ABSTRACT

ELLER, MAGEN STARR. Improving Resistance to Fusarium Ear Rot and Fumonisin Contamination and Increasing Yield with Exotic Maize Germplasm. (Under the direction of James B. Holland and Gary A. Payne.)

Exotic and unadapted varieties contribute advantageous alleles to crop species. Incorporating exotic germplasm into adapted lines has the additional advantage of broadening genetic diversity within the common maize germplasm pool. I explored contributions of unadapted or exotic maize germplasm to improved resistance to Fusarium ear rot and fumonisin accumulation or topcross grain yield (quantitatively inherited traits) using different breeding approaches and population structures.

Topcrosses of $BC_1F_{1:2}$ lines, developed by backcrossing GE440 to FR1064 and selected for divergent levels of resistance to Fusarium ear rot and fumonisin contamination, were used to test the hypothesis that inbred lines with greater resistance to fumonisin contamination produce hybrids with greater ear rot resistance and greater resistance to yield loss under inoculation. Experimental results did not support the hypothesis, but this result may have been due to low levels of infection in the field trials.

Selected $BC_4F_{1:3}$-derived lines representing advanced backcross generations of GE440 alleles into the FR1064 genetic background and their topcrossed hybrids were evaluated in field trials for disease resistance and yield potential. Experimental results demonstrate that advanced backcross generations produced lines comparable to FR1064 for grain yield but with better ear rot and fumonisin resistance, and that indirect selection for reduced fumonisin content by selection for ear rot resistance was partially effective, but that

selection for improved inbred disease resistance again did not result in improved topcross disease resistance.

A random-mated, genetically broad-based population referred to as the Resistant to Fusarium population, was developed to combine alleles for Fusarium ear rot resistance and improved agronomic traits from diverse maize germplasm. One cycle of selection was conducted in this population to test the hypothesis that index selection for reduced Fusarium ear rot, reduced lodging, and increased yield results in reduced fumonisin contamination. Selected lines $S_{0:3}$ were not significantly different than the base population for ear rot percentage or fumonisin content. In $S_{0:2}$ topcrosses, however, selected lines showed significant improvement for ear rot resistance and fumonisin accumulation compared to the unselected Cycle 0 topcross control. Additional cycles are needed to increase favorable allele frequencies for each of the target traits.

By genotyping a segregating $F_{2:3}$ population from a cross between phenotypically distinct F_4-derived sister lines, genome region(s) were identified which confer a topcross yield difference in a nearly-isogenic genetic background and determined that the tropical parent was the source of the favorable allele(s).

Improving Resistance to Fusarium Ear Rot and Fumonisin Contamination and Increasing Yield with Exotic Maize Germplasm

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for a Degree of
Doctor of Philosophy

Crop Science

Raleigh, North Carolina

2009

APPROVED BY:

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DEDICATION

To all my family, for encouraging, supporting, and believing, even when I didn't, and for making sure that I never take myself too seriously. I thank God for each of you. To my Cita, who was so dedicated to her role as little sister that she moved to North Carolina to inoculate corn and stayed! Most importantly, to Jon: you are unbelievable, and I am so blessed to daily receive the gift of your strength and love. Thank you for being my "Favorite". After putting up with this dissertation, you *deserve* a new banjo!

BIOGRAPHY

Magen Starr Eller was raised on a grain and livestock farm in western Illinois. By the time she reached 5th grade she knew she wanted to be a scientist, and by 8th grade, a plant breeder. After graduating high school, Magen attended the University of Illinois.

As a sophomore pursuing a Crop Science degree, Magen sought employment within the department, and began working with maize *macrohairless1* and *glossy15* mutants in the laboratory of Dr. Stephen Moose. She enjoyed the work and continued as an undergraduate assistant in his lab through her graduation in May of 2004.

Magen then accepted an IFAFS training grant fellowship from North Carolina State University, where she worked in the laboratory of Dr. Jim Holland. Under his direction, and that of Dr. Gary Payne, she explored the resistance to fusarium ear rot, and accumulation of the mycotoxin fumonisin in maize caused by the fungal pathogens *Fusarium verticillioides* and *Fusarium proliferatum* for her Ph.D. research. Her interest in the possible sources of resistance within diverse maize germplasm led to a yield QTL discovery project in a tropical by temperate maize population.

Her decision to pursue a degree in North Carolina proved life changing when in 2006 she married local residential builder Jonathan Eller. After graduation, Magen and Jon will be relocating to Indiana, where Magen has accepted a commercial breeding position, and Jon will begin a campaign to broaden the influence of North Carolina barbeque and sweet tea.

ACKNOWLEDGEMENTS

As with any project that spans five years, there are too many people who deserve thanks to mention them all. From farm staff to classmates, there have been so many people who worked with me, or encouraged my learning. However, I will express my gratitude first for my co-advisors, Drs. Jim Holland and Gary Payne. Jim, I am sure there were times that you despaired over my meager grasp of statistics, or the number of times you had to read the same manuscript. Thank you. Gary, thank you for being an optimistic voice of reason when fungus and "environment" would not cooperate.

Many thanks also to Stella Salvo, Wayne Dillard, David Rhyne, and Josie Bloom. Without you corn would never be planted and DNA would never be extracted. You have provided invaluable assistance and advice many times. To the laboratory and field crew, "team corn" from 2004-2009 (http://www4.ncsu.edu/~jholland/homepage.htm); thank you all. To Scott Reed, Heather Hunt, Teagen Gray, and Kathleen Hamby; you went above and beyond the call of duty every time.

To Drs. Leilani Robertson-Hoyt, Marco Oropeza-Rosas, Randy Wisser, Paul Nelson, Carrie Jacobus, John Zwonitzer and Jennifer Tarter, and to Kristen Kump, Hsiaoyi Hung, and Andrea Dolezal thank you for making the experience rich with friendship.

Thanks is also due to Dr. Major Goodman, Dr. Gina Brown-Guedira, Dr. Susanna Milla-Lewis, and Dr. Bob Patterson of NCSU, and Dr. Jim Hawk of the University of Delaware.

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OVERVIEW

Exotic and unadapted plant varieties have long been recognized as important sources of advantageous alleles not commonly found in domesticated crop species. In addition to contributing alleles for disease resistance to many crop species (Lenné and Wood, 1991), exotic germplasm has been shown to contribute superior alleles for many traits, including fruit size (Bernacchi *et al.*, 1998; Rau *et al*., 2003), fruit shape (Monforte *et al*., 2003), seed protein content (Sullivan and Bliss, 1983), oil content and milling and baking qualities (Kunert *et al*., 2007). Incorporating exotic germplasm into adapted lines has the additional advantage of broadening the genetic diversity within the common germplasm pool for a crop species.

Unadapted and exotic germplasm is not a major contributor to commercial maize hybrids (Goodman, 1999), although many studies have documented the usefulness of unadapted maize material for improving temperate U.S. material (Crossa and Gardner, 1987; Goodman *et al*. 2000; Lewis and Goodman, 2003; Tarter and Holland, 2006). The following thesis explores contributions of unadapted or exotic maize germplasm to improving resistance to Fusarium ear rot and fumonisin accumulation or topcross grain yield (all quantitatively inherited traits) using different breeding approaches and population structures. Fusarium ear rot is an economically important disease found in maize growing regions around the world, and topcross grain yield is the most economically important trait evaluated by maize breeders.

Fusarium ear rot is caused by the ascomycete *Fusarium verticillioides* (Saccardo) Nirenberg (teleomorph: currently *Gibberella moniliformis,* formerly *G. fujikuroi* mating population A) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: currently *G. intermedia,* formerly *G. fujikuroi* mating population D) (Munkvold *et al.*, 1997; White, 1999). *Fusarium spp*. overwinter in corn debris from previous seasons (Cotten and Munkvold, 1998) and can be spread by windblown spores during mechanical harvest and by rain splash and insect larvae during the growing season. *Fusarium verticillioides* and *F. proliferatum* most commonly infect maize ears through the silk channel, but *F. verticillioides* is also known to grow systemically throughout the maize plant as an endophyte (Kedera *et al.* 1992). Conditions contributing to the shift from endophyte to pathogen are unknown. Both species produce mycotoxins, of which fumonisin is the most significant (Leslie and Summerell, 2006). Fumonisin is associated with cancer (Prelusky *et al*., 1994) and a number of animal diseases (Morgavi and Riley, 2007).

This study evaluated a backcross population in which the unadapted temperate maize line GE440 was backcrossed to FR1064, a commercially important improved B73-type inbred. GE440 exhibits relatively high levels of resistance to Fusarium ear rot and to fumonisin accumulation, both of which FR1064 lack. $BC_1F_{1:2}$ topcrosses, $BC_4F_{1:3}$-derived lines and $BC_4F_{1:3}$ topcrossed hybrids from this population (referred to as the GEFR population) were evaluated.

The 10 $BC_1F_{1:2}$-derived lines from the GEFR population with lowest fumonisin content and sufficient seed availability, and the 10 lines with highest fumonisin content and

sufficient seed availability were evaluated as topcrosses to the single cross tester FR615 × FR697. Grain yield and Fusarium ear rot were measured under inoculated and noninoculated conditions to test 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 when challenged with the pathogen. The two groups of hybrids did not have significantly different levels of ear rot or grain yield, but this experiment did not prove a suitable test of the hypothesis because generally low levels of ear rot were observed in the testing environments, minimizing the expression of variation for resistance to ear rot.

The 10 GEFR population $BC_1F_{1:2}$-derived lines with lowest fumonisin levels were advanced without selection to the BC_4 generation, BC_4F_1 families were evaluated for ear rot, and 20 most resistant families were selected based on mean ear rot. Selected BC_4F_1s were advanced to the $BC_4F_{1:3}$ and evaluated under inoculated conditions as lines *per se* and as topcrosses onto the single cross tester FR615 ×FR697. Three hypotheses were tested: that advanced backcross generations would produce lines comparable to FR1064 for grain yield but with better ear rot and fumonisin resistance, that indirect selection for reduced fumonisin content could be accomplished by selection for ear rot resistance, and that selection for improved inbred disease resistance would result in improved topcross disease resistance.

GEFR BC_4-derived lines comparable to the recurrent parent for many agronomic traits, but exhibiting greater ear rot and fumonisin resistance than FR1064 were recovered. Selection at the BC_4F_1 generation reduced ear rot levels when $BC_4F_{1:3}$ lines were compared

to an unselected BC_4F_3 control, even though selection was carried out in an environment with low rot levels. Additional selection among $BC_4F_{1:3}$ lines in second year trials produced lines with significantly lower ear rot and fumonisin content than the unselected BC_4F_3 controls, demonstrating that when selection is conducted under appropriate environmental conditions, indirect selection for fumonisin content can be achieved by selection against ear rot. In topcross trials, selected $BC_4F_{1:3}$ lines were lower in ear rot than the unselected control under inoculated conditions. No differences for fumonisin content were observed.

These results demonstrate that backcross breeding is an effective way to improve quantitative ear rot resistance in a genetically elite, commercially important genetic background, but backcross breeding limits the potential for long-term improvement for target traits because most loci are fixed for recurrent parent alleles, and loci that are segregating do so for a maximum of only two alleles. Another approach to improving Fusarium ear rot and fumonisin resistance and incorporating exotic germplasm into temperate programs with less short-term effectiveness but greater long-term potential is to combine quantitative resistance alleles from multiple germplasm sources via recurrent selection.

The Resistant to Fusarium (ReFus) population was developed to combine alleles for Fusarium ear rot resistance with alleles for improved agronomic traits from diverse maize germplasm. The ReFus population was then used to test the hypothesis that index selection for reduced Fusarium ear rot, reduced lodging, and increased yield results in reduced fumonisin contamination.

ReFus Cycle 0 $S_{0:1}$ lines underwent index selection based on percentage ear rot, percent lodging, and grain yield *per se*. Selected lines were evaluated as $S_{0:3}$s and early generation $S_{0:2}$ topcrosses to the ear rot susceptible tester FR1064. The selected lines yielded significantly higher than the base population, but even with greater gains from selection than expected, selected lines were not significantly different than the base population for ear rot percentage or fumonisin contents. In topcrosses, however, selected lines showed significant improvement for ear rot resistance and fumonisin accumulation compared to the unselected Cycle 0 topcross control. Although direct and indirect selection responses are favorable, additional cycles of recurrent selection are needed to increase favorable allele frequencies for each of the target traits.

Yield drag due to linkage of favorable target alleles with unfavorable alleles is often associated with incorporating unadapted and exotic germplasm into elite breeding gene pools. In the third exotic × temperate-derived population discussed here, my objective was to identify the genome region(s) conferring a topcross yield difference in a nearly-isogenic genetic background and to determine if the exotic or temperate parent was the source of the favorable allele(s). Phenotypically distinct F_4-derived sister lines were crossed to develop a segregating $F_{2:3}$ population. A sample of 39 $F_{2:3}$ lines was genotyped at polymorphic markers, and topcrosses were evaluated for grain yield and other agronomic traits. QTL mapping revealed a grain yield QTL in chromosomal bin 3.06, at which the alleles derived from the tropical hybrid founder were associated with increased yield. This result demonstrates directly the yield advantage conferred by an allele originating in an exotic

hybrid over an allele from an elite commercial U.S. inbred line in U.S. growing environments.

References

Bernacchi, D., T. Beck-Bunn, Y. Eshed, J. Lopez, V. Petiard, J. Uhlig, D. Zamir and S. Tanksley. 1998. Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon*. Theoretical and Applied Genetics. 97: 381-397.

Cotten, T.K., and G.P. Munkvold. 1998. Survival of *Fusarium moniliforme, F. proliferatum*, and *F. subglutinans* in maize stalk residue. Phytopathology. 88: 550-555.

Crossa, J. and C.O. Gardner. 1987. Introgression of an Exotic Germplasm for Improving an Adapted Maize Population. Crop Sci. 27:187-190.

Goodman, M.M. 1999. Broadening the genetic diversity in Maize breeding by use of exotic germplasm. In J. G. Coors and S. Pandey, Eds. The Genetics and Exploitation of Heterosis in Crops. pp. 139 -148.

Goodman, M.M., Moreno, J., Castillo-Gonzalez, F., Holley, R., and Carson, M.L. 2000. Using tropical maize germplasm for temperate breeding. Maydica. 45:221–234.

Kedera, C.J., Leslie, J.F., and Claflin, L.E. 1992. Systemic infection of corn by *Fusarium moniliforme*. (Abstr.) Phytopathology. 82:1138.

Kunert, A., A. Ahmad Naz, O. Dedeck, K. Pillen and J. Léon. 2007. AB-QTL analysis in winter wheat: I. Synthetic hexaploid wheat (*T. turgidum* ssp. *dicoccoides* × *T. tauschii*) as a source of favourable alleles for milling and baking quality traits. Theoretical and Applied Genetics. 115: 683-695.

Lenne, J.M., and D. Wood. 1991. Plant Diseases and the Use of Wild Germplasm. Annual Review of Phytopathology. 29: 35-63.

Leslie, J.F. and B.A. Summerell. 2006. Fusarium Laboratory Manual. Blackwell Publishing Professional. Ames, IA.

Lewis, R.S. and M.M. Goodman. 2003. Incorporation of tropical maize germplasm into inbred lines derived from temperate × temperate-adapted tropical line crosses: agronomic and molecular assessment. Theoretical and Applied Genetics. 107: 798-805.

Monforte, A.J., M. Oliver, M.J. Gonzalo, J.M. Alvarez, R. Dolcet-Sanjuan and P. Arús. 2003. Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.) Theoretical and Applied Genetics. 108: 750-758.

Morgavi, D.P., R.T. Riley. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. Animal Feed Science and Technology. 137: 201–212.

Munkvold, G.P., D.C. McGee, and W.M. Carlton. 1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology. 87:209-217.

Prelusky, D., B. Rotter, and R. Rotter. 1994. Toxicology of mycotoxins. p 359-403. In Mycotoxins in Grain: Compounds other than Aflatoxin. Edited by J. Miller & H. Trenholm. St. Paul: The American Phytopathological Society.

Rao, G.U., A. Ben Chaim, Y. Borovsky, and I. Paran. 2003. Mapping of yield-related QTLs in pepper in an interspecific cross *of Capsicum annuum and C. frutescens*. Theoretical and Applied Genetics. 106:1457–1466.

