Time of Infection of Maize Kernels by *Fusarium moniliforme* and *Cephalosporium acremonium*

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ABSTRACT

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Maize kernels were assayed for endogenous fungi at weekly intervals from 1 wk before the midsilk stage to 9–10 wk after midsilk. *Fusarium moniliforme* was first isolated 2 wk after midsilk and infection increased each week to 35–66% at final assay. *Cephalosporium acremonium* was first detected 3–4 wk after midsilk and infection increased weekly to 30–45% at final assay. Kernel infection by *F. moniliforme* in the tip half of corn ears before maturity and by *C. acremonium* in the butt half at maturity was most common.

Fusarium moniliforme Sheld. commonly infects a wide range of crops throughout the world, particularly in tropical and subtropical regions (2). It is the fungus most frequently isolated from maize kernels in the United States (8,12,14,16) and elsewhere (1,9,15). Although *F. moniliforme* is known to cause a kernel rot (18,22,23), the pathogen is often associated with kernels that appear not to be diseased or damaged (10,12,20,21). *Cephalosporium acremonium* Corda (= *Acremonium strictum* Gams) also is commonly found in sound kernels (7,14,21). In addition, *C. acremonium* and *F. moniliforme* are frequently isolated from maize stalks and are capable of causing stalk rot (3). *F. moniliforme* produces a heat-stable toxin that inhibits root growth of maize seedlings under laboratory conditions (6,17).

The mode and time of entry of *F. moniliforme* and *C. acremonium* into kernels are not clearly understood. Koehler (12) indicated that kernels become infected by *F. moniliforme* from infected silks, and Warren (23) increased kernel infection by applying a *F. moniliforme* spore suspension to silks. The reported systemic distribution of *F. moniliforme* in maize plants (5), however, also may contribute to kernel infection. Hesseltine and Bothast (7) reported a study involving a weekly assessment of fungi associated with developing maize kernels in Illinois, but data obtained for the first 8 wk did not distinguish between contamination and infection of kernels.

The purpose of the present investigation was to determine when maize kernels become infected by *F. moniliforme* and *C. acremonium* under conditions of natural inoculation.

MATERIALS AND METHODS

Maize kernels were assayed weekly for endogenous fungi starting with the blister stage, about 1 wk before the midsilk stage, and continuing to 9–10 wk after midsilk. Two commercial hybrids (A and B) were assayed in 1977 and one (B) in 1978. Plants were grown in single-row plots 5 m long and 1 m apart on the Plant Science Farm at Mississippi State, MS. A randomized block design with

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three replications was used. At each assay time, 60 kernels were randomly selected from each of five randomly selected ears in each of three plots. Thus, 900 kernels from 15 ears of each hybrid were assayed each week, and 30,600 kernels from 510 ears were assayed during the course of the study. In 1978, the 60 kernels from each ear were selected to include 30 from the butt half and 30 from the tip half.

Kernels were surface sterilized by being dipped in 70% ethyl alcohol, soaked for 3 min in 1.6% NaOCl (Clorox), and rinsed in sterile distilled water. A less severe surface sterilization involving 2 min in 1.1% NaCl was used for assay periods 1 through 4. Surface sterilized kernels were placed on Difco Czapek solution agar in petri plates (10 kernels per plate). Owing to difficulty in handling the very immature kernels during the first two assay periods, groups of 6–10 kernels were sliced intact from 12 randomly selected areas on the ear surface. These were handled as intact groups during subsequent surface sterilization and plating. After 5 days of incubation at 28 C, fungi growing on or from kernels were identified and enumerated. Care was taken to examine microscopically (×100 magnification) all kernels having *F. moniliforme* growth for coincident growth by *C. acremonium*.

Mean percent internally infected kernels over the five ears sampled from a plot on a given day were used as plot means in analyzing the data that were subjected to a standard analysis of variance to detect differences greater than those expected by chance alone.

