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GRAIN YIELD AND FUSARIUM EAR ROT OF MAIZE HYBRIDS DEVELOPED FROM LINES WITH VARYING LEVELS OF RESISTANCE

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ABSTRACT - Fusarium ear rot, caused by *Fusarium verticillioides* and other *Fusarium* spp. is found in all U.S. maize (*Zea mays* L.) growing regions. Affected grain often contains carcinogenic mycotoxins called fumonisins. We tested the hypothesis that inbred lines with greater resistance to fumonisin contamination would produce hybrids with greater ear rot resistance and greater resistance to yield loss under artificial inoculation with *Fusarium* spp. Grain yield and Fusarium ear rot were measured under artificially inoculated and noninoculated conditions in two groups of hybrids created by topcrossing lines which exhibited either high or low levels of ear rot and fumonisin accumulation as early generation backcross lines *per se* in a previous study. Our results demonstrated that our hypothesis is not universally valid: the two groups of hybrids did not have significantly different ear rot or yield, perhaps because of generally low levels of ear rot observed in the testing environments.

KEY WORDS: Maize; Fusarium ear rot; Fumonisin.

INTRODUCTION

Fusarium verticillioides (Sacc.) Nirenberg (formerly *F. moniliforme* Sheldon) (teleomorph *Gibberella moniliformis*) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*) can colonize maize ears and cause Fusarium ear rot. Fusarium ear rot is prevalent in the warm, dry conditions common in the southern United States and lowland tropics but *F. verticillioides* and *F. proliferatum* can be found worldwide in grain or crop residue of mature maize fields (VAN EGMOND *et al.*, 2007). Fusarium ear rot generates additional concern because high levels of resistance are not present in commercial hybrid maize (MUNKVOLD and DESJARDINS, 1997) and *F. verticillioides* and *F. proliferatum* can produce mycotoxins called fumonisins that contaminate maize grain. Fumonisins are suspected carcinogens (GELDERBLOM *et al.*, 1988; PRELUSKY *et al.*, 1994; MILLER, 1994) and cause a number of human and animal diseases (COLVIN and HARRISON, 1992; ROSS *et al.*, 1992; HENDRICKS, 1999; MISSMER *et al.*, 2006; MORGAVI and RILEY, 2007).

Selection for resistance to both ear rot and mycotoxin contamination are important objectives to improve grain quality and reduce fumonisins in hybrid maize to acceptable levels. The United States Food and Drug Administration's *Guidance for Industry* recommends that fumonisin concentrations should not exceed 2 parts per million (ppm = μg g^{-1}) for many milled maize products used for human consumption (CFSAN, 2001a,b). European Union regulations limit fumonisin concentration to less than 1 ppm for human foods, and to less than 0.2 ppm for baby foods (COMMISSION OF THE EUROPEAN COMMUNITIES, 2007). Ear rot and fumonisin contamination are distinct aspects of the disease with low to moderate phenotypic correlations, but they are highly positively genetically correlated in both partly and highly inbred lines (ROBERTSON *et al.*, 2006). ROBERTSON *et al.* (2006) also reported moderate to high family mean heritabilities for both fumonisin contamination and Fusarium ear rot (between .47 and .89), suggesting that phenotypic selection against ear rot should be effective at improving resistance to these traits in inbreds. The relationships between these disease resistance traits and important agronomic traits also impact the development of cultivars with improved resistance.

ROBERTSON-HOYT *et al.* (2007) evaluated agronomic potential of 213 topcrosses of BC1F1 lines from the backcross of resistant parent GE440 to the commercial inbred FR1064. An unrelated non-Stiff Stalk hybrid (FR697×FR615) was used as the tester,

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and yields were evaluated without artificial inoculation. Their results suggested that backcrossing GE440 into FR1064 would not significantly reduce the agronomic features of that line, except in the case of grain moisture, which was predicted to increase slightly. The small positive correlation observed by ROBERTSON-HOYT *et al.* (2007) between ear rot and hybrid yield might have resulted from lines with fewer GE440 alleles having higher yield potential, despite their lower levels of ear rot resistance. Ear rot was not observed in the hybrids, so it was not clear if ear rot resistance alleles could contribute to higher yield under higher disease pressure.