Sullivan, J.G., and F.A. Bliss. 1983. Expression of enhanced seed protein content in inbred backcross lines of common bean. J Am Soc Hort Sci. 108: 787-791.

Tarter, J.A., and J.B. Holland. 2006. Gains from selection during the development of semiexotic inbred lines from Latin American maize accessions. Maydica. 51:15-23.

White, D.G., ed. 1999. Compendium of Corn Diseases, 3rd ed. APS Press. St. Paul, Minnesota.

CHAPTER 1: Breeding for Improved Resistance to Fumonisin Contamination in Maize

Citation

Eller, M.S., G.A. Payne, and J.B. Holland. 2008. Breeding for Improved Resistance to Fumonisin Contamination in Maize. Toxin Reviews. 27:1–19.

Breeding for Improved Resistance to Fumonisin Contamination in Maize

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Abstract

Maize grain infected by *Fusarium verticillioides* may contain the mycotoxin fumonisin which causes disease in livestock and is a suspected carcinogen. To reduce levels of fumonisin in grain efforts are underway to identify sources of maize with increased resistance to the fungus, and lower levels of accumulated fumonisins. This effort reviews field and laboratory techniques that more accurately measure these two aspects of the disease, application of these techniques to the identification of resistant maize lines, and dissecting the quantitative inheritance of ear rot and fumonisin accumulation resistance. We review recent quantitative trait loci and their application to breeding for resistance to fumonisin accumulation.

Key words: Quantitative Trait Loci (QTL), fumonisin, *Fusarium verticillioides*

Introduction

The fungal toxin fumonisin is a common contaminate of maize grain in the United States, and world wide (van Egmond *et al.*, 2007). Fumonisin may be produced when *Fusarium verticillioides* (formerly *F. moniliforme*, teleomorph *Gibberella moniliformis*) or the related species *F. proliferatum* colonize the maize ear. This mycotoxin is of particular concern because it is suspected of being carcinogenic (Miller, 1994; Prelusky, 1994), is linked with neural tube defects in humans (Hendricks, 1999; Missmer *et al.*, 2006), and causes severe diseases in a variety of livestock (Colvin and Harrison, 1992; Ross *et al.*, 1992; Morgavi and Riley, 2007). Furthermore, it is commonly found at biologically significant concentrations in corn grain produced throughout the United States (Shelby et al, 1994) and in processed foods (Sydenham et al, 1991).

Although this disease is prevalent in warm, dry conditions, like those common to the southern United States, *F. verticillioides* is found in grain or crop residue of virtually all mature corn fields in the United States. As no- till acreage increases across the United States (Conservation Technology Information Center, 2006), incidence of *Fusarium* infection is expected to increase because spores survive longer on surface residue than on residue that is plowed under (Cotten and Munkvold, 1998). *F. verticillioides* is also frequently found colonizing symptomless maize plants (Munkvold and Desjardins, 1997) and can grow systemically without causing visible symptoms (Kedera *et al.* 1992).

Resistance to fumonisin accumulation and fungal growth exists among maize lines, but high levels of resistance have not been identified and adequate resistance is not present in commercial hybrid varieties (Munkvold and Desjardins, 1997). *F. verticillioides* and *F. proliferatum* also cause Fusarium ear rot and stalk rot in corn (White, 1999). The symptoms of fusarium ear rot can range from mild "starbursting" of mycelia in kernels to full blown destruction of kernels and cob tissue. Severe incidence of fusarium ear rot is associated with reduced grain yield (Presello, 2008). Ear rot and fumonisin contamination are distinct aspects of the disease, but, as will be discussed below, they are at least partly related.

Research and breeding efforts aimed at improving resistance to these two aspects of the disease have focused on accurately measuring Fusarium ear rot and fumonisin concentrations, identifying sources of resistance, and characterizing the inheritance of ear rot and fumonisin accumulation. Recently, quantitative trait loci (QTLs) were identified for both resistance to ear rot (Pérez-Brito *et al.*, 2001; Robertson-Hoyt *et al.*, 2006), and resistance to accumulation of fumonisin (Robertson-Hoyt *et al.*, 2006). Techniques to ensure accurate phenotyping of Fusarium ear rot and fumonisin contamination also have been developed and validated, and DNA marker technologies have matured and become economically feasible for some DNA marker-assisted selection programs. We consider the application of these two approaches (which are not exclusive) to breeding for reducing susceptibility to Fusarium ear rot and fumonisin contamination.

Measuring Fusarium Ear Rot and Fumonisin – Field and Laboratory Techniques

Optimal phenotyping of ear rot and fumonisin concentration rely on accurate assays in the field and laboratory. Bush *et al.* (2004) studied the onset of fungal growth and toxin accumulation and determined that maximum infection occurs around 20% kernel moisture, while fumonisin first appears in the dent stage of development at 35 to 40% moisture. In hybrids, the peak for kernel infection is at seven weeks after pollination and the peak for fumonisin concentration is nine weeks after pollination (Bush *et al.*, 2004). This is the optimal time for measurement because fumonisin levels can fluctuate unpredictably if harvest is delayed (Bush *et al.*, 2004).

Ears and kernels are a mixture of tissues that are genotypically 100% maternal (cob, glume, and aleurone), 50% maternal and 50% paternal (embryo), and 67% maternal and 33% paternal (endosperm), so we conducted a study to determine if paternal (pollen) genotype could affect ear rot severity and fumonisin content. Previous studies indicated that Fusarium ear rot resistance is controlled by the maternal parent (Scott and King, 1984; Headrick and Pataky, 1991; Nankam and Pataky, 1996), but no information on the effect of pollen source on fumonisin content was available. We compared Fusarium ear rot and fumonisin content of self-fertilized ears and open-pollinated ears in a study of 143 recombinant inbred lines of the NC300×B104 population in a lattice design with two replications in one environment (Starr, *et al.* 2006). Each plot was subdivided into sub plots, one of which contained open-pollinated plants and one of which contained self-pollinated plants; all plants were artificially inoculated twice with a mixture of *F.*

verticillioides and *F. proliferatum* isolates (Robertson *et al.*, 2006). Our results indicated that pollen source did not significantly affect either Fusarium ear rot or fumonisin concentration, nor was there a significant genotype-by-pollen source interaction due to imperfect genetic correlation between the two types of measurements (Starr *et al.*, 2006). Therefore, Fusarium ear rot and contamination resistance evaluations can be effectively performed on open-pollinated plants.

Because *F. verticillioides* can over-winter in the soil (Cotten and Munkvold, 1998) and is predominately spread by wind, rain splash and insect larvae, artificial inoculation is needed to ensure equal distribution of the pathogen among plants throughout the field. For *F. verticillioides*, infection through the silks is a more important pathway for kernel infection than through the seeds, stalk, or crown; therefore, silk inoculation is best for evaluating genetic resistance to Fusarium ear rot (Munkvold, McGee, and Carlton, 1997). However, not all ear and silk inoculation techniques are equally effective. A comparison of four techniques found that only inoculum injection through the husk significantly increased rot severity and fumonisin concentration. This technique also effectively differentiated levels of susceptibility and resistance between lines (Clements *et al.*, 2003). Bush (2001) conducted a similar study to compare the potential of five inoculation techniques for determining resistance or susceptibility of a variety. Two inoculation techniques, inoculation by penetrating husks with pin bars, and injecting inoculum down the silk channel, were best able to discriminate different levels of resistance to fungal infection and

fumonisin accumulation. Husk penetration mimics natural inoculation by insect and silk channel injection mimics spores splashed onto silks by rain, or carried by the wind.

Good field techniques are important for accuracy and consistency in both ear rot and fumonisin scores, but fumonisin analysis also requires accurate laboratory assays that are cost-efficient for the large numbers of samples evaluated by genetics and breeding programs. High-performance liquid chromatography (HPLC) can be used to very accurately estimate fumonisin concentrations (Bush *et al.*, 2004), and differentiate between structural forms of fumonisin, but this is too expensive to for use in large-scale breeding programs. For example, a laboratory at NC State University charges at least $30 per sample for HPLC analysis of fumonisin. An alternative to HPLC are ELISA assays which detect fumonisins B1, B2, and B3 (structurally different toxins in the fumonisin family of compounds). We have used ELISA assays based on antibodies to fumonisin B1 developed by Chris Maragos of the USDA-ARS Mycotoxin Research Unit in Peoria, IL and a technique optimized by the Immunological Resource Center at the University of Illinois (Clements *et al*., 2003). In addition, we have used commercially available quantifiable ELISA kits (e.g., from Diagnostix Laboratories) to analyze the fumonisin content of ground corn samples in our program. There are many similar kits available in individual sample or 96 well format that can be used as qualitative or quantitative assays.

Identifying Sources of Resistance

Resistance to Fusarium ear rot is under genetic control (King and Scott, 1981), but no complete resistance has been identified in maize. Similarly, Shelby *et al.* (1994) reported significant variation among commercial maize hybrids for fumonisin content, but no hybrid was found to be immune. Furthermore, the hybrid with lowest mean fumonisin content across 11 locations grown without the use of artificial inoculum in Shelby *et al.*'s (1994) study had 5.78 parts per million (PPM, $\mu g\ g^{-1}$), which is above the threshold content level for human food or horse feed provided by the FDA's guideline for industry (Anonymous, 2001). To incorporate resistance to fumonisin accumulation into commercial maize, lines with acceptable resistance levels must first be identified.

Transgenic maize varieties with European Corn Borer resistance derived from *Bacillus thuringiensis Cry* genes have good resistance to insect feeding damage on maize ears (Magg *et al.*, 2001), which is an important route for *Fusarium* infection. Transgenic maize exhibited reduced incidence and severity of Fusarium ear rot (Munkvold, Hellmich, and Showers, 1997), as well as reduced levels of mycotoxins compared to near-isogenic non-transgenic lines (Munkvold *et al.*, 1999; Pabst *et al.*, 2005). While transgenic lines are important for reducing fumonisin accumulation (Wu, 2006), in these studies, transgenic lines still exhibited infection and mycotoxin production, indicating that natural sources of resistance are also needed to protect the corn crop in combination with transgenic insect resistances.

The United States hybrid maize crop is based almost entirely on crosses between proprietary commercial inbred lines (Mikel and Dudley, 2006). Many of the commercial inbred lines are derived from older publicly developed inbred lines, representing a relatively narrow sampling of the available maize gene pool (Mikel and Dudley, 2006; Nelson et al, 2008). Therefore, because commercial hybrids do not appear to provide adequate levels of resistance, it is reasonable to search for higher levels of resistance in germplasm not closely related to the progenitors of modern commercial hybrids. Two important alternative sources of diverse maize germplasm are older public inbred lines and tropical germplasm. Older public inbred lines are often better adapted to typical USA corn production environments, but tend to have lower inherent yield potential, and often poor stalk strength. Tropical germplasm may have higher yield potential, but it is poorly adapted to temperate production environments, limiting its immediate utility to breeding programs in the United States (Goodman *et al.*, 2000).

To identify sources of resistance among older public inbred lines, Clements *et al.* (2004) evaluated Fusarium ear rot and fumonisin accumulation in testcrosses of 1,589 public inbred lines to the susceptible tester, FR1064. They identified several inbreds with superior resistance to production or accumulation of fumonisin. Resistance in these lines appears to be dominant, as it is evident both in the inbreds and the hybrids between 35 selected resistant lines and FR1064 (Clements *et al.*, 2004).

To supplement the germplasm screens conducted by Clements *et al.* (2004), we have been conducting screening trials for both Fusarium ear rot and fumonisin concentration

using material selected by Dr. Mike Blanco from the Germplasm Enhancement of Maize (GEM) project (Pollak and Salhuana, 2001) and advanced lines from Dr. Major Goodman's breeding program at N.C. State University (Goodman *et al.*, 2000). Both of these programs are focused on identifying superior tropical maize germplasm, adapting it to temperate growing conditions, and breeding for maximum hybrid combining ability (Betran *et al.*, 2004). Each year we screen approximately 50 lines, in two replications at one location. Each plant in these trials is inoculated twice with a mixture of *F. verticillioides* and *F. proliferatum* isolates, similar to the methods described by Robertson *et al.* (2006) with modifications. The first inoculation is conducted about 10days post mid-silk and involves injecting a consistent quantity of spore suspension down the silk channel. The second inoculation is performed about 7 days later by injecting a spore suspension through the husk. Promising lines are retested in subsequent years to verify results in multiple environments. Using Best Linear Unbiased Prediction (BLUP; Piepho *et al.*, 2007) this screen has identified several lines with good levels of resistance across years to both Fusarium ear rot and fumonisin accumulation (Table 1.1). Importantly, these lines have unique genetic backgrounds, and may therefore carry unique alleles for disease resistance that can complement those present in public inbred lines and elite commercial maize gene pools.

Inheritance of Fusarium Ear Rot and Fumonisin Contamination

Once suitable sources of resistance have been identified, inheritance of resistance should be considered before selecting a breeding strategy. Clements *et al.* (2003) found a positive, but only moderate, phenotypic correlation between incidence of Fusarium ear rot and fumonisin concentration, and concluded that the two traits should be considered separately in breeding programs, since improvement of ear rot resistance may not result in gains in the resistance to fumonisin content.

Phenotypic correlation estimates include both genetic and non-genetic effects and cannot be used to predict the correlated response in some trait, Y, to selection on a different trait, X. Therefore, Robertson *et al.* (2006) conducted experiments to specifically estimate the correlation between genetic effects on Fusarium ear rot and fumonisin content. Two populations were grown in three to four environments, were inoculated as described above, and evaluated for fumonisin concentrations by quantitative ELISA. Multivariate analysis was used to partition the genotypic variances and covariances from the phenotypic variances and covariances and to estimate the genotypic correlation between Fusarium ear rot and fumonisin content. Estimated genotypic correlations were surprisingly high, r_g = 0.96 and 0.87, in the two populations, even though phenotypic correlations were not (Table 1.2; Robertson *et al.*, 2006). The high genotypic correlations suggest that genotypes with greater resistance to Fusarium ear rot also tend to have greater resistance to fumonisin contamination. Further, it suggests that the genetic components of resistance are largely the same for the two traits, even though they are not highly phenotypically correlated

(Robertson *et al.*, 2006). Although surprising, this does not contradict observations that fumonisin sometimes accumulates to high levels in kernels with little ear rot (Munkvold and Desjardins, 1997). Though the genetic controls of resistance seem to be similar, environmental factors which promote ear rot do not appear to promote fumonisin production to the same extent.

The high genetic correlations between Fusarium ear rot and fumonisin in these two populations lead to the question: how effective would selection aimed solely at reducing ear rot be at reducing susceptibility to fumonisin contamination? Response of fumonisin levels to indirect selection against ear rot is predicted to be less effective than direct selection against fumonisin accumulation because fumonisin concentration had a higher heritability than resistance to Fusarium ear rot in both populations (Table 1.2). This is likely because fumonisin assays are more precise than visual scores of percent ear rot. However, ear rot can be scored quickly in the field, whereas assaying fumonisin content requires harvesting the inoculated ears, shelling grain, grinding grain to a precise particle size, weighing samples, performing fumonisin extractions in the laboratory, and finally conducting ELISA or HPLC assays. Considering the labor required to conduct these additional steps, and the price of ELISA or HPLC assays, indirect selection against ear rot could be a more economically efficient way to reduce fumonisin content in the grain than direct selection against fumonisin contamination. Because ear rot is easier to evaluate, population sizes could be increased, permitting higher selection intensity, or the number of evaluation

environments and replicates could be increased, resulting in a greater entry mean heritability for ear rot compared to fumonisin concentration.

For the nearly 200 lines we evaluated in the GEM fusarium screening trials that between 2003 and 2007 (Table 1.1), the estimated genetic correlation between percent ear rot and fumonisin content was $r_g = 0.56$ ($p < .0001$). This value is lower that those estimated by Robertson *et al.* (2006) but these lines represent a wider sample of genetic diversity than the two populations in that study. Data from the GEM screening trials suggest that there are more trait-specific genes than pleiotropic effects in this diverse set of material. Nevertheless, even across this very broad sample of germplasm, the genetic correlation between the two traits was highly significant and of at least moderate positive magnitude, indicating that selection against ear rot is expected to have favorable consequences for reducing susceptibility to fumonisin contamination.