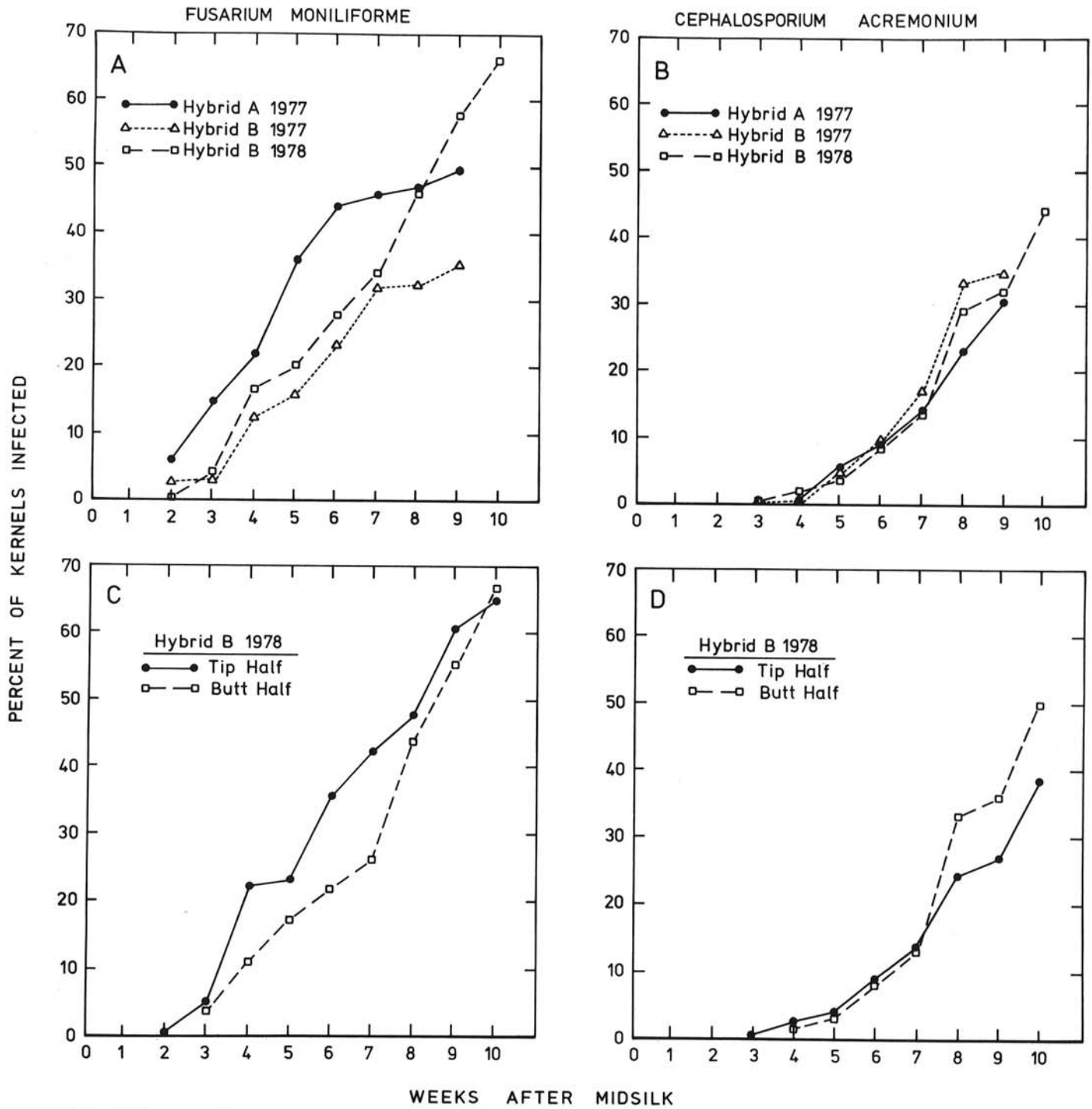

Fig. 1. Relationship between maize kernel maturity and percentage of kernels infected by *Fusarium moniliforme* and *Cephalosporium acremonium*. Kernels randomly selected from entire ear and infected by **A,** *F. moniliforme* and **B,** *C. acremonium*. Kernels randomly selected from tip or butt halves of ears and infected by **C,** *F. moniliforme* and **D,** *acremonium*.