The objective of this study was to determine the direct effect of ear rot resistance on hybrid yield by measuring yield of each genotype under higher and lower levels of Fusarium ear rot. This would allow direct estimation of the effect of resistance on yield under inoculated conditions, and to determine if resistance to ear rot in early generation backcross lines is indicative of hybrid tolerance under high levels of ear rot. For this study, we selected early generation backcross lines demonstrating highest or lowest levels of resistance to fumonisin contamination as lines *per se* in a previous study (ROBERTSON *et al*., 2006). Topcross hybrids of these lines were evaluated under both inoculated and noninoculated conditions. Our working hypothesis was that lines with greater levels of resistance to fumonisin would produce hybrids with greater ear rot resistance and yield tolerance to artificial inoculation with *Fusarium* spp.

MATERIAL AND METHODS

Population development

Fusarium ear rot resistant inbred GE440 (derived from the open-pollinated variety Hasting's Prolific) was crossed and backcrossed once to susceptible inbred FR1064 (an improved B73 type). BC_1F_1 plants were self pollinated to form 213 $BC_1F_{1:2}$ families. The ten most resistant and ten most susceptible families were selected based on mean fumonisin content in replicated trials in four environments in a previous study (ROBERTSON *et al*., 2006). The two groups also differed significantly for percentage of Fusarium ear rot incidence under inoculated conditions in the same study. $BC_1F_{1:2}$ families and the population parents, GE440 and FR1064, were topcrossed to an unrelated single-cross tester, FR615 x FR697, which represents the non-Stiff Stalk heterotic group. Two commercial hybrids, Pioneer brands 31G66 and 31G98, were included as checks. 31G98 is a 117 comparative relative maturity (CRM) hybrid that was recently popular in North Carolina which exhibits average Fusarium ear rot resistance and average staygreen (Robertson-Hoyt, personal communication), while 31G66 has a 118 CRM, exhibits fast dry-down and some tolerance to Fusarium ear rot (PIONEER HI-BRED, 2007). Twenty-nine genotypes were included in this study: topcrosses of the 20 selected lines, four additional lines were used as some of the original selected lines were short on seed, the topcrossed parents GE440 x (FR615xFR697) and FR1064x (FR615xFR697), the tester itself (FR615xFR697), and two commercial checks (Pioneer 31G98 and 31G66.).

Field evaluation

The experiment was conducted in both 2005 and 2006 in four North Carolina environments: the Central Crops Research Station at Clayton, the Tidewater Research Station at Plymouth, the Peanut Belt Research Station at Lewiston, and the Sand Hills Research Station at Jackson Springs. Soils at the experiment sites are classified as Portsmouth Fine Sandy Loam (fine-loamy over sandy or sandy-skeletal, mixed, thermic, Typic Umbraquult) at Plymouth, Norfolk Sandy Loam (fine-loamy, siliceous, thermic Typic Kandiudult) at Lewiston, and Candor Sand (sandy, siliceous, thermic Arenic Paleudult) at Jackson Springs.

A randomized split-plot design was used, with three replications at each location. The whole-plot factor was genotype, and the sub-plot factor was inoculation treatment. Each whole-plot was six rows of a common genotype. Each row was 3.66 m in length, with a 1.22-m alley between ranges of plots. Inter-row spacing was 0.914 m between plots in Lewiston, NC and 0.9652 m in Clayton, Plymouth, and Jackson Springs, NC. Plots were overseeded and thinned to target population densities of 44 plants per plot (62,288 plants ha^{-1} in Clayton, Plymouth, and Jackson Springs, NC or 65,750 plants ha^{-1} in Lewiston, NC). The sub-plot factor was inoculation treatment with *Fusarium* spp.; three rows of the plot received inoculation and three did not. Of the six rows in a whole-plot, the outer rows were hand harvested to score percent ear rot from each of the sub-plots and the inner four rows were mechanically harvested as two separate subplots of two rows each to measure grain yield and grain moisture. No border rows separated the inoculated and noninoculated plots, but *F. verticillioides* spreads very little during the growing season (YATES and SPARKS, 2008).

