Kernel Infection and Aflatoxin Production in Maize by *Aspergillus flavus* Relative to Inoculation and Harvest Dates

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ABSTRACT

Scott, G. E., and Zummo, N. 1994. Kernel infection and aflatoxin production in maize by *Aspergillus flavus* relative to inoculation and harvest dates. Plant Dis. 78:123-125.

Preharvest kernel infection by *Aspergillus flavus* and subsequent aflatoxin contamination of maize (*Zea mays*) grain is a chronic economic problem in the southern part of the United States. Studies were conducted to determine the time of inoculation and harvest date most effective for identifying maize genotypes resistant to kernel infection and aflatoxin contamination by *A. flavus*. Inoculation of ears with *A. flavus* 6 days after midsilk resulted in as many or more infected kernels than inoculation of ears 12 and 18 days after midsilk. Multiple inoculations did not increase incidence of kernel infection or aflatoxin contamination. The percentage of infected kernels in inoculated resistant and susceptible hybrids was similar at 46 and 50 days after midsilk, but the percentage of infected kernels was greater in susceptible hybrids at later harvest dates. Thus, resistant hybrids differed significantly from susceptible hybrids for infection levels for harvest dates of 54–62 days after midsilk. One susceptible hybrid had markedly higher aflatoxin contamination than the other three hybrids. Selection for resistance to *A. flavus* should be more effective at harvest dates around 60 days after midsilk than when grain reaches physiological maturity.

Preharvest kernel infection by *Aspergillus flavus* Link:Fr. and subsequent aflatoxin contamination of maize (*Zea mays* L.) grain is a chronic economic problem in the southern part of the United States. Kernel infection with *A. flavus* and aflatoxin contamination also occur sporadically in the Corn Belt. Information concerning time of infection of kernels by the fungus and commencement of aflatoxin production would help maize breeders determine proper times to inoculate maize ears and evaluate the grain for genotypic response to this fungus.

Zummo (9) double inoculated ears in certain combinations with green-and-white isolates of *A. flavus* from 1 to 10 days after midsilk. Inoculation of ears with the white isolate, then with the green isolate reduced the amount of infection obtained with the green isolate; whereas inoculation first with the green isolate, then with the white isolate did not influence the amount of infection obtained with the white isolate.

Widstrom et al (6) inoculated maize ears at 20 and 40 days after midsilk by three inoculation methods. Visible *A. flavus* occurred on 83% of the ears when inoculations were made 20 days after midsilk, compared to 35% with inoculations made 40 days after midsilk.

Wilson et al (8) reported that the percentage of ears containing *A. flavus* increased from 42 to 95% when the ears were harvested 10–56 days after full silk in 1975, and from 41 to 90% for harvest dates 25–56 days after full silk the following year. Widstrom et al (7) found that aflatoxin concentration increased through harvest dates of 30, 40, 50, and 60 days after full silk in two hybrids tested. However, the increase was much higher in one hybrid than in the other. Payne et al (4) reported that both kernel infection by *A. flavus* and aflatoxin concentration increased from about 1 wk after inoculation and peaked at grain moistures of 18–21% with generally some decrease after this time. Lillehoj et al (3)

Approved for publication as Journal Article No. J-8333 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

Accepted for publication 7 October 1993.

reported that tests conducted in Illinois, Missouri, Texas, and Georgia on maize that was inoculated with *A. flavus* and harvested 15, 30, 45, and 70 days after silking had increases in aflatoxin concentration at later harvest dates.

Research plots used to evaluate maize for the extent of kernel infection or aflatoxin contamination are often reported to have been harvested "at maturity" or "after physiological maturity." Other plots are harvested a given number of days after midsilk or full silk. Plots in tests in Mississippi are routinely harvested 60 days after midsilk, whereas those in Louisiana are usually harvested 49 days after midsilk (1).

In Mississippi, we generally needle inoculate maize ears with *A. flavus* 6 days and harvest ears 60 days after midsilk (10). The objectives of these studies were to determine what modifications in inoculation or harvest date(s) would increase effectiveness in separating resistant and susceptible maize genotypes.

MATERIALS AND METHODS

Single cross Mo18W × Mp313E (resistance to kernel infection by *A. flavus*) (5) and a susceptible single cross maize hybrid, Mp68:616 × SC212M, were grown in 1991 and 1992. Twenty plants were grown in single-row plots 5 m long spaced 0.96 m apart. Eight inoculation treatments of 6, 12, and 18 days after midsilk and combinations of these inoculation times plus a noninoculated check for each of the two hybrids were grown in a randomized complete-block design with six replications. The top ear of each plant was needle inoculated as described below. Ears were harvested 60 days after silking, dried, and machine shelled, and grain was bulked from each treatment within a replication.