RESULTS

F. moniliforme was first detected in surface-sterilized kernels 2 wk after midsilk (Fig. 1A). In 1977, Hybrid A showed a rapid and steady rate of kernel infection (5–14%/wk) during the second through the sixth week after midsilk. This was followed by an increase of only 5% during the last 3-wk period for a total of 50% kernel infection 9 wk after midsilk. On the other hand, infection in Hybrid B was slow during the first 2 wk (3%), more rapid (3–9%/wk) during the next 4 wk, and again slow during the last 2 wk before final harvest when total kernel infection was 35%. In 1978, infection in Hybrid B was again slow during the first 2 wk (4%) and generally rapid (4–13%/wk) during the remaining weeks until it reached 66% at final harvest 10 wk after midsilk.

The pattern of *C. acremonium* kernel infection was similar to *F. moniliforme* kernel infection, but with a few differences (Fig. 1B). Initial *C. acremonium* infection was delayed 1–2 wk longer than initial *F. moniliforme* infection, and infection during this time was slower for *C. acremonium* than for *F. moniliforme.* As with *F. moniliforme*, there was a weekly increase in total infection, but the final *C. acremonium* infection level (30–45%) was generally lower than the final *F. moniliforme* infection level (35–66%).

For any given assay time, no statistically significant ($P = 0.05$) differences in percentage kernel infection by *F. moniliforme* or *C. acremonium* were found between hybrids. This was due to the high level of variability in kernel infection among ears. Variability among ears generally became greater as kernels approached maturity than at earlier stages of kernel development. For example, Hybrid A showed ranges in *F. monilforme* kernel infection among ears of 7–50, 17–63, and 20–92% at 4, 6, and 9 wk after midsilk, respectively. Similarly, and for the same assay times, variability for *C. acremonium* infection among ears of Hybrid A was 0–7, 2–50, and 10–78%.

In 1978, kernels from the tip half and butt half of ears were assayed separately. *F. moniliforme* infection was greater in the tip half during most of the assay periods, but it was about the same in both halves at the last assay (Fig. 1C). Infection by *C. acremonium* was about the same in both halves until ears approached maturity and then it became more frequent in the butt half (Fig. 1D). Although differences in levels of kernel infection by *F. moniliforme* or *C. acremonium* between tip and butt halves of ears were not statistically significant ($P = 0.05$) for any given assay time, there were some significant differences between halves when data of several assay times were combined and analyzed. Total percentage *F. moniliforme* kernel infection during the period from 6 to 9 wk after midsilk was significantly greater in the tip half than in the butt half of ears. Total percentage *C. acremonium* infection was significantly greater in the butt half than in the tip half of ears during the assay period from 10 to 12 wk after midsilk.

Numerous other fungi were isolated from surface sterilized kernels, although frequencies (recorded only for the 1978 test) were much lower than those of *F. moniliforme* and *C. acremonium.* Species of *Cladosporium*, *Mucor*, *Papularia*, and *Rhizopus* were found primarily in immature kernels. They were each present in at least three of the first five assay times following the midsilk stage; infection levels for any one of these fungi were generally under 2%, although they ranged from a trace to 6.5%. *Aspergillus flavus* and species of *Curvularia* and *Penicillium* were first isolated from kernels 1 wk after midsilk and each week thereafter. Infection by any one of these fungi ranged from a trace to 4.4%. *Fusarium roseum* and species of *Alternaria*, *Aspergillus* (other than *A. flavus*), *Chaetomium*, *Epicoccum*, *Helminthosporium*, *Nigrospora*, *Phoma*, and *Trichoderma* also were isolated at one or more assay times after midsilk, but frequencies were generally under 1%.

DISCUSSION

In both years of this study, *F. moniliforme* and *C. acremonium* by far were the fungi most frequently isolated from maize kernels. These two fungi often infected the same kernels and, because of the more rapid growth of *F. moniliforme*, *C. acremonium* might not have always been detected, even with microscopic examination. Hence, the values for *C. acremonium* infection frequency may be somewhat low.