Inoculation technique

Three isolates of *F. verticillioides* (ISU95082, ISU94445, and ISU94040) and three isolates of *F. proliferatum* (310, 37-2, and 19) were cultured separately on PDA (Potato Dextrose Agar, Fisher Scientific Pittsburg, PA). Conidia were collected by washing the cultures with distilled water and diluting the conidia suspension of the six isolates to approximately $2x10^6$ mL^{-1} in water. Two inoculations were conducted seven days apart to reduce escapes and simulate common methods of natural infection.

The primary ear of each plant was injected with 10 ml of 1 x 10^6 conidial suspension in 2005 at each of two inoculation times. In 2006, 5 mL of 2 x 10^6 conidial suspension was injected using a 5mL Allflex draw-off injection syringe (Allflex Inc, Dallas, Tx) fitted with a 16 gauge needle that had the point filed off. One drop of undiluted Tween-20 was added to each liter of inoculum suspension to break the surface tension of the suspension. A silk channel inoculation 10 to 14 days post mid-silk was followed by a direct ear inoculation seven days later. In the rows of sub-plots designated for inoculation and hand harvest the first 15 ears were inoculated.

Phenotypic data collection

Stand count four to six weeks after planting was determined in the two rows of each sub-plot designated for mechanical har-

TABLE 1 - *F-tests for significance of genotype, inoculation treatment, and genotype-by-treatment interaction effects in the combined analysis of variance across environments, excluding checks and parental line hybrids.*

Source of Variation	Grain yield[1]	Fusarium ear rot[1]	Grain moisture[1]	Erect plants[1]	Silking date[2]
Genotype	3.0 *	1.2 ns	4.9 ***	2.5 **	1.8 *
Treatment	12.6 *	4.1 ns	3.1 ns	0.8 ns	1.0 ns
Genotype x Treatment	1.2 ns	1.4 ns	0.7 ns	0.7 ns	0.8 ns

[1] Data from four of eight environments. Clayton 2006; Lewiston, Plymouth, and Jackson Springs 2005 were excluded because each averaged <5% ear rot.
[2] Silking data from Clayton, NC environment in 2005 and 2006.
*, **, *** = significant at P = 0.05, 0.01, and 0.001, respectively.
ns not significant at P = .05.

vest. A maximum stand count of 44 was maintained by thinning overpopulated plots. Silk date and tassel date for each line were recorded at Clayton, NC. Silk date was recorded when half of the ears in each plot had reached 50% silk emergence. Anthesis date was recorded when approximately 50% of the pollen in the plot had been shed.

When all plants reached physiological maturity, 10 primary ears from the outside row of each whole plot were hand harvested and air dried to approximately 140g kg^{-1} moisture. Individual ears were visually rated for the percent of kernels displaying visible symptoms of Fusarium ear rot. Ear rot ratings were estimated to 5% increments. The center four rows of each whole-plot were mechanically harvested to collect grain moisture and yield data.

Statistical analysis

Yields for each plot were adjusted to 155 g kg^{-1} grain moisture. Traditional analyses of variance and spatial analyses were performed on the data for each environment separately to estimate genotypic least square means for each trait within each environment for the variable yield (Brownie *et al*., 1993). Models with up to fourth-order polynomial effects of row and columns in the field layout were tested. Trend effects were maintained if significant in the model at P < 0.01 (Brownie *et al*., 1993). The following models were compared using PROC MIXED in SAS version 9.1 (SAS Institute, 2004): a model including complete and incomplete block effects, a model with significant row and column trend effects, a model with correlated errors, and a model with both significant trend effects and correlated errors (Brownie *et al*., 1993). Percent stand was included as a covariate if significant at P = 0.01. For each environment, the model that minimized Akaike's Information Criterion was chosen (Akaike, 1974).

Environments with an average ear rot of less than 5% after spatial analysis were discarded. Four locations were not conducive to *Fusarium* infection and fungal growth and were discarded, leaving four locations for further analysis; Clayton in 2005, and Lewiston, Plymouth and Jackson Springs in 2006. Analysis of anthesis and silk date was performed on data collected in both years from Clayton.