QTLs for Fusarium Ear Rot and Fumonisin Contamination

Phenotypic evaluations of Fusarium ear rot and fumonisin concentration present some practical difficulties. The traits must be scored on grain harvested from mature ears; two inoculations with calibrated spore suspensions are needed to obtain consistent ear rot ratings (Clements *et al.*, 2004); and evaluation of fumonisin content requires an expensive, laborious, toxin assay on precisely ground and weighed samples. In addition, Fusarium ear rot and fumonisin contamination are often strongly affected by environmental factors.

Given the difficulties of phenotypic evaluation of Fusarium ear rot and fumonisin contamination and the reasonably high heritability of both traits, marker assisted selection may be a more efficient selection strategy than phenotypic evaluation if PCR-based DNA markers linked to genes with moderate effects on resistance can be identified (Holland, 2004; Robertson *et al.*, 2005). Therefore, Robertson-Hoyt *et al.* (2006) mapped QTLs for ear rot resistance and fumonisin contamination in the same two populations used for heritability and genetic correlation estimation by Robertson *et al.* (2006). They identified seven QTLs for Fusarium ear rot resistance in the GEFR population (derived from backcrossing resistant line GE440 into susceptible line FR1064), and five Fusarium ear rot resistance QTLs in the NCB population (NC300xB104, in which both parental lines exhibit some resistance). They also identified nine and six QTLs for resistance to fumonisin accumulation in the GEFR and NCB populations, respectively (Robertson-Hoyt *et al.* 2006). Despite the very high correlations between Fusarium ear rot and fumonisin contamination, the QTLs identified were associated with only 65% (GEFR) and 31% (NCB) of the genetic covariance between the traits. The relatively low proportions of genotypic covariance that were associated with QTLs suggest that not all QTLs were identified. It is possible that a large number of genes with small effects that are hard to detect (i.e., the polygenic background) may explain much of the remaining genetic covariance. Supporting this idea is our observation that the combined effect of QTLs accounted for as little as 39% of genotypic variation for ear rot (Robertson-Hoyt *et al.*, 2006), suggesting that, at least for some traits, some QTLs were not identified. Greater power to detect QTLs and estimate

QTL effects could be gained by increasing population size, improving ear rot phenotyping methods, increasing the number of environments for phenotyping, and increasing the number of markers used (Robertson *et al.*, 2006).

Comparison of QTLs detected for ear rot across two populations studied by Robertson-Hoyt *et al.* (2006) and two populations studied by Pérez-Brito *et al.* (2001) revealed few QTL regions in common across populations (Robertson-Hoyt *et al.*, 2006). Similarly, comparison of QTLs detected for fumonisin concentration across two populations revealed limited congruence in the genetic controls across populations (Robertson-Hoyt *et al.*, 2006).

Having reliable estimates of trait heritabilities and genetic correlations, and QTL positions and effects would allow accurate prediction of the relative value of traditional phenotypic selection versus marker-assisted selection. Marker-assisted selection could offer several advantages over either indirect selection on ear rot or direct selection on fumonisin concentration. Both phenotypic traits require multiple plants per plot and multiple replications and environments to obtain accurate data. In contrast, if QTLs have been accurately mapped, selection on marker loci flanking QTLs could be effective on individual plants (Robertson *et al.*, 2005). Furthermore, marker-assisted selection could be conducted in greenhouses and off-season nurseries without concern for genotype-by-environment interaction that would likely reduce (or eliminate entirely) the response to phenotypic selection in these environments.

Unfortunately, QTL effects on highly polygenic traits cannot be reliably estimated in typical mapping population sizes (Schon *et al.*, 2004; Holland, 2007), such as those used by Robertson-Hoyt *et al.* (2006) and Pérez-Brito *et al.* (2001). If moderate-to-large effect QTLs exist in these populations, they have been reliably mapped, but if only small-effect QTLs exist, their effects may be overestimated to appear as moderate-effect QTLs (Schon *et al.*, 2004). Therefore, until these QTL have been validated in independent studies, we cannot be certain as to the reliability of their effect estimates. Furthermore, the substantial variation for QTLs segregating in different mapping populations indicates a high level of genetic heterogeneity for both Fusarium ear rot and fumonisin contamination resistances. This situation, in combination with complex genetic control by numerous genes, each with relatively small effects, inhibits the widespread application of QTL information for marker-assisted selection across broader arrays of breeding crosses (Holland, 2004; 2007).

Encouragingly, specific genes putatively providing resistance to Fusarium pathogens have recently been identified. Gao *et al.* (2007) reported that a defective lipoxygenase mutation (*lox3*) in maize reduces fumonisin B1 contamination and *F. verticillioides* conidiation as well as providing resistance to several other pathogens of maize. In addition, Yuan *et al.* (2007) reported a maize guanylyl cyclase gene associated with resistance to Gibberella ear rot, caused by the related fungus, *F. graminearum*. Two of the multiple copies of this gene in maize map near QTL positions for ear rot resistance, the gene transcript is up-regulated in response to pathogen inoculation, and the more resistant parent has a higher level of expression of the gene family (Yuan *et al.*, 2007). If the effects of these

genes on resistance can be validated in independent studies and if markers can be developed to distinguish the resistant alleles consistently across breeding populations, then such markers would be ideal tools for implementing marker-assisted selection on a broader scale in maize breeding programs. Each gene contributes only partial resistance, so marker-assisted selection would still need to be complemented by phenotypic selection for resistance.

Application of Inheritance and QTL Studies for Improving Ear Rot and Fumonisin Contamination Resistance

As mentioned previously, the high genotypic correlation between resistance to Fusarium ear rot and fumonisin implies that indirect selection on ear rot could be used to improve resistance to fumonisin contamination in an economically efficient manner. Because at least some of the QTLs for the two traits appear to be different, and because the genetic correlation is lower in more diverse genetic material, however, we suggest that ear rot evaluations for large numbers of early generation breeding materials should be followed by combined ear rot and fumonisin content analysis of fewer selected late-generation lines and hybrids.

Experiments are underway to determine the effectiveness of selection against ear rot for improving resistance to fumonisin contamination. In one experiment, we are testing this in advanced backcross lines containing alleles from GE440 (which has good resistance but poor agronomic quality) introgressed into an elite genetic background that lacks effective

resistance (FR1064). A second, independent test of the hypothesis that selection against ear rot will improve fumonisin contamination resistance is being conducted in a genetically broad-based population that we created and in which we have initiated recurrent S1 selection. The results of these two experiments should provide more definitive insights into the genetic relationship between ear rot and fumonisin contamination resistances and will help guide future breeding efforts.

The results of these two ongoing experiments will also provide insight into a number of other unresolved issues related to resistance to fumonisin contamination. Among these is the question of the effects of improving disease resistance on other key traits related to agronomic performance, such as hybrid combining ability for yield, lodging resistance, and grain dry down. Some predictions about these effects were made by Robertson-Hoyt *et al.* (2007), who crossed each of the GEFR mapping population to a common unrelated tester and evaluated the resulting hybrids for yield and other agronomic characters across eight environments. Hybrid grain yields were correlated to a small extent with increased ear rot, suggesting that selection for increased ear rot resistance may result in small decreases in grain yield potential. In addition, both Fusarium ear rot and fumonisin content were negatively correlated (r = -0.22 to -0.30, $p < 0.001$) with grain moisture, suggesting that improving resistance to fumonisin contamination and ear rot may result in slower grain dry-down (Robertson-Hoyt *et al.*, 2007). We will be able to directly evaluate the effect of selection for improved resistance to Fusarium ear rot on agronomic potential in the two selection populations described.

The advanced backcross lines selected in the GEFR population will also represent a valuable resource for developing near-isogenic lines (NILs) for finer-scale genetic analysis of the resistance QTLs previously mapped. By homogenizing the genetic background and isolating the effects of individual QTL, these NILs will improve resolution of the positions and effects of QTLs (Zamir, 2001; Szalma *et al.*, 2007). They will also provide an independent test to validate the QTLs originally mapped by Robertson-Hoyt *et al.* (2006) and a higher resolution test to determine to what extent genes for Fusarium ear rot have pleiotropic effects on fumonisin contamination.

Finally, experiments are underway with both of these populations to test the relationship between Fusarium ear rot and fumonisin contamination resistance measured in inbred lines and in hybrids created from those lines. If the relationships are strong, this validates the use of selection and genetic analysis in inbred lines for the improvement of resistance to fumonisin contamination. If resistance in inbreds is not highly correlated with resistance in hybrids, however, this will greatly complicate breeding methods for improving resistance.

References

Anonymous. 2001. Guidance for industry: Fumonisin levels in human foods and animal feeds. Online: http://www.cfsan.fda.gov/~dms/fumongu2.html. Food and Drug Administration, Washington, D.C. (verified 24 April, 2008).

Betran, J., M. Menz, and M. Bänziger. 2004. Corn breeding. p. 305–395. *In* C.W. Smith, J. Betran, and E.C.A. Runge (ed.) Corn: Origin, history, technology, and production. John Wiley & Sons, Hoboken, NJ.

Bush, B.J. 2001. *Fusarium verticillioides* infection, fumonisin contamination and resistance evaluation in North Carolina maize. M.S. thesis. North Carolina State Univ., Raleigh, NC.

Bush, B.J., L.M. Carson, M.A. Cubeta, W.M. Hagler, and G.A. Payne. 2004. Infection and Fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology. 94: 88-93.

Clements, M. J., C.E. Kleinschmidt, C.M. Maragos, J.K. Pataky, and D. G.White. 2003. Evaluation of inoculation techniques for Fusarium ear rot and fumonisin contamination of corn. Plant Disease. 87:147-153.

Clements, M. J., C.M. Maragos, J.K. Pataky, and D.G. White. 2004. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopathology. 94:251-260.

Colvin, B.M. and L.R. Harrison. 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia. 117:79-82.

Cotten, T.K., and G.P. Munkvold. 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. Phytopathology. 88: 550-555.

Conservation Technology Information Center. 2006. 2006 Crop Residue Management Survey. Online: http://www.conservationinformation.org/pdf/2006CRMSurvey SummaryLoRes.pdf (verified May 7, 2008).

Gao, X., W.B. Shim, C. Göbel, S. Kunze, I. Feussner, R. Meeley, P. Balint-Kurti, and M. Kolomiets. 2007. Disruption of a maize 9-Lipoxygenase results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin Fumonisin. Molecular Plant-Microbe Interactions. 20: 922–933.

Goodman, M.M., J. Moreno, F. Castillo-Gonzalez , R. Holley, M.L. Carson. 2000. Using tropical maize germplasm for temperate breeding. Maydica. 45:221-234.

Headrick, J. M. & Pataky, J. K. 1991. Maternal influence on the resistance of sweet corn lines to kernel infection by *Fusarium moniliforme*. Phytopathology. 81: 268-274.

Hendricks, K. 1999. Fumonisins and neural tube defects in south Texas. Epidemiology. 10:198-200.

Holland, J.B. 2004. Implementation of molecular markers for quantitative traits in breeding programs - challenges and opportunities. *In*: T. Fischer *et al.* (ed.) New directions for a diverse planet: Proceedings for the 4th International Crop Science Congress, Brisbane, Australia, 26 September – 1 October 2004. Available online at: http://www.cropscience.org.au/icsc2004/symposia/3/4/203_hollandjb.htm (verified 24 April, 2008).

Holland, J.B. 2007. Genetic architecture of complex traits in plants. Current Opinion in Plant Biology. 10:156-161.

Kedera, C. J., Leslie, J. F., and Claflin, L. E. 1992. Systemic infection of corn by *Fusarium moniliforme*. (Abstr.) Phytopathology. 82:1138.

King, S. B. and G.E. Scott. 1981. Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. Phytopathology. 71: 1245-1247.

Magg, T., A.E. Melchinger, D. Klein, and M. Bohn. 2001. Comparison of Bt maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. Plant Breeding. 120:397-403.

Mikel M.A. and J.W. Dudley. 2006. Evolution of North American Dent Corn from Public to Proprietary Germplasm. Crop Science. 46:1193-1205.

Miller, J.D. 1994. Epidemiology of *Fusarium* ear diseases of cereals. p. 19-36. *In*: J.D. Miller and H.L. Trenholm (ed.) Mycotoxins in grain. Eagan Press, St. Paul, MN.

Missmer, S.A., L. Suarez, M. Felkner, E. Wang, A.H. Merrill, Jr., K.J. Rothman, and K.A. Hendricks. 2006. Exposure to Fumonisins and the Occurrence of Neural Tube Defects along the Texas–Mexico Border. Environ Health Perspectives. 114: 237–241.

Morgavi, D.P., R.T. Riley. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. Animal Feed Science and Technology. 137: 201–212.

Munkvold, G.P., and A. Desjardins. 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Disease. 81:556-565.

Munkvold, G. P., Hellmich, R. L., and Showers, W. B. 1997. Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology. 87:1071-1077.

Munkvold, G.P., D.C. McGee, and W.M. Carlton. 1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology. 87:209-217.

Munkvold, G. P., Hellmich, R. L., and Rice, L. G. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Disease. 83:130-138.

Nankam, C., J.K. Pataky. 1996. Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL125b. Plant Disease. 80: 593-598.

Nelson, P.T., N.D. Coles, J.B. Holland, D.M. Bubeck, S. Smith, and M.M. Goodman. 2008. Molecular characterization of maize inbreds with expired U.S. Plant Variety Protection. Crop Science. 48:1673-1685.

Papst, C,. HF Utz, A.E. Melchinger, J. Eder, T. Magg, D. Klein, and M. Bohn. 2005. Mycotoxins produced by *Fusarium spp*. in isogenic Bt vs. non-Bt maize hybrids under European Corn Borer pressure. Agronomy Journal. 97:219-224.

Pérez-Brito, D., D. Jeffers, D. González-de-León, M. Khairallah, M. Cortés-Cruz, G. Velázquez-Cardelas, S. Azpíroz-Rivero, and G. Srinivasan. 2001. QTL mapping of *Fusarium moniliforme* ear rot resistance in highland maize, Mexico. Agrociencia. 35:181–196.

Piepho, H.-P., J. Mohring, A.E. Melchinger, and A. Buscher. 2007. BLUP for phenotypic selection in plant breeding and variety testing. Euphytica. 161:209-228

Pollak L.M., W. Salhuana. 2001. The germplasm enhancement of maize (GEM) project: Private and public sector collaboration. pp. 319-329. *In:* H.D. Cooper, C. Spillane, T. Hodgkin (Eds.), Broadening the Genetic Base of Crop Production. CABI Publ., Wallingford, Oxon, UK.

Prelusky, D., B. Rotter, and R. Rotter. 1994. Toxicology of mycotoxins. p 359-403. *In* Mycotoxins in Grain: Compounds other than Aflatoxin,. Edited by J. Miller & H. Trenholm. St. Paul: The American Phytopathological Society.

Presello, D.A., G. Botta, J. Iglesias and G.H. Eyhérabide. 2008. Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with *Fusarium verticillioides.* Crop Protection. 27:572-576.

Robertson, L.A., G.A. Payne, and J.B. Holland. 2005. Marker assisted breeding for host resistance to mycotoxin contamination. p. 423-435. *In* H.K Abbas (ed.) Aflatoxin and Food Safety. Marcel Dekker, Inc., New York, NY.

Robertson, L.A, C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, and J.B. Holland. 2006. Heritabilities and correlations of Fusarium ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Science. 46:353-361.

Robertson-Hoyt L.A., M.P. Jines, P.J. Balint-Kurti, C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, T.L. Molnár, and J.B. Holland. 2006. QTL mapping for Fusarium ear rot and fumonisin contamination resistance in two maize populations. Crop Science. 46:1734–1743.

Robertson-Hoyt, L.A, C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, and J.B. Holland. 2007. Relationships of Resistance to Fusarium Ear Rot and Fumonisin Contamination with Agronomic Performance of Maize. Crop Science. 47:1770-1778.

Ross, P.F., L.G. Rice, G.D. Osweiler, P.E. Nelson, J.L. Richard, and T.M. Wilson. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia. 117:109-114.

Schön, C.C. ., H.F. Utz, S. Groh, B. Truberg, S. Openshaw, and A.E. Melchinger. 2004. Quantitative Trait Locus Mapping Based on Resampling in a Vast Maize Testcross Experiment and Its Relevance to Quantitative Genetics for Complex Traits. Genetics. 167: 485-498.

Scott, G.E., and S.B. King. 1984. Site of action of factors for resistance to *Fusarium moniliforme* in maize. Plant Disease. 68: 804-806.