Two hybrids, Mo18W × Mp313E and SC54 × Tx601, previously classified as resistant to kernel infection by *A. flavus* (5), were grown with two susceptible single crosses, Mp68:616 × SC212M and GT106 × T202, in two field tests in 1990 and 1991. Each of the four hybrids was harvested at five 4-day intervals beginning 46 days after midsilk. The 20 hybrid × harvest date treatments were grown in a randomized complete-block design with six replications. In each year, plants in one experiment were pinbar inoculated, and plants in the other experiment were needle inoculated. At harvest, only the top ear of each plant was harvested and dried at 42 C for 7 days. Ears that had been pinbar inoculated were hand shelled, and undamaged kernels on both sides of the inoculated row of kernels were removed and bulked for assay (2). Ears that had been needle inoculated were shelled and sampled as stated above.

Inoculum, inoculation techniques, and assay. *A. flavus* isolate NRRL 3357, obtained from Stephen W. Peterson, USDA-ARS, Northern Regional Research Center, Peoria, Illinois, was used to produce inoculum for all studies. Cultures of the fungus were grown on corn cob grits in 500-ml Erlenmeyer flasks, each containing 50 g of grits and 100 ml of H_2O. After 12–14 days, conidia of *A. flavus* were washed from the surface of the grits with sterile, distilled water containing 2 drops of Tween 20 per 100 ml. Inoculum was prepared daily and kept on ice in the field until applied.

Ears were needle inoculated 6, 12, or 18 days after midsilk using a tree-marking gun with a 14-gauge, 35-mm-long hypodermic needle (10). The tip opening of the needle had been plugged, and three 1-mm holes had been drilled 6, 8, and 10 mm from the tip. The needle was inserted through the husks, and 3.4 ml of inoculum containing 9×10^6 conidia per ml was injected over the kernels without visibly damaging them.

The pinbar-inoculation technique (2) utilizes a plastic bar with a single row of 35 stainless steel pins. The pinbar was dipped in the conidial suspension and placed on the ear parallel with the kernel rows, and the pins were pushed through the husk into a single row of kernels. Only a single row of kernels was damaged, and the cob was not penetrated. Inoculations were made 20 days after midsilk.

Undamaged kernels (390) from each plot were dipped momentarily in 70% ethanol, submerged in 1.25% NaOCl for 3 min, and rinsed in sterile, distilled water to eliminate surface microbes. Kernels were plated on Czapek solution agar amended with 7.5% NaCl (CSA-S) in 100-ml petri dishes (13 kernels per plate). The plates were incubated in the dark for 7 days at 28 C, then examined for fungal growth and the percentage of kernels infected with *A. flavus*. A technique recommended by the Vicam Company, Summerville, Massachusetts, was used to determine the aflatoxin concentration in the grain as described by Zummo and Scott (11).

Plant population levels were low in some plots in 1991. Sufficient grain was not available for aflatoxin analyses from pinbar-inoculated plots in 1991, and kernels for assaying the incidence of kernel infection were available in only four replications. Because of some missing plots in 1991, these data, plus data combined over years, were analyzed by SAS GLM. Otherwise, data were analyzed by SAS ANOVA. Letter differences for mean separation are based on least squares difference (LSD) among the mean comparisons with SAS GLM, or the letter differences given by SAS ANOVA based on LSD values. Prior to analyses, aflatoxin (ng g^{-1}) concentrations were converted to LN(ng g^{-1} + 1). The values presented are the antilogs of the mean values obtained in the analyses, but the letter designations associated with these antilog values are those given by the analyses of the log values.

RESULTS AND DISCUSSION

The incidence of kernel infection by *A. flavus* averaged over 1991 and 1992 was 3% for Mo18W × Mp313E and 8% for Mp68:616 × SC212M. Aflatoxin concentration in 1992 averaged 2 ng g^{-1} for the resistant hybrid and 207 ng g^{-1} for the susceptible hybrid.

The incidence of kernel infection by *A. flavus* averaged over hybrids did not differ significantly among inoculations made singly from 6 to 18 days after midsilk in 1991, but it did differ significantly in 1992 (Table 1). Needle inoculation of ears at 6 and 12, 6 and 18, or 6, 12, and 18 days after midsilk did not significantly increase the incidence of kernel infection over a single inoculation at 6 days after midsilk. Thus, needle inoculations made 6 days after midsilk would be as effective as those made at other times tested.

The overall percentages of kernels infected with *A. flavus* in the pinbar- and needle-inoculated experiments in 1990 were 10.5 and 10.7, respectively. These values were approximately double the 5.5% level of infection in the 1991 needle-inoculated test.