Least square means for each combination of hybrid, inoculation treatment, and environment were estimated using the most appropriate statistical model and used as the basis for a combined factorial analysis of variance (ANOVA) across all environments. Combined ANOVAs across environments were implemented with SAS Proc MIXED, considering inoculation treatment and hybrid as fixed effects, and environment as a random effect. Satterthwaite (SAS Institute, 2004) or Kenward-Rodger (Kenward and Rogers, 1997) methods were used to estimate the denominator degrees of freedom for tests of fixed effects and for treatment comparisons. One form of the combined ANOVA included all entries and was used to estimate genotypic means across environments for each inoculation treatment. A second combined ANOVA was performed, excluding the check and parental hybrids, to determine the significance of genotype, inoculation, and genotype-by-inoculation interaction effects for the experimental hybrids only.

RESULTS AND DISCUSSION

The combined ANOVA across environments excluding check hybrids indicated that genotypes varied significantly for grain yield ($P \leq 0.0005$), grain moisture ($P \leq 0.0001$), erect plants (P = .01) and silking date (P = .025). Percent ear rot did not vary significantly across genotypes (Table 1). Inoculation treatment significantly affected yield (P < 0.03), but not ear rot, grain moisture, erect plants, or silking date in the combined analysis (Table 1). The interaction of inoculation treatment and genotype was not significant for grain yield or any other trait (Table 1).

The topcross of resistant parent GE440 had significantly lower ear rot than the topcross of susceptible parent FR1064 under both inoculated and non-inoculated conditions (Table 2). Inoculation more than doubled the difference in ear rot between the two topcrosses from 2.9% to 6.9% (Table 2), but this difference was not statistically significant. The overall levels of ear rot were much lower in this experiment than in our previous studies on early generation lines from this population. For example, the mean ear rot percentage under inoculation for FR1064 x (FR615xFR697) was 11.6% in this study (Table 2), but inbred FR1064 per se had 22% in a

TABLE 2 - *Trait means measured on topcrosses of $BC_1F_{1:2}$ lines to FR615xFR697 in four North Carolina environments. Assignment of lines to groups of highest and lowest fumonisin contaminated families was based on their per se performance in* ROBERTSON *et al.* (2006).

	Fusarium Ear Rot			Grain Yield			Erect Plants[3]	Days To Silk	Grain Moisture
	Inoculated	Noninoculated	Difference Non - Inoc[1]	Inoculated	Noninoculated	Difference Non - Inoc[1]	Average across treatments		
	% of ear	% of ear	% of ear	Mg Ha-1	Mg Ha-1	Mg Ha-1	%	DAP[2]	%
Low Fumonisin Group (Resistant)	7.6	4.3	-3.3	6.0	6.5	0.5	84	75.9	16.1
High Fumonisin Group (Susceptible)	9.1	5.0	-4.1	5.9	6.4	0.5	88	76.2	15.7
Difference between High and Low groups	**1.5** ns	**0.7** ns	**-0.8**	**-0.1** ns	**-0.1** ns	**0.00**	**4.0** ns	**0.3** ns	**-0.40****
GE440xTester (Resistant)	4.6	2.5	-2.1	5.2	5.5	0.3	49	76.3	17.6
FR1064xTester (Susceptible)	11.5	5.4	-6.1	6.0	7.3	1.3	98	75.2	15.5
Difference between FR1064 and GE440	**6.9***	**2.9***	**-4.0**	**0.8** ns	**1.8****	**1.0**	**49.0****	**-1.0** ns	**-2.1***
FR615xFR697 (Tester)	26.7	10.8	-15.9	4.18	5.17	0.99	95	78.0	13.2
31G66	6.2	3.1	-3.1	7.66	8.20	0.54	92	76.1	16.4
31G98	7.7	4.2	-3.5	8.11	9.28	1.16	86	76.0	16.3
LSD‡	7.0	3.1	-	0.94	0.77	-	0.10	1.79	0.54

[1] Difference Non – Inoc, difference between mean value in inoculated and noninoculated treatments.
[2] DAP, days after planting
[3] Lodging was not measured in Sandhills in 2006 because no lodging was observed.
ns not significant at P = .05.
*, * significant at P = 0.05, and .0001, respectively.
‡ The LSD shown is appropriate for comparing pairs of individual hybrid means. Comparisons involving checks may have higher precision.