Shelby, R.A., D.G. White, and E.M. Bauske. 1994. Differential fumonisin production in maize hybrids. Plant Disease. 75:582-584.

Starr, M, L.A. Robertson-Hoyt, J.B. Holland, G.A. Payne. 2006. Improving Resistance to Fumonisin Contamination in Maize. p. 83-92. In: *Proceedings of the 42nd Annual Illinois Corn Breeders School.* Urbana, IL.

Sydenham, E.W. , G.S. Shephard, P.G. Thiel, W.F.0. Marasas, and S. Stockenstrom. 1991. Fumonisin Contamination of Commercial Corn-Based Human Foodstuffs. J. Agric. Food Chem. 39*:*2014-2018.

Szalma S.J., B.M. Hostert, J.R. LeDeaux, C.W. Stuber and J.B. Holland. 2007. QTL mapping with near-isogenic lines in maize. Theoretical and Applied Genetics. 114: 1211-1228.

van Egmond, H.P., R.C. Schothorst, M.A. Jonker. 2007. Regulations relating to mycotoxins in food; Perspectives in a global and European context. Anal Bioanal Chem. 389:147–157.

White, D. G. 1999. Compendium of Corn Diseases 3rd Edition. D. G. White ed. APS Press. St. Paul, MN.

Wu. F. 2006. Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. Transgenic Research. 15:277–289.

Yuan, J. , M. Liakat Ali , J. Taylor, J. Liu , G. Sun, W. Liu, P. Masilimany, A. Gulati-Sakhuja, K. P. Pauls. 2007. A guanylyl cyclase-like gene is associated with Gibberella ear rot resistance in maize (*Zea mays* L.).Theoretical and Applied Genetics. 116:465-479.

Zamir, D. 2001. Improving plant breeding with exotic genetic libraries. Nature Reviews Genetics 2:983-989.

Table 1.1**. Maize inbred lines from NC State University and USDA Germplasm Enhancement of Maize breeding programs with highest and lowest predicted genetic values (BLUPs) for ear rot and fumonisin accumulation in screening trials at Clayton, NC from 2003 to 2007[1].**

Fusarium ear rot (% rotted kernels)				Fumonisin content (ppm)			
Material	BLUP	Standard Error	No. years tested	Material	BLUP	Standard Error	No. years tested
				10 most resistant lines			
GEMS-0136 (Dekalb Brazilian hybrid XL380)	0.8	4.5	1	**GE440**[2] (Hastings Prolific)	**5.1**	**7.5**	**4**
NC358 (Tropical Hybrid derived line)	1.7	4.5	1	BR51675:N0620-033-001 (unreleased GEM line)	6.6	11.2	1
UR11003:S0302-937-1 (unreleased GEM line)	**3.0**	**2.9**	**3**	GEMS-0002 (Florida Synthetic)	6.7	9.3	2
NC406 (SC76^4×B52)	3.2	4.5	1	DK212T:S11-2088-01 (unreleased GEM line)	7.1	9.3	2
NC444 (NC258×NC296^2)	3.4	2.6	4	DKXL370A:N11-1883-002 (unreleased GEM line)	8.4	11.2	1
1128-1/94 (NC350sister×NC258)	3.4	4.5	1	1801-1/94 (KUI2301×NC296)	8.7	11.2	1
NC458 (KU2301×PM703)	3.7	2.4	5	BR51675:N0620-053-001 (unreleased GEM line)	9.7	11.2	1
DK888:S11-2132-03 (unreleased GEM line)	3.7	3.5	2	3170b-1/98 (NC320×NC258A)	9.9	8.1	1
GE440 (Hastings Prolific)	**3.7**	**2.7**	**4**	**UR11003:S0302-937-1** (unreleased GEM line)	**10.2**	**11.2**	**3**
DKXL370:N11a20-199-2 (unreleased GEM line)	3.7	4.5	1	6959-2.3564-2 (1478-1×NC354)×NC458 derived line)	10.5	6.7	1
				5 most susceptible lines			
3520-blk/03 (NC262A sister×NC298)[3]	*28.9*	*4.5*	*1*	*3520-blk/03 (NC262A sister×NC298)*	*59.4*	*11.2*	*1*
AR17056:N2035-421-001 (unreleased GEM line)	31.9	4.5	1	*MDI022:N2120-333-001(unreleased GEM line)*	*64.0*	*11.2*	*1*
1005-9/04 TAMU (NC300^4×CML288)	38.1	4.5	1	NC378 [(PX105A×P306B)B73] × [Fla Syn×Va35^4]	65.4	11.2	1
UR13085:N0215-021-001 (unreleased GEM line)	44.7	4.5	1	NEI9004:S2818-025-001 (unreleased GEM line)	65.6	11.2	1
MDI022:N2120-333-001(unreleased GEM line)	***50.3***	***4.5***	***1***	6513-1/95 (NC258×NC296^4)	69.3	11.2	1
				Susceptible check			
FR1064 (B73 type)	12.5	2.6	4	FR1064	46.9	7.3	4

[1] Each year, at least 50 experimental lines are grown in randomized complete block experiments with two replications at one location in North Carolina and artificially inoculated with *F. verticillioides* and *F. proliferatum*. Lines with best or worst ear rot or fumonisin contamination may be retested in additional years. Data were analyzed as a single unbalanced experiment across years with mixed models, considering year, replication, and genotype effects all to be random. BLUPs are best estimate of line values, adjusted for environmental differences across tests.

[2] Lines in bold font appear in group of lowest 10 BLUPs for both Fusarium ear rot and fumonisin content.

[3] Lines in italic font appear in group of highest five BLUPs for both Fusarium ear rot and fumonisin content.

Table 1.2. Estimates of heritability on a line mean basis for Fusarium ear rot and fumonisin content resistance, of the genotypic and phenotypic correlations between ear rot and fumonisin content, and of the predicted ratio of response of fumonisin content to indirect selection on ear rot to response to direct selection on fumonisin content in two maize populations (adapted from Robertson *et al.*, 2006).

Parameter estimate	GEFR population	NCB population
Ear rot line mean h^2	0.47	0.86
Fumonisin line mean h^2	0.75	0.88
Genotypic correlation (r_g)	0.96	0.87
Phenotypic correlation (r_p)	0.40	0.64
Indirect selection response ratio	0.76	0.86

CHAPTER 2: Grain Yield and Fusarium Ear Rot of Maize Hybrids Developed from Lines with Varying Levels of Resistance

Citation

Eller, M.S., L.A. Robertson-Hoyt, G.A. Payne, and J.B. Holland. 2008. Grain Yield and Fusarium Ear Rot of Maize Hybrids Developed from Lines with Varying Levels of Resistance. Maydica 53: 231-237.

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. occurs 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 *et al.*, 1997) and *F. verticillioides* and *F. proliferatum* can produce mycotoxins called fumonisins that contaminate maize grain. Fumonisins are suspected carcinogens (Gelderblom *et al.*, 1988; Miller *et al.*, 1994; Prelusky, 1994) and cause a number of human and animal diseases (Ross *et al.*, 1992; Colvin and Harrison, 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 = $\mu g\ g^{-1}$) 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 BC_1F_1 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_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 × (FR615 × FR697) and FR1064 × (FR615 × FR697), the tester itself (FR615 × FR697), 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 Marlboro Loamy Sand (clayey, kaolinitic, thermic Typic Paleudult) at Clayton, 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 over seeded 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 sub plots 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 from plant to plant 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 harvest. 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 140 g 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-Rogers (Kenward and Roger, 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 2.1). Inoculation treatment significantly affected yield ($p < 0.03$), but not ear rot, grain moisture, erect plants, or silking date in the combined analysis (Table 2.1). The interaction of inoculation treatment and genotype was not significant for grain yield or any other trait (Table 2.1).

The topcross of resistant parent GE440 had significantly lower ear rot than the topcross of susceptible parent FR1064 under both inoculated and noninoculated conditions (Table 2.2). Inoculation more than doubled the difference in ear rot between the two

topcrosses from 2.9% to 6.9% (Table 2.2), 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 × (FR615 × FR697) was 11.6% in this study (Table 2.2), but inbred FR1064 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 2.2). 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 2.2). 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, FR615 × FR697 (Table 2.2). 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 2.2). On average, the topcrosses of the 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 2.2). 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 was not supportive of 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 2.2), 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 2.3). 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 scores under inoculated and noninoculated conditions were not correlated (Table 2.3). 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 2.3), 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 2.3). 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 2.2), and that the differences between inoculated and noninoculated treatments measured in yield and ear rot were not significantly correlated (Table 2.3).

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 affect 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.

Acknowledgements

The authors would like to acknowledge Dr. Don White, University of Illinois, for beginning the backcross work with GE440 and FR1064, and Illinois Foundation Seeds for granting permission to use FR1064 in these studies. We also gratefully acknowledge Scott Reed, Kathleen Starr, Stella Salvo, Marco Oropeza-Rosas, Nate Coles and Andrea Dolezal for field assistance, and valuable discussion.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. AC-19: 716-23.

Bacon, C.W., D.M. Hinton. 1996. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. Can. J. Bot. 74: 1195-1202.

Brownie, C., D.T. Bowman, J.W. Burton. 1993. Estimating spatial variation in analysis of data from yield trials: a comparison of methods. Agron. J. 85: 1244-1253.

Bush, B.J., M. L. Carson, M.A. Cubeta, W.M. Hagler, G.A. Payne, 2004. Infection and fumonisin production by *Fusarium verticillioide*s in developing maize kernels. Phytopathology. 94: 88-93.

CFSAN. 2001a. Background paper in support of fumonisin levels in corn and corn products intended for human consumption. Available online at http://www.cfsan.fda.gov/~dms /fumonbg3.html (verified 5 June 2008). USFDA Center for Food Safety and Applied Nutrition and the Center for Veterinary Medicine.

CFSAN. 2001b. Guidance for Industry: Fumonisin levels in human foods and animal feeds. Available online at http://www.cfsan.fda.gov/~dms/fumongu2.html (verified 5 June 2008). USFDA Center for Food Safety and Applied Nutrition and the Center for Veterinary Medicine.

Clements, M.J., C.E. Kleinschmidt, C.M. Maragos, J.K. Pataky, D. G.White. 2003. Evaluation of inoculation techniques for Fusarium ear rot and fumonisin contamination of corn. Plant Dis. 87: 147-153.

Clements, M. J., C.M. Maragos, J.K. Pataky, D.G. White. 2004. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopath. 94: 251-260.

Colvin, B.M., L.R. Harrison. 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia 117: 79-82.

Commission of the European Communities. 2007. Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. Official J. Europ. Union. 255: 14-17.

Diener, U.L., R.J. Cole, T.H. Sanders, G.A. Payne, L.S. Lee, M.A. Klich. 1987. Epidemiology of Aflatoxin formation by *Aspergillus flavus*. An. Rev. Phytopath. 25: 249-270.

Gelderblom, W. C. A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar, N.P.J. Kriek. 1988. Fumonisins- novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microb. 54:1806-1811.

Hendricks, K. 1999. Fumonisins and neural tube defects in south Texas. Epidemiol. 10: 198-200.

Kenward, M.G., J.H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics. 53: 983–997.

Miller, J.D. 1994. Epidemiology of *Fusarium* ear diseases of cereals. p. 19-36. *In*: J.D. Miller and H.L. Trenholm (ed.) Mycotoxins in grain. Eagan Press, St. Paul, MN.

Missmer, S.A., L. Suarez, M. Felkner, E. Wang, A.H. Merrill, Jr., K.J. Rothman, and K.A. Hendricks. 2006. Exposure to Fumonisins and the Occurrence of Neural Tube Defects along the Texas–Mexico Border. Environ. Health Perspectives. 114: 237–241.

Morgavi, D.P., R.T. Riley. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. Animal Feed Science and Technology. 137: 201–212.

Munkvold, G.P., A. Desjardins. 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Dis. 81: 556-565.

Oren, L., S. Ezrati, D. Cohen, A. Sharon. 2003. Early events in the *Fusarium verticillioides*-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. Appl. Environ. Microbiol. 69: 1695-1701.

Payne, G. A., D. K. Cassell, C.R. Adkins. 1986. Reduction of aflatoxin contamination in corn due to irrigation and tillage. Phytopathology. 76: 679-684.

Pioneer Hi-Bred International, Inc. 2007. http://www.pioneer.com/web/site/portal/template.MAXIMIZE/menuitem.8bd903dc0c895618bc0c0a03d10093a0/?javax.portlet.tpst=3edaf1c3d7b1b9c956072557d10093a0_ws_MX&javax.portlet.prp_3edaf1c3d7b1b9c956072557d10093a0_viewID=productListView&beanID=1849867011&viewID=productListView&javax.portlet.begCacheTok=com.vignette.cachetoken&javax.portlet.endCacheTok=com.vignette.cachetoken&nonav=true Verified 5 June 2008.

Prelusky, D., B. Rotter, R. Rotter. 1994. Toxicology of mycotoxins. In *Mycotoxins in Grain: Compounds other than Aflatoxin*, pp. 359-403. Edited by J. Miller & H. Trenholm. St. Paul: The American Phytopathological Society.

Robertson, L.A., C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, J.B. Holland 2006. Heritabilities and correlations of Fusarium ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Science. 46: 353-361.

Robertson-Hoyt, L.A., C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, J.B. Holland. 2007. Relationships of resistance to Fusarium ear rot and fumonisin contamination with agronomic performance of maize. Crop Science. 47: 1770-1778.

Ross, P.F., L.G. Rice, G.D. Osweiler, P.E. Nelson, J.L. Richard, T.M. Wilson. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 117: 109-114.

SAS Institute Inc. 2004. *SAS/IML 9.1 User's Guide*. Cary, NC: SAS Institute Inc.

Shelby, R.A., D.G. White, E.M. Bauske. 1994. Differential fumonisin production in maize hybrids. Plant Disease. 78: 582-584.

Starr, M.R., L.A. Robertson-Hoyt, G.A. Payne, J.B. Holland. 2006. Improving resistance to fumonisin contamination in maize. pp. 83-92. In: *Proceedings of the 42nd Ann. Illinois Corn Breeders' School*. Univ. of Illinois, Urbana, IL.

Yates, I.E., D. Sparks. 2008. *Fusarium verticillioides* dissemination among maize ears of field-grown plants. Crop Protection. 27: 606–613.

Van Egmond, H.P., R.C. Schothorst, M.A. Jonker. 2007. Regulations relating to mycotoxins in food; Perspectives in a global and European context. Anal. Bioanal. Chem. 389:147–157.

Table 2.1 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 (Mg ha^{-1})[1]	Fusarium ear rot (µg g^{-1})[1]	Grain moisture (%)[1]	Erect plants (%)[1]	Silking date (DAP)[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.
DAP =Days after planting
ns not significant at p=.05.

Table 2.2. Trait means measured on topcrosses of $BC_1F_{1:2}$ lines to FR615 × FR697 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.

Table 2.3. 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

CHAPTER 3: Selection for Reduced Fusarium Ear Rot and Fumonisin Content in Advanced Backcross Lines and Their Topcross Hybrids

Prepared for submission to Crop Science

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Abstract

Backcross breeding is an important method to improve elite cultivars for traits controlled by a small number of loci but has been used less frequently to improve quantitatively controlled traits. Resistance to Fusarium ear rot and contamination by the associated mycotoxin fumonisin in maize are quantitatively inherited. We backcrossed the highly resistant but unadapted inbred GE440 for four generations to the more susceptible but agronomically elite commercial inbred FR1064 to test improved resistance to Fusarium ear rot and fumonisin contamination. A selected set of 19 $BC_4F_{1:3}$ lines had greater resistance to ear rot and fumonisin content that their recurrent parent FR1064. Compared to

an unselected set of BC_4 lines derived from the same initial BC_1 families, these selected lines had increased resistance to ear rot, but not to fumonisin contamination. Topcrosses of the selected lines had greater resistance to Fusarium ear rot and similar grain yield compared to the topcross of the recurrent parent FR1064. The selected line topcrosses were not significantly improved for resistance to fumonisin contamination compared to FR1064, nor were they significantly improved for resistance to ear rot or fumonisin contamination compared to the topcrossed random BC_4 control. We also genotyped selected lines at DNA markers linked to ear rot and fumonisin resistance quantitative trait loci (QTL) identified in the BC_1 generation of this cross to determine which QTL demonstrated allele frequency shifts due to selection. Markers for QTL on the long arm of chromosome four, and the short arm of chromosome four inherited the GE440 allele significantly more often than expected by random chance.

