicates of 260 kernels over 2 yr. Values within a column not followed by the same letter differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test.

ergosterol levels observed.

There were few differences in percent germination of maize hybrid kernels at physiological maturity or later harvest dates. The higher germination of certain hybrids at 11 wk after mid silk may actually reflect variations in physiological maturity. Significant pathogenic effects of seedborne *F. moniliforme* and other fungi have been previously reported (9,14,15). Aulakh et al (3) observed poor stands of maize after sowing seed infected by *F. moniliforme*. Because many of the hybrids tested in this study had a high percentage of internal *F. moniliforme*, pathogenic effects may be expressed after germination.

Trojan TXS114 was the only maize hybrid with a significant ( $P = 0.05$ ) reduction in germination percentage 14 wk after mid silk. This hybrid also had one of the highest percentages of kernel infection at the final harvest. Maize kernels free of insect damage were used exclusively for fungal isolations, because the role of insects, particularly earworms (*Heliothis* spp.), in providing ports of entry for kernel-invading fungi is well known (7,10,11).

We concluded that the presence of *F. moniliforme* in maize increases as harvest is delayed beyond physiological maturity. The relative susceptibility of the hybrids tested to this fungus remained the same over time. Those hybrids with high or low infection levels 8 wk after mid silk had correspondingly high or low infection 14 wk after mid silk. The ergosterol assay was considered suitable only for detecting extreme differences in fungal invasion of

maize hybrid kernels. Furthermore, the assay did not discriminate between fungal species or distinguish between preharvest or postharvest infections. Finally, the minor differences encountered in germination of maize hybrid kernels either at physiological maturity or later maturity stages showed that the hybrids used in this study may not be affected deleteriously by *F. moniliforme* infection before germination (mean germination 95%).

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