previous study in North Carolina and Illinois environments by ROBERTSON *et al.* (2006). Similarly, the mean ear rot percentages for the 10 experimental lines with lowest fumonisin contamination and of the 10 lines with highest fumonisin contamination were 9 and 26%, respectively, in the previous study by ROBERTSON *et al.* (2006), whereas the ear rot percentages of their respective topcrosses were 8 and 9% in the current study (Table 2). Although the difference in mean ear rot for the two groups of early generation backcross lines was significant (ROBERTSON *et al.*, 2006), the differences between the corresponding two groups of topcrosses was not significant under either inoculation condition in this study (Table 2). The resistant parent GE440 topcross had the lowest ear rot among entries, but this was not significantly lower than either of the check hybrids, Pioneer brand hybrids 31G66 and 31G98.

The genotype with greatest ear rot percentage was the sister-line hybrid tester, FR615xFR697 (Table 2). This entry also had the lowest yield because of the limited heterosis expressed in the cross between related non-Stiff Stalk lines. This suggests that ear rot is easier to induce in plants with lower vigor, which is one explanation for the generally lower ear rot percentages in this study compared with previous studies on partly or completely inbred lines (ROBERTSON *et al.*, 2006).

The topcross of commercial line FR1064 had significantly greater yield than the GE440 topcross under noninoculated conditions, but its yield advantage was reduced from 1.8 Mg ha^{-1} to 0.9 Mg ha^{-1} with inoculation and the difference was not significant. As predicted under our hypothesis, resistance genes from GE440 reduced yield loss under inoculation (Table 2). On average, the topcrosses of the

TABLE 3 - *Pearson's correlations and associated P-values among traits measured in inoculated or noninoculated plots, excluding check entries.*

	Ear rot: noninoculated	Ear rot: inoculated	Difference in ear rot (N-I)[1]	Grain yield: noninoculated	Grain yield: inoculated	Grain yield: loss (N-I)	Silking Date
Ear rot: noninoculated	1	NS[2]	NS	NS	NS	NS	r = -0.5 P = 0.024
Ear rot: inoculated		1	r = -0.71 P = 0.0004	NS	NS	NS	NS
Difference in ear rot (N-I)			1	NS	NS	NS	NS
Grain yield: noninoculated				1	r = 0.797 P = <0.0001	r = 0.547 P = 0.013	NS
Grain yield: inoculated					1	NS	NS
Grain yield loss (N-I)						1	NS
Silking Date							1

[1] N-I, Difference between trait observed in noninoculated and inoculated treatments.
[2] NS, non significant at P = 0.05.

lines with lowest fumonisin content had yields similar to those of the topcrosses of the lines with highest fumonisin content under both inoculation treatments (Table 2). Neither topcrosses of low fumonisin accumulating lines, or the topcrosses of high fumonisin accumulating lines had a yield advantage in this study, suggesting that disease resistance *per se* did not confer a yield advantage. This result did not support our hypothesis and contrasts with the yield response to inoculation observed in the parental lines. The topcrosses of lines with lowest fumonisin content had significantly higher moisture than topcrosses of lines with the highest fumonisin content (Table 2), in agreement with the previous study (ROBERTSON-HOYT *et al.*, 2007). No other significant differences were observed between the two groups.

Silking date was significantly correlated (r = -0.5; P = 0.02) with ear rot under noninoculated conditions (Table 3). This agrees with studies by CLEMENTS *et al.* (2003) which show that time of inoculation is important for fungal growth. However, silking date was not significantly correlated with ear rot under inoculated conditions. It appears that the inoculation techniques used in these experiments were sufficient to overcome the effect of the slight correlation due to flowering time.

Ear rot under inoculated and noninoculated conditions were not correlated (Table 3). This result, in addition to the correlation between flowering time and ear rot in noninoculated conditions and the low levels of ear rot observed, suggests that environmental and developmental effects on ear rot masked genetic contributions to resistance, particularly in the noninoculated plots. In contrast, yield was highly correlated between the two inoculation treatments (Table 3), suggesting that it was reliably measured under both conditions.