Introduction

The backcross breeding method has been used by breeders for many years to improve an elite genetic background which is deficient for a particular trait (Allard 1960). It is particularly efficient breeding method when an advantageous trait under simple genetic control has been discovered in exotic or unadapted material because it has the advantage of limiting the introduction of deleterious alleles from the unadapted material while retaining the beneficial allele(s) for the trait of interest. It has been used less often for improving

quantitatively inherited traits because, by intention, it limits the introgression of donor parent alleles to a small proportion of the genome (Fehr, 1987). Fehr (1987) suggested that backcrossing for quantitatively inherited characteristics is most successful when the environment has limited impact on trait expression.

Bliss (1981), however, demonstrated the inbred backcross line (IBL) method, a modified backcrossing method for quantitatively inherited traits. Bliss (1981) conducted at least two generations of backcrossing and selfed before imposing selection on advanced lines. With this method improvement for quantitative traits without loss of recurrent parent phenotype was demonstrated in common bean (Sullivan and Bliss, 1983a, 1983b) and cucumber (Owen *et al*. 1985a, 1985b) using an unadapted or exotic donor parent.

Tanksley and Nelson (1996) proposed a similar approach called advance backcross QTL (AB-QTL) analysis in which materials are advanced to the BC_2 or BC_3 generations with only minimal culling before mapping QTL and implementing marker-assisted selection. Both Sullivan and Bliss (1983b) and Tanksley and Nelson (1996) suggested that backcross breeding is very effective with unadapted donor varieties because of the rapid reduction of the donor parent germplasm, and the segregation of the remaining donor alleles among different inbred backcross lines.

An important quantitatively inherited trait of maize is resistance to Fusarium ear rot, caused by *Fusarium verticillioides* (Sacc.) Nirenberg (teleomorph *Gibberella moniliformis*). *Fusarium verticillioides* infects maize in many parts of the world (van Egmond *et al.*, 2007), but is most severe in warm, dry climates like those found in the southeastern United States

and lowland tropics. In addition to damage from rotten kernels, *F. verticillioides* and related species *F. proliferatum* produce the mycotoxin fumonisin, a suspected carcinogen (Gelderblom *et al.*, 1988; Prelusky *et al.*, 1994; Miller, 1994, Ehrlich *et al.*, 2002), which contaminates the grain. Fumonisin contamination causes a number of animal diseases, (Colvin and Harrison, 1992; Ross *et al.*, 1992; Morgavi and Riley, 2007) human diseases, and birth defects (Hendricks, 1999; Missmer *et al.*, 2006). Treatment of grain for food and feed consumption to remove fumonisins is not commercially viable and cultural practices which reduce fumonisin levels in grain are often impractical or logistically difficult to implement in areas where fumonisins are most problematic.

Ear rot and fumonisin contamination are distinct aspects of the disease, with fairly low phenotypic correlations, but high positive genetic correlations in both partially and highly inbred lines (Robertson *et al.*, 2006). Resistance to both Fusarium ear rot and fumonisin accumulation is quantitative in nature, and no sources of complete resistance have been identified for either trait (Munkvold and Desjardins, 1997).

Whereas ear rot can be scored fairly rapidly with a visual rating scale, measuring fumonisin content requires High Performance Liquid Chromatography (HPLC) or Enzyme Linked Immunosorbent Assay (ELISA). Sample preparation is expensive, time consuming and labor intensive for both of these analyses. Robertson *et al.* (2006) reported moderate to high entry mean heritabilities for both fumonisin contamination (0.75) and Fusarium ear rot (0.47) in the GEFR (GE440 × FR1064) population, suggesting that phenotypic selection against ear rot should be an effective way to improve resistance to both ear rot and

fumonisin contamination in backcross-derived lines. Although fumonisin concentration is more highly heritable than resistance to Fusarium ear rot (Robertson *et al.*, 2006) and response in fumonisin levels to indirect selection is predicted to be less effective than direct selection, indirect selection on the basis ear rot is much less expensive and time consuming. Therefore, selection against ear rot susceptibility is a potentially more economically efficient method of producing maize lines with decreased fumonisin content.

The goal of this research was to test if backcross breeding using unadapted germplasm as a donor parent is an effective strategy for improving quantitative disease resistance in maize. The donor parent used was GE440; an older public inbred line with poor yield potential and limited range of adaptation, but with high levels of resistance to Fusarium ear rot and fumonisin contamination (Clements *et al.*, 2004; Robertson *et al.*, 2006, Robertson-Hoyt *et al*., 2006). The recurrent parent was a commercially important but more susceptible proprietary line, FR1064. Specific objectives of this study were to test the following hypotheses: (1) that backcross breeding will prove to be an effective way to introgress quantitatively inherited traits from unadapted donor material into elite germplasm, (2) that selection for reduced ear rot will result in lines with greater resistance to both ear rot and fumonisin accumulation, (3) that selection for improved ear rot resistance in inbred generations will result in favorable indirect responses in topcross generations selected lines, and (4) that donor parent alleles at markers linked to previously identified Fusarium ear rot and fumonisin contamination resistance QTL will be recovered more often than expected by chance in selected lines.

Material and Methods

Population Development

Inbred GE440 (derived from the open-pollinated variety Hasting's Prolific) is relatively tall, highly susceptible to lodging, and produces small white-seeded ears. It was identified by Clements *et al.* (2004) as conferring a relatively high level of resistance to Fusarium ear rot and fumonisin contamination in topcross to the susceptible commercial inbred line FR1064 (an improved B73 type). Dr. Don White (University of Illinois) developed a set of families derived from the first backcross (BC_1) generation of GE440 to recurrent parent FR1064, followed by one generation of self-fertilization ($BC_1F_{1:2}$ lines. Robertson *et al.* (2006) evaluated these families under artificial inoculation with *Fusarium verticillioides* and *Fusarium proliferatum* in replicated trials in four environments. The 10 $BC_1F_{1:2}$ families with lowest mean fumonisin contents identified in these trials were selected for additional backcrossing to FR1064. A modified single seed backcrossing method was used to advance the 10 BC_1F_1 lines with seed that exhibited lowest mean fumonisin content (Robertson, 2006) to the BC_4F_1 generation (Figure 1). In this method 10 backcrossed ears were harvested from each $BC_1F_{2:3}$ family and two seeds were chosen from each ear to create a balanced BC_2F_1 bulk of 20 seeds per family. Each of these bulks was planted in a single row, and backcrossed to FR1064 the following season; 10 backcrossed ears were harvested from each row (BC_2F_1 family) and the process repeated. A balanced remnant bulk was also

created each generation. No intentional selection was applied during these backcrossing steps. Each original BC_1 family was represented in the BC_4 generation.

Field Evaluation of BC_4F_1 Families

Selection against susceptibility to Fusarium ear rot was conducted among the 455 BC_4F_1 families generated by the backcrossing program. The 455 BC_4F_1 families were evaluated at two locations (the Central Crops Research Station in Clayton, NC and the Peanut Belt Research Station in Lewiston-Woodville, NC) in summer, 2006. In addition to the 455 BC_4F_1s, the recurrent parent, FR1064, was added five times, to bring the total number of entries to 460. The experimental design was a 20 x 23 α-lattice with two replications, with a single replication planted in each location. The α-lattice was then augmented by adding FR1064 to each block in a random position to bring the total number of experimental plots to 483. At both locations plots were 3.05 m in length, with 0.965 m between plots in Clayton, and 0.914 m between plots at Lewiston-Woodville. Plots were overplanted and thinned to a uniform density of 23 plants per row, resulting in population densities of 65,100 plants hectare^{-1} at Clayton and 68,750 plants hectare^{-1}at Lewiston.

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, loosening conidia by brushing with a paint brush, straining the suspension through cheese cloth, and diluting the conidia suspension of the six isolates to approximately 2×10^6 mL^{-1} in water. Two inoculations were conducted seven days apart to reduce escapes and simulate common methods of natural infection. A silk channel inoculation ten to fourteen days post mid-silk was followed by a direct ear inoculation seven days later. The primary ear of the first 10 plants in each row at Lewiston-Woodville, and the first 15 plants in each row at Clayton was injected with 5 mL of 2×10^6 conidial suspension using a 5 mL Allflex draw-off injection syringe (Allflex Inc, Dallas, TX) fitted with a 16 gauge needle that had the point filed down. One drop of undiluted Tween-20 was added to each liter of inoculum suspension to break the surface tension of the suspension.

Ear rot was scored as percent of kernels exhibiting kernel rot or infection symptoms per ear; 10 ears were scored per row, and the 20 families with lowest mean ear rot scores were selected for advancement.

Advancement to $BC_4F_{1:3}$ Generation

Four self-pollinated ears (each representing a $BC_4F_{1:2}$ line) were harvested from each of the 20 selected families, except one family which had only three selfed ears available for advancement. Each line was advanced in bulk to form 79 $BC_4F_{1:3}$ lines, which were evaluated as lines *per se* and as topcross hybrids using a common single-cross tester FR615 × FR697. At the time selections were made, one kernel was also collected from each of four self-pollinated ears from all 455 BC_4F_1 families to form a control BC_4F_2 unselected

bulk. A random sample of 360 seeds of this bulk was advanced to the BC_4F_3 generation and bulked to serve as a control for selection evaluation experiments.

Backcross Derived Line Evaluation

To determine if selection for Fusarium ear rot resistance in advanced backcross lines resulted in lines with greater ear rot resistance and reduced fumonisin accumulation, selected $BC_4F_{1:3}$ lines and the unselected control $BC_4F_{1:3}$ bulk population were evaluated under artificial inoculation for ear rot and fumonisin content in 2007 and 2008. Seventy-nine $BC_4F_{1:3}$ lines, the population parents (GE440 and FR1064), the two inbred lines used to make the single cross tester used in hybrid evaluations (FR615 and FR697), and a random BC_4F_3 control bulk of the population were evaluated in two environments (Central Crops Research Station and Peanut Belt Research Station) in the summer of 2007. The random BC_4F_3 control population bulk was included as an entry seven times per replication to make a total of 90 entries per complete replication. Two replications were evaluated in each location with entries arranged in a 10×9 α-lattice.

The trial was modified in 2008 to contain only the 20 best $BC_4F_{1:3}$ lines based on mean ear rot from the 2007 trial, five $BC_4F_{3:4}$ sub lines from each of the top four $BC_4F_{1:3}$s from the previous year, the population parents, and the random BC_4F_3 control repeated seven times in each replication. Entries were tested in two replications at the same two locations in a 7×7 α-lattice.

The line GEFR402-3-7-5-1 was included in the field trial, but dropped from the analysis in 2007 and 2008, and its sub lines were dropped in 2008 because it has a red cob, and therefore is likely contaminated. This reduced the number of selected $BC_4F_{1:3}$ lines to 19, and number of $BC_4F_{3:4}$ sub lines to 16.

Topcross Hybrid Evaluation

$BC_4F_{1:3}$ lines were topcrossed to the commercial non-Stiff Stalk single cross F_1 tester (FR615 × FR697), and topcross yield trials were performed to assess hybrid performance of backcross derived lines. Evaluations were conducted at each of four North Carolina locations in 2007 and 2008; 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 near Jackson Springs.

In 2007 the 90 entries included topcrosses of the 79 $BC_4F_{1:3}$ families and the population parents, GE440 and FR1064, to (FR615 × FR697).. The topcross tester (FR615 × FR697) itself was included as a check. A random bulk of BC_4F_3 seed, advanced in each generation without evaluation for ear rot, and topcrossed to (FR615 × FR697), was included six times in each replication as a repeated control. Two commercial hybrids, Pioneer brand 31G66 and 31G98, were also 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).

In 2008, entries were limited to hybrids of the 20 best lines based on mean ear rot scores from the previous year, the topcrossed parents, and the check entries (Pioneer 31G66, Pioneer 31G98, (FR615 × FR697) and five entries composed of the topcrossed GEFR Random BC_4F_3 bulk). The topcrossed random BC_4F_3 bulk was later dropped from analysis in 2008, because the wrong tester was used when increasing the seed. The topcross of GEFR402-3-7-5-1 was dropped from the analysis in 2007 and 2008 because the line was contaminated.

The experimental design to evaluate yield and ear rot under inoculated and noninoculated conditions was a split-plot design with two replications within each environment. The whole plot factor was genotype and the sub plot factor was inoculation treatment. Each whole plot consisted of six 3.66-m rows of a common genotype planted 0.94 or 0.97 m apart. Whole plots were randomized across replications as a 5 × 6 lattice design. Plots were over seeded and thinned to target population densities in the two rows of each sub plot for mechanical harvest (62,288 plants ha^{-1} in Clayton, Plymouth, and Jackson Springs, NC or 65,750 plants ha^{-1} in Lewiston, NC). Sub plots consisted of three rows which were assigned an inoculation treatment: inoculation with *F. verticillioides* or noninoculated. Ten ears from the outer rows of each whole plot (one from each sub plot) were hand harvested, dried, and scored for percent ear rot. The inner four rows (two rows of each sub plot) were mechanically harvested to measure grain yield and grain moisture.

Border rows were not needed to separate inoculated and noninoculated plots, because *F. verticillioides* spreads very little from plant to plant during the growing season (Yates and Sparks, 2008).

Inoculation Technique - Hybrids

The inoculation protocol used for hybrid evaluation trials was the same as that used when conducting selection with the following exceptions. Six isolates of *F. verticillioides* (NC-i6, NC-i7, NC-i9, NC-n16, NC-n17, and NC-n22) originally collected from maize in North Carolina and deposited with the Fusarium Research Center collection (http://frc.cas.psu.edu/) were selected for use based on their superior prolificacy and higher fumonisin production levels in culture. Only plants in the inoculation treatment whole plots were inoculated. The first 12 primary ears in the rows designated for hand harvest were inoculated, while all primary ears were inoculated in the rows of inoculated topcross sub plots designated for mechanical harvest.

Phenotypic Data Collection - Backcross Derived Lines

Stand count was recorded four to six weeks after planting, and plots were not thinned. Silk and anthesis 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. Plant and ear heights were recorded after grain fill to the nearest 5 cm. Plant height was measured to

the height of the flag leaf, and ear height was measured at the node from which the ear emerged.

Primary ears of the first 12 plants per row that were inoculated were hand-harvested and air-dried to approximately 140 g 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. In 2008, ears with less than 5% ear rot were given scores of 0%, 1% or 3%. After rating, ears were shelled and a sub-sample of shelled grain was ground on a Romer II Series Mill (Romer Labs, Union, MO). A sample of 20 g of ground grain was weighed out and tested for fumonisin content (μg g^{-1}) using Diagnostix fumonisin ELISA assay kits (Diagnostix, Mississanga, ON, Canada).

Phenotypic Data Collection –Topcross Hybrids

Phenotypic data were collected on the hybrids using the methods described for the backcross derived lines, with the following exceptions. Stand counts were taken as described above following thinning to target planting densities. The row designated for hand harvest in each sub plot was not thinned. When all plants reached physiological maturity, two rows of each sub plot (representing the center four rows of each whole-plot) were mechanically harvested to collect grain moisture and yield data. Lodging data were collected just before harvest on the mechanically harvested rows; the number of plants in each plot exhibiting root lodging (leaning more than 30 percent from vertical) or stalk lodging (with a stalk broken below primary ear or with a dropped primary ear) were

recorded separately and later summed and reported as percent erect plants, or percent of the stand count that did not exhibit lodging.

Ten primary ears were hand-harvested from the outside row of each whole plot, air dried, and rated for ear rot. Hand-harvested ears from each inoculated sub-plot were shelled after rating and a sub-sample of shelled grain was ground and assayed for fumonisin content ($\mu g\ g^{-1}$) while ears from noninoculated sub plots were discarded after rating.

Statistical Methods - Backcross Derived Lines

PROC MIXED in SAS Version 9.1.3 (SAS Institute, Cary, NC) was used to analyze percent ear rot, fumonisin content, flowering time, plant height and ear height data on the backcross derived lines. A standard model with fixed genotypes and random location, replication and incomplete block effects was used on the full dataset for each trait. This model was compared to a model with heterogeneous error variances within each environment, and a heterogeneous errors model weighted by number of ears rated (when appropriate). The best model was selected based on improved Akaike's Information Criterion (AIC; Akaike, 1974). A heterogeneous errors model, weighted by the number of ears scored was the best model for ear rot. A heterogeneous errors model, with no weighting was the best model for fumonisin. The standard model was used for plant and ear height and flowering time. Pearson's correlation coefficients among genotype means across all locations for different traits were estimated using PROC CORR.