Harvesting ears 46 days after midsilk did not separate the genotypes into resistant and susceptible groups for kernel infection (Table 2). One of the susceptible

Table 1. Average percentage of kernels infected by *Aspergillus flavus* after needle inoculation on different days after midsilk in 1991 and 1992[x]

Inoculation[y]	Year		Mean
	1991	1992	
6 DAM	5.1 a[z]	8.7 a	6.9 a
12 DAM	7.5 a	5.7 bc	6.6 a
18 DAM	4.1 a	5.5 bc	4.8 a
6 and 12 DAM	5.7 a	6.2 bc	6.0 a
6 and 18 DAM	3.9 a	7.2 ab	5.6 a
12 and 18 DAM	7.4 a	7.1 ab	7.3 a
6, 12, and 18 DAM	6.5 a	5.1 c	5.8 a
CHECK (uninoculated)	2.4 a	2.1 d	2.3 b

[x]Values are the average of two hybrids (Mo18W × Mp313E and Mp68:616 × SC212M).
[y]DAM = Days after midsilk.
[z]Means within a column not followed by the same letter differ significantly from each other at the 0.05 level of probability.

hybrids, GT106 × T202, differed significantly from the other three hybrids when harvested 50 days after midsilk. The incidence of kernel infection for the two susceptible hybrids was significantly higher than for the two resistant hybrids beginning 54 days after midsilk.

Kernel infection increased over harvest dates in susceptible but not in resistant hybrids (Table 2). Regression coefficients for incidence of infection on days of harvest were −0.04 for the resistant hybrids compared to 0.36 for the susceptible hybrids. That is, the predicted increase in incidence of infection over the harvest times was zero for resistant hybrids compared to 5.7% for susceptible hybrids. This emphasizes that separation of resistance from susceptibility was much more feasible at the later harvest periods, especially 62 days after midsilk.

Differences among hybrids were significant for aflatoxin contamination (Table 3). SC54 × Tx601 consistently had the lowest aflatoxin contamination, and GT106 × T202 always had the highest level of contamination. However, aflatoxin contamination of the two hybrids resistant to kernel infection by *A. flavus* differed significantly from the two susceptible hybrids only at the final harvest date.

Mo18W × Mp313E is the latest maturing hybrid in this test. It reached the midsilk stage of growth about 7 days later than the other three hybrids, which only differed by a day. This later maturity did not appear to be the reason Mo18W × Mp313E responded differently to inoculation with *A. flavus* than did the susceptible hybrids. Its performance at the different harvest dates was very similar to the other resistant hybrid, SC54 × Tx601. The two resistant hybrids averaged 20% grain moisture at 62 days after midsilk, compared to an average of 18% for the two susceptible hybrids. However, the susceptible hybrids differed from the resistant hybrids in percentage of infected kernels before the final harvest date, so this difference in grain moisture may or may not have had a effect on the difference in kernel infection of the two groups of hybrids.

These results show that selection for resistance to kernel infection by *A. flavus* should be done somewhat later than physiological maturity (assumed to be around 50 days after midsilk in these tests); however, it should not be delayed until too much field deterioration occurs. Our present procedure of harvesting at 60 days after midsilk offers a reasonable compromise.

From a producer's viewpoint, these data indicate that maize should be harvested as early as possible after physiological maturity when drying facilities are available to reduce the grain moisture to a safe storage level. However, aflatoxin contamination can increase rapidly in early-harvested grain that is not dried properly.

Table 2. Average percentage of kernels infected by *Aspergillus flavus* on two resistant and two susceptible maize hybrids when ears were needle inoculated in both 1990 and 1991, and with pinbar inoculation in 1990

Hybrid	Classification[y]	Days after midsilk					Mean
		46	50	54	58	62	
Mo18W × Mp313E	R	7.8 ab[z]	7.9 b	7.0 b	7.3 b	6.5 b	7.3 c
SC54 × Tx601	R	6.2 b	6.7 b	7.9 b	6.6 b	6.3 b	6.8 c
Mp68:616 × SC212M	S	7.0 b	7.0 b	10.4 a	10.2 a	15.9 a	10.1 b
GT106 × T202	S	9.5 a	11.6 a	10.8 a	11.8 a	13.3 a	11.4 a
Mean		7.6 C	8.4 BC	9.0 B	9.0 B	10.5 A	

[y] R and S = resistant and susceptible to kernel infection by *A. flavus*.
[z] Means within a column (or row for overall means) not followed by the same letter (column = lowercase, row = uppercase) differ significantly from each other at the 0.05 level of probability.

Table 3. Aflatoxin concentration of four maize hybrids at five harvest dates when needle inoculated with *Aspergillus flavus* 6 days after midsilk in 1990

Hybrid	Classification[y]	Days after midsilk					Mean
		46	50	54	58	62	
Mo18W × Mp313E	R	21 b[z]	67 b	111 b	42 b	12 b	37 b
SC54 × Tx601	R	8 b	13 c	13 c	11 b	4 b	25 c
Mp68:616 × SC212M	S	41 b	35 bc	155 b	74 b	161 a	77 b
GT106 × T202	S	706 a	525 a	821 a	745 a	1,091 a	757 a
Mean		47	64	116	72	54	

[y] R and S = resistant and susceptible to kernel infection by *A. flavus*.
[z] Means within a column not followed by the same letter differ significantly from each other at the 0.05 level of probability.

ACKNOWLEDGMENTS

We thank Jack Haynes and Lee Scruggs for assistance in handling these tests in the field and laboratory.

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