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 Egmmond 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,
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$_2$F$_1$ plants were self polinated to form 213 BC$_3$F$_2$ 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-HOYT 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.

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 to breakpoint the surface tension of the suspension. A silk brush was added to each liter of inoculum suspension to break the surface tension of the suspension. A silken channel inoculation to 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-
**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 ($P = 0.01$) and silking date ($P = 0.025$). 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
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>
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<th>Trait means measured on topcrosses of BC₁F₁ 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).</th>
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<tr>
<td><strong>Fusarium Ear Rot</strong></td>
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<tr>
<td>% of ear</td>
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<td>Low Fumonisin Group (Resistant)</td>
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<td><strong>Difference between High and Low groups</strong></td>
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<td>GE440xTester (Resistant)</td>
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<td><strong>Difference between FR1064 and GE440</strong></td>
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<td>LSD‡</td>
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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.
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 \( (r = 0.5; P = 0.024) \) 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 non-inoculated 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 material with various combining abilities, and ear rot resistance levels. Drought stress is known to be associated with aflatoxin contamination (Payne et al., 1986; Dener 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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