Comparison of Normal and High-Lysine Maize Inbreds For Resistance to Kernel Rot Caused by Fusarium moniliforme

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ABSTRACT

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Quantitative differences in resistance to *Fusarium moniliforme* occurred in both opaque-2 (o_2) maize inbreds and in their normal counterparts. *Fusarium moniliforme*/maize-inbred interactions were observed among eight o_2 and normal endosperms. Ear rot caused by *F. moniliforme* was more severe on susceptible o_2 maize inbreds than on their normal counterparts. However, there was little or no difference in kernel rot between resistant o_2 and normal isogenic lines. The correlation coefficient between susceptibility to *F. moniliforme* in o_2 and in normal inbreds was 0.62. Susceptibility or resistance in the o_2 endosperm was positively correlated with susceptibility or resistance in the normal endosperm. Five mutants (o_2, o_2 modified, floury-2 (fl_2), and double mutant o_2/fl_2) of inbred W64A were inoculated at different times after pollination. Floury-2 and o_2/fl_2 were most susceptible to *F. moniliforme* 1 and 7 days past pollination. The other mutants were most susceptible 7 and 14 days past pollination. A method for inoculation of maize ears with *F. moniliforme* for differentiation of reactions to Fusarium kernel rot is described. A high incidence of infection and clear differential effects were observed when ears were inoculated by spraying the silk 6-18 days past pollination. Inoculations made 23 days or more past pollination resulted in only a small amount of kernel rot.

Additional key words: *Zea mays*.

Fusarium moniliforme Sheld. is prevalent throughout the world on many crops and is isolated frequently from corn (*Zea mays* L.) stalks (1, 3, 11) and kernels (4, 10, 15, 19). The fungus penetrates stalks directly (8) or enters through wounds made by insects (5, 13) or hail (11). Roots are infected by soilborne inoculum (8) and kernels by airborne spores. Foley (4) considered infection to be systemic and to originate from infected kernels or leaf bases.

High-lysine [opaque-2 (o_2)] corn lines were more susceptible to ear rot caused by *F. moniliforme* than were their normal counterparts (16). The disease is becoming more destructive as planting of high lysine (o_2) corn hybrids increases and have been severely damaged in some years (16). In addition, *F. moniliforme* is isolated from corn kernels more consistently and frequently than any other fungus (6, 15). Smith and Madson (14) reported that of 60 inbred lines of normal corn tested in California, all had some kernels infected with *F. moniliforme*.

The mode of entry of *F. moniliforme* into the kernel and the factors associated with infection have not been studied. These experiments were done to ascertain the relative susceptibility of kernels of normal and o_2 isogenic maize inbreds to *F. moniliforme* and to determine the period of susceptibility of kernels to this disease.

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MATERIALS AND METHODS

Four isolates of *F. moniliforme* were mixed and used for inoculum. Cultures were isolated originally from infected kernels placed on moistened filter paper in petri plates. Single-spore isolates were grown on potato-dextrose agar (PDA) under 16-hr of light per day for 7 days. Conidial suspensions from the four isolates were combined, filtered through cheesecloth and adjusted to 1×10^5 conidia/ml. One drop of Tween 80 (polyoxyethylene sorbitan monolaurate) per 100 ml of spore suspension was added as a wetting agent. The inoculum was sprayed onto the maize silk with an atomizer at approximately 5 ml per plant or until run-off. The silk was covered immediately after inoculation with two moist paper towels. A waxed (glassine) shoot cap was placed on the ear followed by a pollinating bag. The paper towel, shoot cap, and pollinating bag remained on the ear until harvest.

Eight inbreds with normal (N) and opaque (o_2) endosperms were replicated three times in each plot at the Agronomy Farm, Lafayette, Indiana, in 1973 and 1974. Each plot consisted of two rows, 5.6 m long and 1 m apart. Each plant was selfed (hand pollinated) and the ear was covered with a glassine shoot cap and a pollinating bag until harvest time. In this trial, inoculations were made when 99% of the plants of each genotype had been selfed. The time required for all inbreds to reach this stage was

approximately 25 days. Thus, silks were of different ages at the time of inoculation.

The ears were harvested in October when the moisture content of the kernels was about 20%, and then dried to 15.5%. Percentage infection was obtained in 1973 by counting kernels with visual signs of *F. moniliforme* or by visually estimating the percentage of diseased kernels. Results with both methods were similar, so the latter method was used in 1974. Inbreds were evaluated based on their reaction to *F. moniliforme*.