The ESTIMATE statement in PROC MIXED was used to test the null hypotheses of no significant differences between the parental lines, between the 78 $BC_4F_{1:3}$ lines advanced from the selected BC_4F_1s and each parent, and between the 78 $BC_4F_{1:3}$ lines and the control random BC_4F_3 bulk. The ESTIMATE statement was then used to test the null hypotheses of no significant differences between the parental lines, between the 19 $BC_4F_{1:3}$ lines selected after the 2007 trial and each parent, and between the 19 selected lines and the control random BC_4F_3 bulk.

Statistical Methods - Comparison of $BC_4F_{1:3}$*s to* $BC_4F_{1:4}$ *Sub lines*

Data from the 2008 season, in which four sub lines of the four $BC_4F_{1:3}$s with the lowest ear rot were grown, were used to test for variation within and among the BC_4F_1lineages. Checks were removed from the dataset and analysis of variance was conducted using the MIXED procedure in SAS. Two models were analyzed. The first model excluded the sub-lines and analyzed the 19 tested $BC_4F_{1:3}$ lines as fixed effects, and location, replication within location, incomplete block, and location × mother line as random. This model was used to test the null hypothesis of no variation among the 19 $BC_4F_{1:3}$ lines. The second model excluded the $BC_4F_{1:3}$ line data and included only the five BC4F3:4 sub lines randomly chosen from the four most ear rot resistant BC4F1:3 mother lines. This model treated mother-line lineage and sub-lines within mother lines as fixed, and location, replication within location, incomplete block, location × mother line and location × sub line within mother line as random. This second model was used to test the null

hypotheses of no variation among the four selected mother line lineages and of no variation among sub-lines within mother-line lineages.

Statistical Methods – Topcross Hybrids

A mixed model analysis of variance was conducted on the topcross hybrid dataset combined across the two years of testing using ASReml Version 2.0 software (Gilmour *et al.*, 2002). Genotype, treatment, and the genotype × treatment interaction were considered fixed effects, and environment, environment-by-treatment, environment-by-genotype interaction, environment-by-treatment-by-genotype interaction, replication, and incomplete block effects were random. A model with heterogeneous error variances among environments was compared to a model with homogeneous error variance, and the model with the most significant F-test for genotype main effect was chosen for each trait. Models including heterogeneous error variances were selected for analyses of grain yield, grain moisture, and fumonisin. A model with homogeneous error variance was used for percent ear rot, plant height, and ear height. Contrasts were used to test the null hypotheses of no significant differences between the topcrossed parents, between the BC_4F_3 random control and topcrosses of the 78 $BC_4F_{1:3}$ lines, the FR1064 topcross and topcrosses of the 78 $BC_4F_{1:3}$ lines, the BC_4F_3 random control and the 19 selected $BC_4F_{1:3}$ lines, and between the FR1064 topcross and the 19 selected $BC_4F_{1:3}$ line topcrosses.

A correlation between the magnitudes of residual and predicted values was observed in the original ear rot data. Square root and natural log transformations were compared and

the square root transformation was used because it most effectively eliminated the dependency of residual values on predicted values.

Inheritance of QTL Regions

To test the null hypothesis that selection for ear rot had no effect on the allele frequency of markers flanking QTL for ear rot and fumonisin content, DNA from the 79 selected BC_4F_1s was analyzed at DNA markers flanking the Fusarium ear rot and fumonisin contamination resistance QTL regions mapped by Robertson-Hoyt *et al.* (2006) in the $BC_1F_{1:2}$ generation of this same population. Leaf or root tissue was collected and bulked from eight plants in each of the seventy nine selected BC_4F_1s, and the GEFR population parents (GE440 and FR1064). DNA was extracted from ground tissue using Invitrogen Charge Switch ® (invitrogen.com) or an alcohol/salt DNA precipitation method adapted from Mogg and Bond (2003). The thirty three Simple Sequence Repeat (SSR) markers identified by Robertson-Hoyt *et al.* (2006) as flanking ear rot resistance and/or fumonisin accumulation resistance QTL (Table 3.1) were assayed using the Polymerase Chain Reaction (PCR) method (Senior, 1998). PCR amplification products were separated by electrophoresis on 4% (v/w) SFR agarose gels (Amresco, Solon, OH). Gels were stained with 0.05% (v/w) ethidium bromide and exposed to ultra-violet light to fluoresce DNA.

Null hypotheses of random inheritance of alleles were tested separately for each marker locus linked to a previously identified QTL. For each marker, the test was conducted only on those lines descended from a heterozygous BC_1 parent. According to the null

hypothesis of no selection for QTL alleles conditional on a heterozygous BC_1 parent, GE440 alleles should be present in 50% of the BC_2 progeny, 25% of the BC_3 progeny and 12.5% of the BC_4 generation. This null hypothesis was tested for each marker with the observed data from BC_4-derived lines at each marker with a Chi - squared test. Expected values were dependant on the number of progeny descended from the parent groups segregating for each marker.

Results and Discussion

Evaluation of Backcross Derived Lines

Analysis of variance of the inbred line *per se* evaluations revealed significant genotype main effects for ear rot ($p = 0.001$), fumonisin content ($p = 0.002$) plant height ($p = 0.0001$), ear height ($p = 0.0001$) and flowering time ($p = 0.005$ for anthesis date and $p = 0.01$ for silk date; Table 3.2). The recurrent parent FR1064 exhibited 25 percentage points greater ear rot and accumulated 33 μg g^{-1} more fumonisin than the donor parent GE440. FR1064 was also 0.65 m shorter than GE440, with ear placement 0.5 m lower, and flowered approximately 7 days earlier than GE440 (Table 3.2).

On average, the 78 $BC_4F_{1:3}$ lines had 12 percentage points greater ear rot than GE440 ($p = 0.0008$), but 13 percentage points less ear rot than FR1064 ($p = 0.0005$; Table 3.2), and otherwise were not significantly different from FR1064. The mean of the 78 $BC_4F_{1:3}$ lines was not significantly different than either parent for fumonisin content.

The 78 $BC_4F_{1:3}$ lines had on average 10 percentage points less ear rot than the unselected BC_4F_3 control ($p = 0.0004$), but exhibited no significant differences from the control for fumonisin content, plant height, ear height or flowering time (Table 3.2). After one generation of truncation selection, lines derived from the selected families showed improvement over the recurrent parent for ear rot, the trait under selection, but not for fumonisin content, the trait under indirect selection. For other traits measured (plant height, ear height, and flowering time) the selected lines were comparable to the recurrent parent FR1064 (Table 3.2).

In the 2006 selection experiment, the 455 BC_4F_1 families ranged from 10.0% ear rot to 37.2%, with and overall population mean of 17.2% ear rot. The 20 selected BC_4F_1 families exhibited 12.0% rot (SE=2.6), whereas the recurrent parent FR1064 exhibited 19.2 % rotten kernels. The selection differential was 5.2% for ear rot, and selection intensity was 0.04. Heritability on an entry mean basis for ear rot was relatively low (0.31) in the 2006 selection experiment, due to relatively low mean levels of ear rot observed and limited replication.

The 19 selected lines chosen after the second round of truncation selection based on the 2007 season data had 8 percentage points greater ear rot than GE440, but 17 percentage points less ear rot than FR1064 ($p < 0.05$; Table 3.2). It is not surprising that the backcross lines did not exhibit the high level of ear rot resistance displayed by GE440 as the $BC_4F_{1:3}$ lines are still segregating at any disease resistance loci they may have inherited from GE440. In addition, since ear rot resistance is quantitatively inherited, it would be unlikely

to recover resistance alleles at all QTL that confer ear rot resistance in GE440. The selected lines accumulated an average of 22 μg g^{-1} less fumonisin than FR1064, and there was no significant difference between fumonisin content in GE440 and the 19 selected lines. These selected lines did not differ significantly from FR1064 in plant height, ear height, and flowering times (Table 3.2). Thus, the backcrossing program achieved much of its primary practical objective to obtain lines with improved resistance to Fusarium ear rot and fumonisin accumulation while recovering the FR1064 phenotype for other agronomic traits.

The 19 selected $BC_4F_{1:3}$ lines were compared to the random BC_4F_3 control bulk for each trait of interest to test the null hypothesis that selection among BC_4F_1 families had no effect. Selected lines exhibited less ear rot than the control (14.34%, $p \geq 0.0001$), and also accumulated lower levels of fumonisin (14.02, $p = 0.056$; Table 3.2). There were no significant differences between the selected lines and the BC_4F_3 controls for plant and ear height, or silk and tassel date. Percent ear rot and fumonisin μg g^{-1} were phenotypically correlated in this population ($r = 0.43$, $p = 0.001$) in close agreement with results reported by Robertson *et al.* (2006). These results demonstrate that indirect selection for resistance to fumonisin accumulation via selection for percent ear rot was effective.

Comparison of $BC_4F_{1:3}$ Lines and $BC_4F_{3:4}$ Sub lines

Analysis of variance based on only the 19 selected lines in the $BC_4F_{1:3}$ generation grown in 2008 showed significant variation among lines for fumonisin ($p = 0.05$), plant height ($p = 0.002$), ear height ($p = 0.0002$), mid silk date ($p = 0.02$), and anthesis ($p =$

0.005), but not for percent ear rot (Table 3.3). When ANOVA was used to partition variation among and within the four selected $BC_4F_{1:3}$ mother lines that were represented in the 2008 evaluation by four $BC_4F_{3:4}$ sub lines each, significant main effects were revealed among mother lines for plant height ($p = 0.01$), and ear height ($p = 0.01$), but not for percent ear rot, fumonisin content, or either measure of flowering time (Table 3.3). Within-mother line variation was significant for percent ear rot ($p = 0.03$) only (Table 3.3). As expected, less variation was observed among the 19 $BC_4F_{1:3}$ lines for traits under selection (ear rot and fumonisin), than for traits that experienced limited selection pressure. Less variation was observed among the best four lines than among the best 19, as expected due to the smaller sample size, and as a result of undergoing truncating selection for ear rot based on mean values from the 2007 evaluation environments. The variation observed among sub-lines within mother lines for ear rot suggests that additional progress could be made from selection within lines, which should be segregating at disease QTL at which they inherited alleles from GE440.

Topcross Hybrid Evaluation

Analysis of variance for the topcross hybrid trial conducted at four environments revealed significant main effects for genotypes for grain yield ($p < 0.001$), ear rot ($p < 0.001$), grain moisture ($p < 0.001$), fumonisin accumulation ($p = 0.015$), erect plants ($p = 0.022$) and plant height ($p < 0.001$). No significant genotype main effects were observed for ear height, days to anthesis, or days to mid silk. Significant inoculation treatment effects

for genotypes were found for grain yield (p = 0.01), and ear rot (p = 0.003; Table 3.4). Inoculation treatment had no effect on grain moisture, erect plants, plant height, ear height, or flowering time. No significant genotype × inoculation treatment interactions were revealed for any of the traits observed (Table 3.4).

FR1064 topcrosses exhibited significantly greater grain yield (0.54 Mg ha^{-1}, p = 0.04) and significantly lower grain moisture (-0.24 g kg^{-1}, p = 0.002) than the GE440 topcross on average across treatments. Under inoculation, the FR1064 topcross had lower plant height (0.26 m, p = 0.001) and greater fumonisin content (24.34 μg g^{-1}, p = 0.001; Table 3.5) than the GE440 topcross (Table 3.5). The parental topcrosses did not differ for ear height, erect plants, or flowering time. Within treatments, grain yield did not differ significantly between the parental topcrosses, but the inoculated FR1064 topcrosses exhibited 10.5 percentage points greater rot (p = 0.02) than the inoculated GE440 topcross, while under non-inoculated conditions the difference was smaller but still significant (3.02 percentage points, p = 0.04; Table 3.5).

The mean value of topcrosses of the 78 $BC_4F_{1:3}$ derived from selected BC_4F_1s exhibited no significant differences from the topcross of the random BC_4F_3control. The 78 $BC_4F_{1:3}$ topcrossed lines exhibited significantly less ear rot than topcrosses of the FR1064 topcross under inoculated conditions (4 percentage points, p = 0.048; Table 3.5). For all other traits, no significant differences were identified between topcrossed FR1064 and topcrosses of the $BC_4F_{1:3}$s.

Topcrosses of the 19 selected lines were 0.14 m shorter on average, and had 0.072 g kg^{-1} lower grain moisture, than the random BC_4F_3 control bulk ($p = 0.001$) but did not differ significantly from the random BC_4F_3 controls for grain yield, percent ear rot, fumonisin content, ear height, erect plants or flowering time (Table 3.5). Thus, two generations of selection among inbred lines resulted in significant improvement in ear rot resistance and nearly significant improvement in fumonisin contamination resistance observed in inbred lines *per se*, but not in topcrosses of those lines. Non-significant trends of improved resistance for ear rot and fumonisin content were observed in topcrosses, but these results suggest that evaluation and selection among topcrosses will be necessary to make significant gains from selection in topcross performance for Fusarium ear rot resistance and fumonisin contamination.

The 19 selected line topcrosses averaged less ear rot than the FR1064 topcross under inoculated conditions (4.12 percentage points, $p = 0.008$) and when averaged across inoculation treatments ($p = 0.04$), but not under noninoculated conditions. The 19 selected lines had slightly lower plant height (-0.03 m, $p = 0.05$; Table 3.5), but did not differ from FR1064 for grain yield, fumonisin content, or ear height (Table 3.5).

Although differences among topcrosses for fumonisin content were not significant, several lines were identified which performed well as both inbreds and hybrids were identified (Table 3.6). Additional evaluations of these lines as topcrosses to multiple testers to evaluate their combining ability for yield and disease resistance is warranted.

Inheritance of QTL Regions

Thirty-three SSR markers which flank QTL for ear rot and fumonisin content were identified in the BC_1 generation of this population (Robertson-Hoyt *et al.*, 2006) and were genotyped in the 78 selected $BC_4F_{1:3}$ lines studied here. The 78 lines selected in the BC_4F_1 generation are offspring of nine of the original 10 selected BC_1 lineages. One marker (phi001) was not reproducible in the BC_4 generation, and three markers were fixed for the recurrent parent allele across all lineages (bnlg1811, umc1134, and umc1594; Table 3.7). Across the 78 $BC_4F_{1:3}$ lines, marker loci at which the GE440 amplicon was most frequently inherited were umc2280, bnlg2244, and dupssr6 with 27, 26, and 24 $BC_4F_{1:3}$ lines carrying the GE440 allele at these loci, respectively (Table 3.7).

The null hypothesis of random inheritance of alleles was rejected at nine markers at $p < 0.001$ (Table 3.7). Robertson-Hoyt *et al.* (2006) identified 7 SSR markers associated with QTL for both rot and fumonisin; at five of these GE440 alleles were recovered at significantly higher frequency than dictated by random chance ($p < 0.001$). These markers are located in bins 2.08, 4.03, 4.09 and 5.05 and the GE440 allele at each of these QTL was estimated by Robertson-Hoyt *et al.* (2006) to reduce ear rot by 3.2, 2.6, 3.1 and 3.1 percentage points respectively and fumonisin content by 4.0, 2.6, 3.3 and 5.8 $\mu g\ g^{-1}$, respectively,. Four SSR markers associated only with fumonisin variation (Robertson-Hoyt *et al.*, 2006) were also inherited at significantly higher frequency than dictated by random chance ($p < 0.001$). Three of these markers are closely linked to the ear rot QTL in bins 4.03, and 4.09). Dupssr06 ($p < 0.001$) is located near a fumonisin content QTL in bin 9.02

(where the GE440 allele estimated to reduce fumonisin content by 1.83 µg g^{-1} by Robertson-Hoyt).

The ear rot QTL with the largest effect detected previously by Robertson-Hoyt *et al.* (2006) (located in bin1.02 with GE440 allele effect of reducing ear rot by 6.08%) is flanked by phi001, the marker we could not reproduce, and bnlg1953, which deviated significantly ($p = 0.01$) from random inheritance. The largest-effect fumonisin QTL was also in this region (bin1.03, at which the GE440 allele reduced fumonisin content by 5.27 µg g^{-1}), flanked by phi001 and bnlg1811, both of which also deviated from the expectation of random inheritance ($p = 0.05$).