The effect of inoculation treatment on individual line yields was correlated with their mean yield only under noninoculated conditions. In contrast, the effect of inoculation on ear rot of individual lines was highly correlated with their ear rot only under inoculated conditions (Table 3). The signs of the significant correlation coefficients indicate that inoculation increased ear rot more on more susceptible lines and decreased yield more for lines with greater yield potential.

This study was designed to test the hypothesis that lines with greater resistance to fumonisin contamination would produce hybrids with greater ear rot resistance and greater resistance to yield loss under artificial inoculation with *Fusarium* spp. The topcrosses made from lines with greater fumonisin contamination resistance had better ear rot resistance on average, but the difference between groups was not significant. The topcrosses of lines with greater fumonisin contamination resistance had better yield than the more susceptible lines, but this difference was also not significant, and occurred

under both inoculation conditions. We predicted two results based on our hypothesis: (1) the decrease in yield due to inoculation would be lower in the topcrosses of the more resistant lines, and (2) the difference in yield between inoculated and noninoculated conditions would be negatively correlated with the difference in ear rot between inoculated and noninoculated treatments because of the protective effect of resistance genes on yield. In fact, we observed that topcrosses of the more resistant lines had the same decrease in yield under inoculation as those of the less resistant lines (Table 2), and that the differences between inoculated and noninoculated treatments measured in yield and ear rot were not significantly correlated (Table 3).

We conclude from these results that our hypothesis is not universally valid, but may be dependent on the level of ear rot disease present. Under the low to moderate levels of ear rot observed in this study, resistance of early generation backcross lines *per se* is not predictive of ear rot tolerance in hybrids. Nevertheless, it is still possible that our hypothesis would hold when comparing yield under conditions amenable to ear rot. The results observed in this study may be due to the lower than expected levels of ear rot encountered.

Low levels of rot in this study likely resulted because weather conditions at the environments sampled were not as favorable to pathogen growth as in previous studies. This effect was observed in a screening trial of experimental and check inbred lines that is grown annually at Clayton, NC under artificial *Fusarium* inoculation (STARR *et al*., 2006). In that trial, mean ear rot percentage for B73 was 11.4% in 2004, but only 7.1% in 2005/2006; similarly FR1064 had 26.7% ear rot in 2004 but 4.9% in 2005/2006, indicating that while this study was being executed we observed lower ear rot percentages compared with previous years when the FR1064/GE440 backcross population was first studied.

Despite these factors, previous reports indicate that commercial hybrids grown in North Carolina can exhibit significant levels of Fusarium ear rot and fumonisin contamination, even without artificial inoculation (SHELBY *et al*., 1994; MUNKVOLD and DESJARDINS, 1997; BUSH *et al*., 2004; CLEMENTS *et al*., 2004). Therefore, future studies on hybrids may require manipulating the environment to promote Fusarium ear rot and fumonisin contamination.

One way to accomplish this may be to induce greater levels of plant stress during the pollination and grain-filling growth stages. Conditions that result in plant stress favor growth and pathogenicity of *F. verticillioides* (BACON and HINTON, 1996; OREN *et al*., 2003). Plant stress was lower than expected in the growing seasons evaluated, possibly reducing the visible symptoms of fungal ear rot. Weather conditions during the 2006 growing season did not create an optimum environment for fungal growth. While temperatures were suitable, late season rainfall was low which reduced dry-down time and likely limited mycelial growth. Plant stress could be induced by reducing fertilizer applications and irrigation. Drought stress is known to be associated with aflatoxin contamination (PAYNE *et al*., 1986; DIENER *et al*., 1987), and may also be conducive to Fusarium ear rot and fumonisin contamination. One possibility would be to restrict irrigation until the later grain filling stages, to permit both drought stress on the host plant and adequate moisture to incite ear rot in developing kernels. Finally, we have also collected more aggressive *Fusarium* spp. isolates from field plots in North Carolina to use in future experiments. Further research is needed to test these ideas.

To better understand the relationship between hybrid vigor, ear rot, and fumonisin accumulation we are evaluating a diallel mating of diverse inbred material with various combining abilities, and ear rot resistance levels.

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