F. moniliforme was first detected 2 wk after midsilk and *C. acremonium* was first detected 3–4 wk after midsilk (Fig. 1A and B). In contrast, Koehler (12) reported that these fungi did not become established in kernels until the ears were approaching maturity, especially in tightly husked ears that had not been damaged by insects. He found that kernels in exposed or damaged ears first became infected by *F. moniliforme* about 20 days earlier than those in well-covered ears, but this apparently did not influence the time of *C. acremonium* infection. In the present study, care was taken to assay kernels from only sound ears whenever possible.

A direct comparison between the present study and that by Hesseltine and Bothast (7) cannot be made because they did not use surface-sterilized kernels in their weekly assessment of kernel-associated fungi until the ninth week after tasseling. They did, however, find *F. moniliforme* and *C. acremonium* associated with kernels as early as 3 and 6 wk after tasseling, respectively. In their study, *C. acremonium* infection in surface-sterilized kernels reached a peak of about 35% at 10 wk after tasseling and *F. moniliforme* reached a peak of about 18% at 11 wk after tasselling, a frequency considerably lower than that found for *F. moniliforme* infection in the present study. In succeeding weeks of their study, they showed a decline in kernel infection by these fungi. The present study was discontinued at 9–10 wk after midsilk. Hence, a similar decline could not be detected; however, because levels of 60–100% infection are commonly encountered at harvest at Mississippi State (*unpublished*) it would not seem reasonable to expect a decline in infection after 10 wk.

Kernel infection by *F. moniliforme* and *C. acremonium* showed a weekly increase from its initial detection until final harvest (Fig. 1A and B), suggesting a continuing kernel susceptibility to these fungi during kernel development. However, only two genotypes were used in the present study, and greater differences in time and frequency of infection might have been found if other genotypes had been tested. Under conditions of artificial inoculation with *F. moniliforme*, Warren (23) reported differences among genotypes in reaction to Fusarium kernel rot that was influenced, at least in part, by the time of inoculation.

Differences in results between those reported here for studies done in Mississippi and similar studies done in Illinois (7,12) should not be entirely unexpected. The environments of these locations are sufficiently different that they could significantly influence the levels of inoculum, the infection process, and possibly competition from other microorganisms. Also, the maize genotypes used in these studies might have differed significantly in reaction to kernel infection by these fungi.

When kernels from the tip and butt halves of ears were assayed separately, there was a tendency for more infection by *F. moniliforme* in the tip half before maturity followed by about equal amounts in both halves at maturity (Fig. 1C). In contrast, infection by *C. acremonium* was about equal in both halves before maturity but became somewhat greater in the butt half at maturity (Fig. 1D). The reason for these differences is not known, but they do differ from reports on similar, though not identical, studies. Koehler (12) reported more kernel infection by *F. moniliforme* in the tip half of ears at harvest (about 10 wk after tasseling) and suggested that this was because *F. moniliforme* entered ears at the tip end through the silk channel. Hesseltine and Bothast (7) reported generally higher levels of both *F. moniliforme* and *C. acremonium* in kernels at the tip end of ears at 9–11 wk after tasseling.

The results further demonstrate the presence of high levels of infection by *F. moniliforme* and *C. acremonium* in maize kernels, but the significance of this infection is not well understood. These fungi are generally not considered to be aggressive pathogens, although they are involved in stalk rot (3), and *F. moniliforme* can cause a preharvest seed rot (18,22,23). There is a possible relationship between *F. moniliforme* infection in planting seed and subsequent infection of seedling plants (5), but this has not been clearly demonstrated (13). Furthermore, it has been suggested that because of the ability of *F. moniliforme* and *C. acremonium* to

grow at high CO_2 and low O_2 levels these fungi might be of potential importance when high-moisture maize is stored hermetically (21). It has been demonstrated that grain infected by *F. moniliforme* can be toxic to animals (11) and that some strains of this fungus are capable of in vitro production of a metabolite, moniliformin, that is toxic to both animals and plants (4,19). More recent work suggests that some strains of *F. moniliforme* are capable of producing other, as yet unidentified, mycotoxins (15).

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