Tests were conducted with an inbred line of maize, W64A the spontaneous mutants W64A homozygous o_2; (o_2) W64A homozygous o_2, modified endosperm (o_2-mod); W64A homozygous floury-2 (fl_2); and the double-mutant homozygous opaque-2/floury-2 (o_2fl_2). The inbred line and all mutants were grown during 1973 and 1974 in four-row plots, replicated three times. The plants were selfed (hand pollinated) as previously described. The ears were inoculated with a spore suspension of *F. moniliforme* by atomization onto the silk, as described, 1, 7, 14, or 21 days after pollination. In 1974 another control group of plants that were selfed but not covered was added to determine the percentage infection from natural inoculum. All ears were harvested and rated for percentage of diseased kernels. All field data were subjected to a computerized analysis of variance and mean values were compared by Duncan's multiple range test.

For weight determinations, kernels were dried to 15.5% moisture content and bulked from plants in each replication. Then 100 kernels were obtained with a Boerner sampler and weighed.

One hundred kernels obtained with the Boerner sampler from each treatment were surface-disinfected with a 1% solution of NaOCl for 2 min, and 10 kernels placed on each of 10 petri plates of PDA. The plates were placed under light for 6 days and the incidence of *F. moniliforme* was recorded.

RESULTS

Host genotype.—Corn genotypes in both the normal and o_2 endosperms differed significantly ($P = 0.01$) in their response to inoculum of *F. moniliforme* (Table 1). Among inbreds with normal endosperms, A545, B14, and Oh43 were significantly more resistant than the other inbreds with normal endosperms and W22 was the most susceptible. Opaque-2 endosperm of the same inbreds also were more resistant to *F. moniliforme* than the other o_2 inbreds tested. Thus, the response of the o_2 endosperms were related to the particular genetic background into which the o_2 gene was incorporated. Inbreds B14 and Oh43 both resistant in their normal endosperm were similar in reaction in their o_2 counterparts.

Variation in susceptibility to *F. moniliforme* was evident within and among both normal and o_2 isogenic lines. Of the eight genotypes of both endosperms tested, five of the o_2 endosperms were significantly more susceptible than their normal counterparts (Table 1). All of the o_2 endosperms, except A545, that were significantly different from their normal counterparts were susceptible in both genotypes. Two other inbreds, B14 and Oh43, were resistant to ear rot in both the normal and o_2 versions and these lines did not show statistically significant ($P = 0.05$) differences between the two endosperms. The correlation coefficient between susceptibility to *F. Moniliforme* in normal and in o_2 inbreds was 0.62.

Effect of time of inoculation on ear rot incidence and development.—To study the effect of time of inoculation on ear rot development, four endosperm mutants of W64A and normal W64A were inoculated at various times after pollination. Previous observations indicated that W64A in the normal and o_2 endosperms are intermediate in reaction to *F. moniliforme* (Table 1). All of the near-isogenic lines had some kernels damaged by *F. moniliforme* at all inoculation periods (Table 2). The mean ratings for disease severity for all inoculation periods on specific mutants ranged from 2.3 to 13.2. In all cases, the pattern of host response to time of inoculation was different for each isogenic line. When the mean percentages of ear infections were analyzed based on time of inoculation; i.e., 1, 7, 14, and 21 days for each mutant, fl_2 had less ear rot than the other mutants. Opaque-2 modified and o_2fl_2 had significantly more infected kernels than the other mutants.

TABLE 1. Comparison of normal and opaque-2 inbreds for susceptibility to ear rot caused by *Fusarium moniliforme* in maize inbreds

Inbred	Infected kernels per ear		Isolation of *Fusarium moniliforme* from undamaged kernels	
	Normal (%)	Opaque-2 (%)	Normal (%)	Opaque-2 (%)
A239	10.5 b[x]	45.0 a *[y]	54[z] a	66 a
A545	2.6 d	10.8 d *	1 d	22 b
B14	4.5 d	3.6 e	8 c	16 b
B37	12.3 b	20.7 c *	4 c	6 c
H60	9.8 c	47.6 a *	21 b	14 b
Oh43	2.1 d	4.7 e	16 b	8 c
W22	19.7 a	40.7 b *	50 a	42 a
W64A	14.6 a	16.7 cd	14 b	10 c

[x]Numbers in each column followed by the same letter are not significantly different ($P = 0.01$).

[y]An asterisk indicates a significant difference ($P = 0.05$) in percentage ear rot between normal inbreds and their respective opaque-2 counterpart.

[z]Kernels with no visible evidence of infection were plated on PDA (100 kernels per inbred).

The o_2 and normal endosperms had significantly more infected kernels when the silk was inoculated 7 and 14 days after pollination than at 1 or 21 days. However, o_2-mod (horney endosperm) was equally susceptible 7, 14, and 21 days after pollination than at 1 day. Infection of fl_2 endosperm was significantly greater when the silk was sprayed 1 day after inoculation and the percentage of diseased kernels decreased with time after pollination. The percentage of infected kernels of the double mutant, o_2fl_2, was significantly higher 1 and 7 days after inoculation.

