Evaluation of Tropical Maize Germ Plasm for Resistance to Kernel Colonization by *Fusarium moniliforme*

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ABSTRACT

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A wide range of maize (*Zea mays*) hybrids were evaluated for resistance to kernel colonization by *Fusarium moniliforme*. Thirty-four tropical inbreds were topcrossed to two corn belt testers and were combined with eight commercial hybrids to form two tests. The hybrids were evaluated at two locations in North Carolina during the summer of 1985. Grain samples were collected during combine harvesting and were dried to <12% grain moisture. Samples were ground and used to form extracts that were placed on a selective medium to determine the number of colony-forming units of *F. moniliforme* per gram of grain. Significant differences were found among hybrids for resistance to colonization by *F. moniliforme*. B73 × Mo17 had the highest mean value for colonization in the study. With the exception of Pioneer Brand 3369A and Pioneer Brand 3055, the commercial hybrids appeared to be more susceptible to pathogen development than the topcross hybrids as a group. The high degree of susceptibility of B73 × Mo17 indicates that the extensive use of hybrids with closely related pedigrees may be an important factor in the increasing incidence of grain contamination by *F. moniliforme* observed over the past 10 yr. The results of this study indicate that tropical germ plasm may be a source of resistance to this pathogen. However, the measurement error was large and indicates the need for further research into the factors affecting infection and colonization.

Fusarium moniliforme Sheld. is a common pathogen of maize (*Zea mays* L.) throughout the world (12). In addition to causing stalk rot, this fungus also causes the most widespread disease of maize ears (18). Contamination of maize kernels with *Fusarium* spp. has increasingly been associated with toxicity problems of grain fed to livestock (1,2,9-13). In addition, the pathogen has been associated with human esophageal cancer in a region where contaminated grain is often directly consumed (14). Over the past 10 yr, reports of *F. moniliforme* in grain samples across the United States have increased dramatically (16), and colony counts of 2-3 million per gram of grain have been reported (15).

The extent to which maize grain contamination can be reduced via the development of resistant hybrids is dependent upon the development of an efficient screening system. Specific mycotoxins have been identified from *Gibberella zea* (Schwein.) Petch (*F. graminearum* Schwabe), and the quantity of mycotoxins present in grain samples has been used along with visual ratings of fungal development for identification of resistant lines (4,6). In the case of *F. moniliforme*, the toxin(s) associated with animal health problems have not been definitively identified, and the maize breeder-plant pathologist must select superior genotypes on the basis of the presence or absence of the fungus. Unfortunately, contaminated kernels frequently appear symptomless, and infection is not evident until they are placed on growth media and/or allowed to germinate (17,20). Some researchers have screened maize genotypes on the basis of the percent of kernels infected, but this technique has proven to be very time-consuming and labor-intensive due to the large numbers of kernels that must be used to show significant differences among genotypes (3,8,17). In addition, measurement of the percent of kernels infected does not allow the breeder to distinguish between genotypes that differ in the degree of pathogen development within the kernels. However, the total amount of pathogen development can be measured by making an extract from a ground sample of grain and determining the number of colony-forming units per unit grain weight via plate counts using a selective medium.

In this study, a wide range of corn genotypes were evaluated for resistance to infection by *F. moniliforme* and its development by determining the number of colony-forming units per gram of grain. The maize genotypes used included a large number of tropical maize inbreds in hybrid combination with corn belt germ plasm, seven commercial maize corn hybrids, and B73 × Mo17. B73 × Mo17 was included due to the extensive use of closely related lines in the production of many currently used commercial hybrids (19). One of the objectives of the study was to determine if the increasing problems associated with grain colonization by *F. moniliforme* might be related to the increasing use of hybrids closely related to B73 × Mo17. A second objective was to determine if tropical maize germ plasm contains genes for resistance. The experiments were conducted at two locations to allow for an evaluation of the relative magnitude of location and the location-by-genotype effects on pathogen development. In addition, by using plate counts of the number of colony-forming units per gram of grain the study examines a different procedure of evaluating germ plasm for resistance to the pathogen.

MATERIALS AND METHODS

A wide range of maize germ plasm was used in this study, including both corn belt and adapted tropical materials. Thirty-four 100% tropical corn inbred lines were crossed to two corn belt single-cross testers (A632Ht × B73 and Mo17 × [H95 × H99^3]). The testcross hybrids were kept separate by tester and were combined with eight commercial hybrids to form two experiments. The tropical inbred lines were generated from progeny of a diallel cross of nine tropical hybrids as part of a germ plasm enhancement project. The tropical hybrids used included Pioneer Brand X105A, X304B, and X306C; Agroceres 155 and 504; and Centro Naccional de Technologia y Agropecuara (CNTA) H5 and H101. These hybrids contain germ plasm representing several different races of maize, including Coastal Tropical Flint, Cuban Flint, Tuson, Cateto, and Tuxpeno. This germ plasm has been fully described by Goodman (5) and Holley and Goodman (7). The commercial hybrids represented a wide range in maturity and included B73 × Mo17, USS 9001, DeKalb 789, Pioneer Brand 3055, 3165, 3369A, 3358, and 3389.