In the subset of 19 selected $BC_4F_{1:3}$ lines, eleven markers linked to ear rot or fumonisin contamination QTL exhibited significant deviations from random inheritance ($p < 0.05$; Table 3.7). GE440 alleles at these loci are present at frequencies higher than expected due to random chance in the group of 78 $BC_4F_{1:3}$ lines as well (Table 3.7). Fewer marker loci were detected with significant deviations in the subset of 19 selected lines compared to larger set of 78 lines because of lower power for detection due to smaller sample size.

The selected 19 lines varied widely for the number of GE440 alleles carried at the surveyed SSR markers. The $BC_4F_{1:3}$ line GEFR399-2-10-4-1 contained GE440 alleles at 11 markers, while GEFR400-9-9-3-3 contained GE440 alleles at only two. The markers at which GE440 alleles occurred in the most lines were dupssr34 and umc2280, at which 10 and nine lines inherited the GE440 allele, respectively. These two loci also carried the

highest frequencies of GE440 alleles across the 79 selected $BC_4F_{1:3}$ lines. The subset of 19 selected lines inherited the recurrent parent allele at a higher frequency than all 80 lines. In addition to bnlg1811, umc1134, and umc1594, the recurrent parent allele has been fixed at markers umc1193 and umc1355.

The three markers which were segregating in the BC_1 generation but were fixed for the recurrent parent in $BC_4F_{1:3}$s (bnlg1811, umc1134, and umc1594) are located in bins 1.03, 7.02, and 3.09 respectively. All three markers were flanking fumonisin QTL. The additional two markers which were fixed for the recurrent parent in the 19 selected lines (umc1193 and umc1355) are located in bins 7.02 and 5.03, and were also flanking fumonisin QTL. This may suggest that indirect selection for lower fumonisin content is not strong enough to maintain these alleles in the population. Another possibility is that, the resistant QTL alleles may still be maintained in the population but cannot be tracked because of recombination between the flanking markers and QTL at these loci in previous generations.

It is possible that lines inheriting GE440 alleles at more loci would have reduced agronomic performance than lines with fewer donor alleles. Our results do not indicate such as relationship, however. For example, GEFR399-2-10-4-1, which carried GE440 alleles at 11 loci performed well in both inbred and hybrid studies (Table 3.6) for ear rot resistance and had low levels of fumonisin in inbred trials. It was not significantly different than the FR1064 topcross for grain yield, flowering time, plant height or ear height.

Conclusions

Is backcrossing a good strategy for improving elite lines for quantitative traits in general? Results reported here demonstrate the effectiveness of a backcrossing program for improving quantitatively inherited disease resistance traits, which are strongly influenced by the environment. Through backcross breeding, we were able to improve an important commercial inbred line, FR1064, for ear rot and fumonisin contamination resistance without significantly lowering its yield potential, even with the use of a donor line with poor agronomic potential.

Following one generation of selection on advanced backcross derived lines, gains were observed for the primary trait of interest in advanced inbred generations. Following two generations of selection, we improved *per se* performance for ear rot resistance and reduced fumonisin accumulation in the 19 selected lines without significantly affecting important agronomic characteristics such as plant height, ear height, or flowering time compared to the recurrent parent, FR1064. The 19 selected lines were also significantly more resistant to ear rot under inoculated conditions than the FR1064 topcross without exhibiting significant reductions in topcross grain yield or other agronomic traits. Several individual lines that were not statistically different that GE440 for ear rot or fumonisin content as inbreds, and that were not statistically different for ear rot from the GE440 topcross were recovered (Table 3.6). These lines exhibited topcross yield comparable to the FR1064 topcross, although they were not competitive with commercial check yields. Thus,

from a practical standpoint, the backcrossing method was effective at improving quantitative disease resistance in an elite commercial line using an unadapted donor parent.

Gains from selection were clearer following two combined generations of inbred line selection than the first generation of selection in the BC_4F_1 generation alone. These results underscore the need to evaluate quantitative traits across multiple environments. Selection in the first generation was less effective at reducing fumonisin levels because the selection environment was not representative of a target environment conducive to the development of Fusarium ear rot. The low mean level of Fusarium ear rot permitted only limited expression of variation for disease resistance. The use of a single replication at only two environments, necessitated by the large population size and limited seed quantities available, was not sufficient to provide highly heritable mean disease resistance scores.

Was selection against susceptibility to Fusarium ear rot effective at indirectly reducing susceptibility to fumonisin contamination? Results on this point were mixed. Two generations of selection for ear rot resistance combined were effective at indirectly reducing fumonisin levels in advanced inbred generations compared to unselected controls at $p = 0.056$. Further, the selected lines were significantly more resistant to fumonisin contamination than the recurrent parent.

Did selection for improved inbred line disease resistance result in correlated gains in topcrosses? No significant differences were observed between the selected and unselected topcrosses, indicating that the desired gains in topcross performance were not achieved by selection for inbred *per se* disease resistance. Eller et al. (2009) observed a similar result

when comparing topcrosses of lines with divergent levels of Fusarium ear rot and fumonisin contamination as inbreds *per se*. They hypothesized that the use of tester hybrid FR615 × FR697 was a major factor limiting the expression of variation for disease resistance, because inbred line FR697 has a relatively high level of resistance (not significantly different from GE440 in inbred trials, Table 3.8). When the susceptible line FR1064 was used as a topcross tester for non stiff-stalk lines, significant variation has been observed among topcrosses (Clements, 2004; Eller, Chapter 3 of this thesis).

Finally, were GE440 alleles recovered more frequently than expected by chance at markers linked to previously identified Fusarium ear rot and fumonisin contamination QTL? Selection among inbred lines appeared to significantly increase the recovery of GE440 alleles at a number of previously identified resistance QTL regions, including the most important QTL identified in the BC1 generation of this same cross. This result confirms the importance of these QTL for conferring disease resistance and indicates the effectiveness of phenotypic selection for enhancing frequencies of favorable alleles at QTL.

Acknowledgments

This research was supported in part by a USDA-IFAFS multidisciplinary training grant to North Carolina State University (award number 2001-52101-11507), and the North Carolina Corn Growers Association. Thanks to Dr. Don White who provided us with the initial GEFR backcross seed and Illinois Foundation Seeds for permission to conduct

studies with FR1064. Thank you also to Kathleen Hamby, Teagen Gray, Heather Hunt, Jessica Smith, and others for assistance with inoculation and sample processing.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Cont. AC-19: 716-723.

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons. New York.

Clements, M. J., C.M. Maragos, J.K. Pataky, D.G. White. 2004. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopath. 94: 251-260.

Colvin, B.M., L.R. Harrison. 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia 117: 79-82.

Eller, M.S., L.A. Robertson-Hoyt, G.A. Payne, and J.B. Holland. 2009. Grain yield and Fusarium ear rot of maize hybrids developed from lines with varying levels of resistance. Maydica 53: 231-237.

Ehrlich, V., F. Darroudi, M. Uhl, H. Steinkellner, M. Zsivkovits, and S. Knasmueller. 2002. Fumonisin B_1 is genotoxic in human derived hepatoma (HepG2) cells. Mutagenesis. 17: 257-260.

Gelderblom, W. C. A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar, N.P.J. Kriek, 1988 Fumonisins- novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microb. 54:1806-1811.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and Thompson, R. 2002. ASReml User Guide Release 1.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK

Fehr, W.R. 1987. Backcross Method. In *Principles of Cultivar Development. Volume 1. Theory and Technique*. MacMillan Publishing Company. New York, NY. p. 360-376.

Fusarium Research Center (http://frc.cas.psu.edu/)

Hendricks, K. 1999. Fumonisins and neural tube defects in south Texas. Epidemiol. 10: 198-200.

Miller, J.D., 1994. Epidemiology of *Fusarium* ear diseases of cereals. p. 19-36. *In*: J.D. Miller and H.L. Trenholm (ed.) Mycotoxins in grain. Eagan Press, St. Paul, MN.

Missmer, S.A., L. Suarez, M. Felkner, E. Wang, A.H. Merrill, Jr., K.J. Rothman, and K.A. Hendricks. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environ. Health Perspectives. 114: 237–241.

Mogg, R.J. and J.M. Bond. 2003. A cheap, reliable and rapid method of extracting high-quality DNA from plants. Mol. Ecol. Notes. 3: 666–668.

Morgavi, D.P., R.T. Riley. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. Animal Feed Science and Technology. 137: 201–212.

Munkvold, G.P., A. Desjardins. 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Dis. 81: 556-565.

Prelusky, D., B. Rotter, R. Rotter. 1994. Toxicology of mycotoxins. In *Mycotoxins in Grain: Compounds other than Aflatoxin*, pp. 359-403. Edited by J. Miller & H. Trenholm. St. Paul: The American Phytopathological Society.

Robertson,L.A., C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos and J.B. Holland. 2006. Heritabilities and correlations of Fusarium ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Sci. 46:353-361.

Robertson-Hoyt L.A., M.P. Jines, P.J. Balint-Kurti, C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, T.L. Molnár and J.B. Holland. 2006. QTL mapping for Fusarium ear rot and fumonisin contamination resistance in two maize populations Crop Sci. 46:1734-1743.

Ross, P.F., L.G. Rice, G.D. Osweiler, P.E. Nelson, J.L. Richard, T.M. Wilson, 1992 A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 117: 109-114.

Senior, M.L., J.P. Murphy, M.M. Goodman, and C.W. Stuber. 1998. Utility of SSRs for determining similarities and relationships in maize using an agarose gel system. Crop Science. 38:1088–1098.

Tanksley, S.D. and J.C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theoretical and Applied Genetics. 92: 191-203.

Van Egmond, H.P., R.C. Schothorst, M.A. Jonker, 2007 Regulations relating to mycotoxins in food; Perspectives in a global and European context. Anal Bioanal Chem. 389:147–157.

Table 3.1. Chromosome and map positions in $BC_1F_{1:2}$ lines , nearest flanking marker loci, effects, and variances associated with QTL identified through multiple interval mapping across four North Carolina environments, or across Clayton, NC and Plymouth, NC (environment with higher levels of rot) for Fusarium ear rot resistance and fumonisin contamination resistance (adapted from Robertson-Hoyt *et al.*, 2006.)

Trait	Chromo-some	QTL position	Bin	Left Marker	Right Marker	QTL Effect	Percent phenotypic variation explained
Across all environments							
Ear rot	1	75.9	1.02	bnlg1953	phi001	6.08‡	18.4
Ear rot	1	221.89	1.10	bnlg1347	bnlg2331	3.37	5.7
Ear rot	2	7.9	2.02	bnlg1017	bnlg1017	3.07	4.4
Ear rot	2	151.9	2.08	bnlg2144	bnlg1662	3.16	5.8
Ear rot	4	52.7	4.03	umc2280	umc2280	2.55	3.4
Ear rot	4	182.3	4.09	umc1086	umc1101	3.1	5
Ear rot	5	99.6	5.05	umc2111	umc2111	3.06	3.8
Fumonisin	1	95	1.03	phi001	bnlg1811	5.27	10.3
Fumonisin	2	177.1	2.08	bnlg1662	bnlg1606	3.97	6.2
Fumonisin	3	189.9	3.09	umc1489	umc1594	4.09	6.1
Fumonisin	4	53.7	4.03	umc2280	umc2061	2.58	5.5
Fumonisin	4	144.8	4.08	bnlg2244	umc1086	3.71	9.2
Fumonisin	4	193.3	4.09	umc1086	umc1101	3.33	7.7
Fumonisin	5	96.6	5.05	umc2111	umc2111	5.8	13.1
Fumonisin	7	24	7.02	umc1193	umc2098	3.71	6.6
Fumonisin	9	36.9	9.02	dupssr6	dupssr6	1.83	0.1
Across Clayton and Plymouth, NC environments							
Ear rot	1	67.9	1.02	bnlg1953	phi001	3.74	6.7
Ear rot	2	189.43	2.08	bnlg1606	bnlg1520	3	4.1
Ear rot	4	35.3	4.03	umc2150	umc2281	3.54	6.9
Ear rot	4	187.33	4.09	umc1086	umc1101	3.64	6.1
Ear rot	5	86.31	5.03	umc1355	umc2111	2.73	3.7
Ear rot	9	136.29	9.05	umc1078	bnlg1270	22.6	3.1
Fumonisin	1	100	1.04	phi001	bnlg1811	0.43	6.7
Fumonisin	1	191.7	1.09	umc1085	umc2047	0.19	1
Fumonisin	2	149.6	2.08	mmc0271	bnlg2144	0.44	8.6
Fumonisin	3	174.9	3.07	umc1489	umc1594	0.32	3.3
Fumonisin	4	53.7	4.03	umc2280	umc2061	0.37	8.7
Fumonisin	4	133.9	4.08	dupssr34	bnlg2244	0.26	5.3
Fumonisin	4	180.3	4.09	umc1086	umc1101	0.32	6.8
Fumonisin	5	94.3	5.05	umc1355	umc2111	0.55	12.2
Fumonisin	7	37.2	7.02	umc2098	umc1134	0.37	6.2
Fumonisin	8	7	8.03	umc1360	umc1778	0.3	3.7

†QTL effect estimated as the difference between homozygous FR1064 genotypes and heterozygous GE440/FR1064 genotypes in the GEFR population. Effects of ear rot resistance are reported in percent rotten kernels, and effect estimates of fumonisin as back-transformed data to approximate their original value of $\mu g\ g^{-1}$.

‡ Positive effects refer to GE440 as the origin of the beneficial allele, and negative effects refer to FR1064 as the beneficial allele.

Table 3.2. F-tests and estimated differences between the recurrent and resistant donor parents, between 19 selected $BC_4F_{1:3}$ lines and each population parent, and between the 19 selected lines and the BC_4F_3 randomly chosen control lines for percent Fusarium ear rot, fumonisin content, plant and ear heights, and flowering times from evaluations of lines per se across four locations in 2007 and 2008.

	Ear Rot (%)	Fumonisin ($\mu g\ g^{-1}$)	Plant Height (m)	Ear height (m)	Anthesis Date (DAP†)	Silk date (DAP)
FR1064 – GE440	25.7***	33.2*	-0.7***	-0.005***	-7.3***	-8.2***
FR1064 – 78 BC4F1:3 lines	13.1***	-15.0ns‡	-0.02ns	-0.05ns	-1.5ns	-1.2ns
GE440 – 78 $BC_4F_{1:3}$ lines	-12.6***	-18.2ns	0.64***	0.46***	5.7***	7.0***
BC_4F_3 control - 78 $BC_4F_{1:3}$ lines	10.2***	7.0ns	-0.03ns	0.003ns	0.05ns	-0.02ns
FR1064 – 19 selected lines	17.18***	22.0*	0.001ns	-0.05 ns	-1.2ns	-0.64ns
GE440 – 19 selected lines	-8.53*	-11.19 ns	0.66***	0.46***	6.08***	7.56***
BC_4F_3 control – 19 selected lines	14.36***	14.02 ns	-0.86 ns	0.19ns	0.39ns	0.51ns
F- test	1.7***	1.6**	7.1***	2.3***	2.6**	2.2*

†DAP = days after planting.

‡ns = not significant at $p = 0.05$.

*,**, and *** = p-values of 0.05, 0.01, and 0.001 respectively.

Table 3.3. Analysis of variance F-tests for variation among all $BC_4F_{1:3}$ lines, among the four $BC_4F_{1:3}$ lines whose $BC_4F_{3:4}$ sub lines were included in the trial, and among sub lines evaluated in two locations in 2008.

	Ear rot (%)	Fumonisin (μg g^{-1})	Plant height (m)	Ear height (m)	Silking date (DAP‡)	Anthesis date (DAP)
All $BC_4F_{1:3}$ lines	1.66^{ns}	2.32*	4.18**	6.27***	2.32*	2.75**
$BC_4F_{1:3}$ Mother lines	1.13^{ns}	1.20^{ns}	23.64**	24.75**	1.36^{ns}	2.78^{ns}
Sub line(Mother)	3.09*	0.64^{ns}	1.64^{ns}	1.81^{ns}	0.87^{ns}	0.92^{ns}

‡DAP = days after planting

*,**,*** significant at p = 0.05, 0.01 and 0.001 respectively

Table 3.4. Analysis of variance F-tests for $BC_4F_{1:3}$ topcross trials over eight environments in 2007 and 2008 in a split plot design where genotype was the whole plot variable and inoculation or non-inoculation was the sub plot treatment.