Some kernels were infected in the control plants despite precautions taken to cover the ears before and after pollination. However, more infection occurred in the noncovered controls than on control ears that remained covered throughout the duration of the experiment.

Effect on kernel weight.—Kernels from inoculated infected ears weighed less than kernels from non-inoculated, apparently healthy, ears. Diseased kernels of fl_2, N, o_2fl_2, o_2, and o_2-mod weighed 10, 12, 15, 20, and 29 percent less, respectively, than noninfected controls for each inbred. These results are significantly different from the controls ($P = .05$). Also, diseased kernels weighed less than apparently healthy kernels from the same ear. Weight differences were less on all endosperms from ears inoculated 1 day after pollination than at the other dates tested, except on fl_2. Weight differences between both diseased o_2, and o_2-mod kernels and apparently healthy kernels of these lines were greatest 21 days after pollination, while differences between fl_2 and o_2fl_2 were greatest 7 and 1 days after pollination, respectively.

Kernel infection related to age of ear.—Kernels were infected 2-27 days after pollination. However, too few silks were available before 6 and after 23 days after pollination to make valid conclusions. Where data are compiled, a minimum of 30 ears were inoculated. In all cases where infection was found before 6 days and after 23 days, damaged kernels never exceeded 3%.

The results shown in Fig. 1-A and 1-B are illustrated as averages of all susceptible and resistant inbreds,

TABLE 2. Effect of time of inoculation on ear rot caused by *Fusarium moniliforme* on isogenic lines of W64A corn inbreds

Inoculated days after pollination	Fusarium kernel rot per isogenic line of W64A					
	N[y]	o_2	o_2 mod	o_2 fl_2	fl_2	Means
1	3.2 b[z]	4.8 b	9.7 b	21.8 a	8.1 a	9.3 b
7	8.8 a	8.3 a	20.9 a	17.1 a	2.0 b	11.4 a
14	7.0 a	14.9 a	22.5 a	9.9 b	1.6 b	13.6 a
21	2.7 b	4.4 b	18.1 a	11.2 a	0.6 d	7.4 c
Noninoculated noncovered	2.8 b	0.4 c	5.1 b	4.4 b	1.4 c	
Noninoculated covered	0.5 c	0.2 c	2.6 c	0.9 c	0.2 d	

[y]N = normal, o_2 = opaque, o_2-mod = opaque modified, o_2fl_2 = opaque-2, floury-2 double mutant, and fl_2 = floury-2 endosperm.
[z]Numbers in each column followed by the same letter are not significantly different ($P = 0.05$).

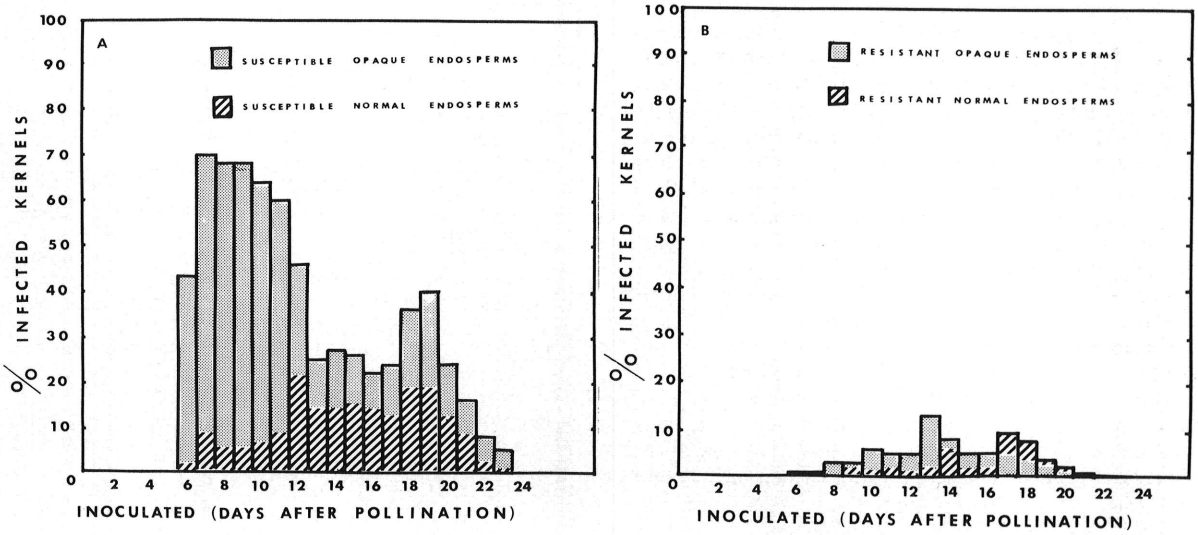

Fig. 1-(A, B). Summary of the reaction of opaque and normal inbred of maize to *Fusarium moniliforme*. **A)** Percentage of susceptible opaque and normal endosperm corn kernels infected after inoculation at various times after pollination (average of A239, B37, H60, W22, and W64A). **B)** Percentage of resistant opaque and normal endosperm corn kernels infected after inoculation at various times after pollination (average of A545, B14, and Oh43).