Tests were conducted at two locations in 1985 using a randomized complete

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block design. Both tests consisted of 42 entries described above with three replications at each location. The two locations were the Peanut Belt Research Station in Lewiston, NC, and the Tidewater Research Station in Plymouth, NC. Plots consisted of two 3.66-m rows with 18–22 plants per row. Row spacing was 0.91 and 0.96 m at Lewiston and Plymouth, respectively. Standard cultural practices were used. The plots were harvested using a modified Gleaner K2 Allis-Chalmers combine, and a random sample of 152 g of grain was taken from each plot for use in laboratory evaluations. All grain samples were placed in paper bags, dried down to 12% moisture, and kept in an air-conditioned room until processing in the laboratory.

Grain samples were ground in a blender and were used to form extracts that were applied to a selective growth media after a series of dilutions. All samples were ground to a uniform particle size. Ground samples (10 g) were placed in dilution bottles with 90 ml of Triton X-100 solution. All samples were then shaken on a wrist-action shaker for 5–10 min. Using sterile pipettes, 1 ml of sample extract was transferred to the first dilution tube. Contents were mixed on a vortex mixer between each transfer. Sample extract (1 ml) was pipetted onto each of two plates at three different dilution rates for a total of six plates. The growth medium was pentachloronitrobenzene (PCNB) (21). After the addition of 15 ml of PCNB media, the plates were incubated at 24 C for 72 hr. The average count from the two plates at the optimum counting density was used as the plot mean.

The statistical analysis was done in two phases using standard analysis of variance. First, the analysis was done by experiment, by location. Then the data were combined by experiment using the model log(no. of colony-forming units per gram) = location + replication (location) + hybrid + location/hybrid + error. Before the analysis, a natural log transformation was used to make the distribution of the data closer to a normal distribution. All experimental effects were considered to be random, with the exception of hybrids.

RESULTS AND DISCUSSION

Significant differences were found among hybrids for resistance to colonization by *F. moniliforme* (Tables 1 and 2). B73 × Mo17 had the highest mean value for colonization in the study (Table 3). From the results it appears that the coincident increases in grain contamination due to *F. moniliforme* and the use of hybrids closely related to B73 × Mo17 (19) may not represent independent events. In general, the commercial hybrids appeared to be relatively susceptible to pathogen development, with the exception of Pioneer 3055 and possibly Pioneer 3369A. Pioneer 3369A was significantly different from B73 × Mo17 in three of the four individual location tests, but not in the overall analysis. The presence of several tropical inbreds that conferred resistance to hybrids formed with either of the two testers is encouraging and suggests that improvement can be made via a straightforward recurrent selection program. The resistance shown by many of the 50% tropical hybrids suggests that tropical maize germ plasm should be a good source of resistance to this pathogen.

Significant differences were found among hybrids in three of the four tests (Table 1) and among hybrid means over locations in experiment 1 (Table 2), but measurement error was large. The reason for the large measurement error remains unclear because the relative magnitude of the components of variance differed greatly among tests. The replication component of variation, reflecting the magnitude of microenvironmental effects, was highly significant in two of the tests and nonsignificant in the other

Table 1. Analysis of variance using natural log-transformed data of colony-forming units of *Fusarium moniliforme* per gram of grain for individual tests by location[y]

		Mean squares			
		Lewiston		Plymouth	
Source	df	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Replications	2	1.88	11.35**[z]	5.88	51.37**
Entries	41	1.74*	2.21*	3.24	11.46**
Residual	82	1.08	1.39	2.21	5.87

[y] Experiments differ in the testers that were used in crosses with tropical inbreds. Experiment 1 contained the A632Ht × B73 testcrosses.
[z] * = Significant at the $P = 0.05$ level, ** = significant at the $P = 0.01$ level.

Table 2. Analysis of variance using natural log-transformed data of colony-forming units of *Fusarium moniliforme* per gram of grain by experiment over locations[y]

		Mean squares	
Source	df	Experiment 1	Experiment 2
Location	1	17.78	280.89*[z]
Replication (location)	4	3.89	31.36**
Entry	41	3.39**	7.75
Entry × location	41	1.60	5.92*
Residual	164	1.65	3.64

[y] Experiments differ in the testers that were used in crosses with tropical inbreds. Experiment 1 contained the A632Ht × B73 testcrosses.
[z] * = Significant at the $P = 0.05$ level, ** = significant at the $P = 0.01$ level.

Table 3. Actual and natural log-transformed hybrid means of colony-forming units of *Fusarium moniliforme* per gram of grain over locations and experiments

	Overall means[y]	
Pedigree[w,x]	Actual	Natural log
X105A × T[z]	26,370	10.18
X105A × T	68,871	11.14
X105A × T	128,027	11.76
X105A × T	112,420	11.63
(X105A × H5) × T	66,171	11.10
(X105A × H5) × T	174,556	12.07
(X105A × H5) × T	70,262	11.16
(X105A × H5) × T	151,751	11.93
(X105A × H5) × T	183,505	12.12
(X105A × H5) × T	135,944	11.82
(X105A × H5) × T	137,310	11.83
(X105A × H5) × T	26,635	10.19
(X105A × H5) × T	59,874	11.00
(X105A × X306C) × T	263,023	12.48
(X105A × X306C) × T	166,042	12.02
(X105A × X306C) × T	75,357	11.23
A155 × T	198,789	12.20
H5 × T	90,219	11.41
H5 × T	130,613	11.78
H101 × T	106,937	11.58
X105A × T	12,835	9.46
(A155 × A504) × T	75,357	11.23
(A155 × A504) × T	144,350	11.88
(H5 × A155) × T	117,008	11.67
(X105A × A155) × T	114,691	11.65
(X105A × A155) × T	39,340	10.58
(X105A × A155) × T	87,553	11.38
(X105A × A155) × T	45,706	10.73
(X304B × A504) × T	299,539	12.61
(X105A × H5) × T	147,266	11.90
(X306C × H5) × T	92,042	11.43
(X105A × H101) × T	119,372	11.69
(X304B × H101) × T	388,481	12.87
(X304B × H101) × T	198,789	12.20
Pioneer Brand 3369A	97,733	11.49
Pioneer Brand 3165	167,711	12.03
Pioneer Brand 3055	94,845	11.46
Pioneer Brand 3389	228,661	12.34
Pioneer Brand 3358	380,789	12.85
USS 9001	293,608	12.59
DeKalb 789	282,095	12.55
B73 × Mo17	455,886	13.03
LSD (0.05)		1.56

[w] Hybrids include commercial hybrids and experimental hybrids derived from crosses of tropical inbreds with two corn belt testers (T).
[x] All entries are representative of different tropical inbreds. In some cases, several tropical inbreds were derived from the same tropical hybrid or tropical hybrid cross.
[y] Means presented are the average for each tropical inbred over testers and locations.
[z] In experiment 1, the tester was (A632Ht × B73) and in experiment 2, the tester was (Mo17 × [H95 × H99[3]]).

two (Table 1). The two pairs of tests reflect differences in the single-cross tester that was used in forming the hybrids and indicates the possibility of different factors being involved in the expression of resistance. However, tester effects are confounded with field location effects, and no clear determination can be made. If the major problem in measurement of the character was associated with microenvironmental variation, then the separation of entries should be greatly improved by combining data over locations. When the data were combined over locations, only one of the two experiments had a significant component of variation associated with entries (Table 2). It is particularly interesting that experiment 2, in which replication effects are most pronounced, is the one that has a nonsignificant entry component of variation in the analysis over locations. The significance of the entry × location interaction component would tend to indicate that resistance rankings are not stable across environments. However, the rank correlation of 0.37 among entry means over locations in experiment 1 is statistically significant at $P = 0.05$. In addition, the error variance in experiment 2 at the Plymouth location was over two times the size of the variance in any of the other tests and was a major factor leading to the significant interaction, via the presence of heterogeneous variances, and the associated nonsignificant entry effects. The test mean for experiment 2 at Plymouth was lower than the means of the other locations. Therefore, there was no apparent reason for the heterogeneity of error variances. The suggestion that the genotype-by-environment interaction may not be due to changes in the rank of hybrids across environments is favorable for the potential for selecting genotypes that are resistant to colonization by the pathogen over a wide range of environments. Its negative implication is that there is a source of variation that cannot be efficiently addressed by simply increasing the number of replications or locations used in the screening process.

The use of laboratory evaluation of the number of colony-forming units per gram of grain appears promising for screening maize germ plasm for resistance to the development of *F. moniliforme*. The measurement error is fairly large, but this is not uncommon when dealing with kernel-infecting pathogens (17). More research is needed to obtain a better understanding of the factors that affect pathogen development. However, the results of this study indicate that the extensive use of highly susceptible hybrids closely related to B73 × Mo17 may have contributed to the increasing problems with contamination of maize grain and that tropical maize germ plasm may represent good source material for resistance to this pathogen.

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