respectively. However, when the averages were compiled for inbreds singly, some exhibited the greatest susceptibility when inoculated 3-14 days after pollination, and others when inoculated 10-19 days after pollination. The two peaks in Fig. 1-A (6-12 and 18-20 days after pollination) reflect the differences in inbred susceptibility at different periods after pollination. In general, kernels of o_2 inbreds were most susceptible when inoculated 6-12 days after pollination and normal inbreds were most susceptible 18-20 days after pollination. At all dates after pollination, susceptible o_2 inbreds had a higher percentage of infected kernels than their normal counterparts. However, this was not always true for the resistant normal and o_2 inbreds.

The infection periods were shorter for kernels from resistant inbreds than from susceptible inbreds. Although sufficient silk was inoculated in the resistant lines, infection did not occur until 6 days after pollination and ceased 21 days after pollination. For the susceptible inbreds, infection began at 6 and ended 23 days after pollination. Some of the resistant normal inbreds (Fig. 1-B) had more kernel damage than their o_2 counterparts. The period when the percentage infected kernels in the resistant normal endosperm was higher than the o_2 resistant endosperm was from 17-20 days after pollination.

DISCUSSION

Mertz et al. (12) showed that the mutant gene o_2 in maize increased the lysine and tryptophan composition of the endosperm. However, it also increased susceptibility to Fusarium ear rot and lowered yield compared to that of normal maize.

Several workers have shown that *F. moniliforme* is the fungus most frequently isolated from stalks and kernels of corn (1, 2, 3, 7, 8, 9, 10). The presence of *F. moniliforme* in corn kernels is not always associated with damaged or apparently diseased kernels, but there is ample evidence that *F. moniliforme* can cause damage to corn kernels (6, 10, 16, 19). No maize inbred yet tested is immune to kernel rot caused by *F. moniliforme*, but there are differences in degree of susceptibility among inbreds. The differences among inbreds are in percentage of kernels damaged as opposed to the number of kernels harboring the fungus.

Variation in susceptibility is evident among both the normal and opaque inbreds. Of eight inbreds tested and representing both endosperm types, five had significantly more infected kernels than their normal counterparts. Susceptibility was intensified in the susceptible o_2 endosperm as compared to its normal counterpart (Table 1). However, resistant o_2 lines were about equal in disease reaction to their normal counterparts. It appears that resistance or susceptibility is related to the particular genetic background into which the opaque-2 gene was incorporated. The increased kernel infection in the o_2 lines may be a phenotypic expression of the kernel. Opaque-2 kernels tend to be softer, which may account for easier penetration by fungi.

There are indications of less disease initiation in resistant genotypes inoculated 18 days past pollination compared to susceptible genotypes and resistant and susceptible genotypes inoculated prior to 18 days. In susceptible inbreds less disease initiation was not evident until 23 days past pollination. This indicates one basis for susceptibility, namely, the maintenance of a favorable condition for infection for a longer period on a susceptible host than on a resistant host. The best differential infections were obtained when inoculations were made 4-17 days after pollination. Inoculations made 3 wk past pollination or later resulted in appreciably less kernel rot. Ullstrup (16, 17, 18) reported that the highest incidence of infection of ears by *Diplodia maydis* occurred in plants inoculated within 2 wk after full silk, and inoculations made 4-5 wk after full silk resulted in less ear rot. Our results with *F. moniliforme* parallel those of Ullstrup; infection was greatest 4-14 days after pollination and least 21 or more days past pollination. The incidence of kernel rot lowered by late inoculations may be due to unfavorable environmental conditions in late summer, the time between inoculation and harvest was too short for disease development, the moisture content of silk and kernel at time of inoculation was unfavorable, or that the kernels became more resistant due to physiologic changes.

Sufficient moisture in the silk and kernel apparently is necessary for the germination of conidia of *F. moniliforme* and for subsequent invasion of the kernel. After silks become dry, little or no infection occurs. Inoculations 5 wk past pollination consistently resulted in less kernel rot, even in years when the temperature and humidity were ideal for infection.

Our technique of spraying ears with a spore suspension is useful in differentiating between resistant and susceptible hosts of *F. moniliforme* (Table 1). Inoculation of the silk closely simulates natural infection because the host is not wounded. The technique augments natural infection and differs only by providing for a greater volume, higher concentration, specific time of application, and more uniform distribution of inocula than a natural infection. Studies on relative resistance of inbreds to *F. moniliforme* and other ear rots and studies on inheritance of resistance could be improved by using this method.

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