Source of Variation	Grain yield	Fusarium ear rot	Grain moisture	Erect plants	Fumonisin‡
Genotype	3.6***	1.9***	7.1***	1.5*	-
Treatment	12.5**	20.3**	0.6ns‡	0.3ns	1.6*
Genotype × Treatment	1.0ns	1.1ns	1.1ns	0.6ns	-

‡Measured on inoculated treatments only.

‡ns = not significant at $p = 0.05$.

*,**,*** = significance at $p = 0.05$, 0.01 and 0.001 respectively

Table 3.5. Least square means within treatments for Fusarium ear rot, grain yield and fumonisin content, and across treatments for grain moisture, plant height and ear height in GEFR $BC_4F_{1:3}$ lines topcrossed onto FR615 × FR697 and evaluated in four locations in 2007 and 2008. Estimates of within-treatment differences between topcrossed parents, between topcrossed random BC_4F_3 control and 78 $BC_4F_{1:3}$ topcross lines, FR1064 and78 $BC_4F_{1:3}$ lines, topcrossed random BC_4F_3 control and 19 selected $BC_4F_{1:3}$ lines, and between FR1064 and 19 selected $BC_4F_{1:3}$ lines.

	Fusarium ear rot (% of ear)[†]			Grain Yield (Mg ha^{-1})		
	Inoculated	Non-inoculated	Difference (I – N) [‡]	Inoculated	Non-inoculated	Difference (I – N)
Average of 78 $BC_4F_{1:3}$ topcrosses	12.07	3.98	8.09	6.39	6.93	-0.54
Average of 19 selected line topcrosses	11.95	3.83	8.12	6.28	6.83	-0.55
FR1064 Topcross	16.07	4.70	1.37	6.23	6.88	-0.65
GE440 Topcross	5.59	1.68	3.91	5.80	6.34	-0.54
Random BC_4 topcross	10.90	3.86	7.04	5.85	6.43	-0.58
Commercial check mean	10.04	2.69	7.35	7.71	8.49	-0.78
FR1064 Topcross -78 $BC_4F_{1:3}$ Topcrosses	4.00*	0.72 [ns§]	-6.72	-0.16 [ns]	-0.06 [ns]	-0.11
Random BC_4F_3 – 78 $BC_4F_{1:3}$ lines	-1.17 [ns]	-0.12 [ns]	-1.05 [ns]	-0.54[ns]	-0.50 [ns]	-0.04 [ns]
FR1064 Topcross -19 selected line topcrosses	4.12**	0.87ns	-6.75	-0.05[ns]	0.05[ns]	-0.01
Random BC_4F_3 -19 selected line topcrosses	-1.05[ns]	0.03[ns]	-1.08	-0.43[ns]	-0.40[ns]	-0.03
Difference between parents (GE440 – FR1064)	-10.48*	-3.02*	-7. 46	-0.43[ns]	-0.54[ns]	0.11
Standard Error of the Difference	1.38	1.38	-	0.40	0.36	-

[†]Backtransformed from the natural log.

[‡] Difference in ear rot percentage or grain yield between noninoculated and inoculated treatments.

[§]ns = not significant at $p = 0.05$.

*,**,*** = significance at $p = 0.05$, 0.01 and 0.001 respectively

Table 3.5 continued

	Fumonisin ($\mu g\ g^{-1}$)	Grain Moisture ($g\ kg^{-1}$)	Plant Height (m)	Ear Height (m)
Average of 78 $BC_4F_{1:3}$ topcrosses	29.80	1.522	1.87	0.92
Average of 19 selected line topcrosses	30.21	1.531	1.87	0.92
FR1064 Topcross	36.35	1.513	1.84	.91
GE440 Topcross	12.01	1.749	2.10	1.00
Random BC_4 topcross	25.72	1.603	2.01	0.92
Commercial check mean	21.24	1.651	2.00	0.93
FR1064 Topcross -78 $BC_4F_{1:3}$ Topcrosses	6.55 ^{ns}	-0.009 ^{ns}	-0.03 ^{ns}	-0.01 ^{ns}
Random BC_4F_3 – 78 $BC_4F_{1:3}$ lines	-4.08 ^{ns}	0.081 ^{ns}	0.14 ^{ns}	0 ^{ns}
FR1064 Topcross -19 selected line topcrosses	6.14^{ns}	-0.018^{ns}	-0.03*	-0.01^{ns}
Random BC_4F_3 -19 selected line topcrosses	-4.49^{ns}	0.072***	0.14***	0.00^{ns}
Difference between parents (GE440 – FR1064)	24.34**	-0.236**	-0.26***	-0.09 ns
Standard Error of the Difference	6.42	0.24	0.005	0.055

[†]Backtransformed from the natural log.

[‡] Difference in ear rot percentage or grain yield between noninoculated and inoculated treatments.

[§]ns = not significant at $p = 0.05$.

*,**,*** = significance at $p = 0.05$, 0.01 and 0.001 respectively

Table 3.6. Least square means of superior GEFR (GE440 × FR1064) lines for fumonisin content and Fusarium ear rot resistance as inbreds per se (four environments) and for fumonisin content, Fusarium ear rot and grain yield. grain moisture, and percent erect plants as topcrosses to the single cross tester, FR615 × FR697 (evaluated in eight locations).

	Inbred		Topcrosses				
Experimental lines	Fumonisin (µg g^{-1})	Ear Rot (%)	Fumonisin (µg g^{-1})	Ear Rot (%)	Grain Yield (Mg ha^{-1})	Grain Moisture (g kg^{-1})	Erect Plants (%)
GEFR 399-1-5-1-3	33.9	17.1	26.75	10.8	6.42	152.3	0.989
GEFR 399-2-10-4-1	31.0	13.0	28.69	9.3	6.79	152.6	0.996
GEFR 399-2-10-4-2	54.1	14.9	26.06	10.3	6.31	151.2	0.998
GEFR 399-3-7-4-2	29.5	19.6	29.13	12.4	6.64	151.4	0.995
GEFR 400-9-9-3-2	36.8	17.2	2983	10.9	6.91	152.8	0.992
Checks							
FR1064	68.3	35.4	36.35	15.1	6.57	151.3	1.000
FR615	17.1	33.9	20.88	-	-	-	-
FR615 × FR697	-	-	-	18.6	5.02	147.6	0.997
GE440	38.0	10.6	12.01	7.7	5.94	174.9	0.951
Pioneer brand 31G66	-	-	17.18	9.0	8.20	169.9	0.993
Pioneer brand 31G98	-	-	25.30	15.0	8.10	160.2	0.996
Mean of 80 selected lines	52.0	18.7	29.80	12.1	6.65	152.2	0.995
LSD (0.05)	36.7	14.6	8.0	6.1	0.36	6.2	0.020

Table 3.7. Recovery of donor parent (GE440) alleles in selected BC_1-derived lines at SSR markers flanking QTL identified previously in the BC_1 generation by Robertson-Hoyt *et al.* (2006). IBM bin position, trait affected by QTL, number of BC_1 founder families that were segregating at a locus, and number of BC_1 lineages still segregating in the BC_4 generation at the locus are presented for each tested SSR locus. For both the initially selected set of 78 BC4-derived lines and the set of 19 lines resulting from two generations of selection against ear rot the excess number of BC_4-derrived lines carrying the GE400 allele over the number expected in the absence of selection, and the chi-square value associated with this excess are presented for each marker is displayed.

Marker locus	IBM bin number †	Trait(s) affected by linked QTL in BC1 generation	Number of founder BC_1 families segregating at locus	Number of BC_1–derived $BC_4F_{1:3}$ lineages segregating at locus	Excess number of lines segregating for GE440 allele above expectation			
					Among 78 selected $BC_4F_{1:3}$ lines		Among 19 selected $BC_4F_{1:3}$ lines	
					Number	χ^2	Number	χ^2
Bnlg1017	2.02	Ear rot	7	4	3.625	1.79	-0.25	0.03
Bnlg1270	9.05	Ear rot	6	4	4.375	2.87	1.5	1.71
Bnlg1347	1.1	Ear rot	8	7	5.125	3.38	2	2.29
Bnlg1520	2.08	Ear rot	6	4	5.25	4.67*	1.375	1.33
Bnlg1606	2.08	Ear rot/fumonisin	7	4	5.5	5.32	2.125	2.75
Bnlg1662	2.08	Ear rot/fumonisin	7	5	8.75	12.07***	3	5.14*
Bnlg1811	1.03	Fumonisin	7	0	-6.875	7.86**	-2	2.29
Bnlg1953	1.02	Ear rot	7	5	7.5	7.56**	4.125	10.37**
Bnlg2144	2.08	Ear rot	7	2	-0.375	0.03	-0.875	0.47
Bnlg2244	4.08	Fumonisin	7	5	19.125	60.80***	5	14.29***
Bnlg2331	1.1	Ear rot	8	6	5.625	4.318*	1.25	1.02
dupssr06	9.02	Fumonisin	6	6	18.375	68.60***	3.5	9.33**
dupssr34	4.08	Fumonisin	8	5	9.5	13.75***	7.625	27.98***
mc0271	2.08	Fumonisin	5	4	5.875	6.44*	1.5	1.71
phi001‡	1.02/1.03	Ear rot/fumonisin	5	-	-	-	-	-
Umc1078	9.05	Ear rot	6	3	0.75	0.09	0.375	0.10
Umc1085	1.09	Fumonisin	6	4	5.375	4.33*	4.375	13.46***
Umc1086	4.08/4.09	Ear rot/fumonisin	6	3	8.25	13.53***	0	0.00
Umc1101	4.09	Ear rot/fumonisin	7	6	8.625	19.43***	2.75	6.91**
Umc1134	7.02	Fumonisin	5	0	-5.375	6.14*	-0.75	0.86
Umc1193	7.02	Fumonisin	8	4	-0.25	0.01	-2.125	2.43

Table 3.7 continued

Marker locus	IBM bin number[†]	Trait(s) affected by linked QTL in BC1 generation	Number of founder BC_1 families segregating at locus	Number of BC_1–derived $BC_4F_{1:3}$ lineages segregating at locus	Excess number of lines segregating for GE440 allele above expectation			
					Among 78 selected $BC_4F_{1:3}$ lines		Among 19 selected $BC_4F_{1:3}$ lines	
					Number	χ^2	Number	χ^2
Umc1355	5.03	Ear rot	5	2	-0.875	0.15	-2.125	2.43
Umc1360	8.03	Fumonisin	5	4	1.5	0.40	-0.5	0.19
Umc1489	3.09	Fumonisin	7	5	-0.375	0.019	-0.625	0.27
Umc1594	3.09	Fumonisin	5	0	-5.375	6.14*	-1.25	1.43
Umc1778	8.03	Fumonisin	4	3	4.875	5.30*	1.75	2.80
Umc2047	1.09	Fumonisin	7	6	7.625	7.93**	4.25	11.80***
Umc2061	4.03	Fumonisin	7	6	10.5	19.38***	2.875	4.45*
Umc2098	7.02	Fumonisin	8	5	2.75	1.048	-0.625	0.27
Umc2111	5.05	Ear rot/fumonisin	6	5	12.875	26.59***	3	5.14*
Umc2150	4.03	Ear rot	5	1	-1.25	0.55	-1	0.57
Umc2280	4.03	Ear rot/fumonisin	7	6	20.125	67.33***	6.625	21.12***
Umc2281	4.03	Ear rot	7	5	4.5	3.56	0.75	0.29

[†]The IBM (Intermated B73 x Mo17) genetic map is divided into 100 bins; each roughly 20cM in size, and designated by the chromosome number and a two digit decimal (www.maizegdb.org).

[‡]We were unable to score phi001 on the BC_4:$F_{1:3}$ progeny.

*,**,*** = significance at $p < 0.05$, 0.01 and 0.001 respectively

Table 3.8. Fusarium ear rot least squared means observed in 2006. Data was collected from two replications in two locations.

Material	Group	Ear rot (%)[†]
FR1064	Susceptible Check	24.8
FR615	Tester Parent	24.0
FR697	Tester Parent	7.0
GE440	Resistant Check	6.0
LSD		9.7

[†]Ear rot was back transformed from the natural log.

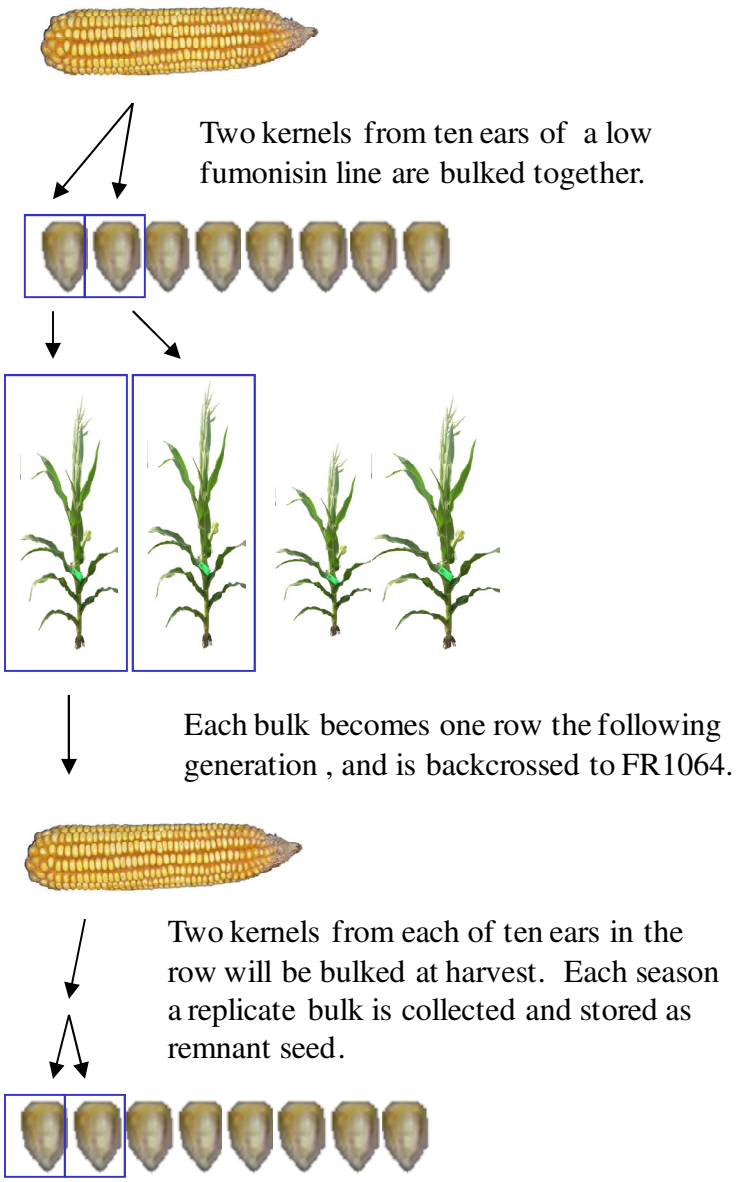

Figure 3.1. Modified Single Seed Backcrossing Scheme. (photographs and editing by Magen Starr Eller.)

CHAPTER 4: Indirect Selection for Reducing Fumonisin Accumulation Susceptibility in a Genetically Broad-based Recurrent Selection Population

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Abstract

Fusarium verticillioides causes Fusarium ear rot of maize (*Zea mays* L.), a disease which is found in all U.S. maize growing regions. Affected grain contains the carcinogenic mycotoxin fumonisin. We developed a recurrent selection population with twenty two publicly available parental lines to test the hypothesis that index selection to improve resistance to ear rot, standability, and grain yield would indirectly improve resistance to fumonisin accumulation in the grain. Two hundred- six Cycle 0 $S_{0:1}$ lines were evaluated for a selection index incorporating information on ear rot, lodging and grain yield *per se*. We evaluated selected lines as partially inbred lines ($S_{0:3}$s) and as early generation $S_{0:2}$ topcrosses onto the commercially important but relatively ear rot susceptible tester FR1064. The selected lines were significantly better than the base population for grain yield *per se*, but although gains from selection were greater than expected, selected lines were not significantly different than the base population for ear rot. Selected lines were also not significantly different than the base population for fumonisin content. In topcrosses,

however, selected lines showed significant improvement for ear rot resistance and fumonisin accumulation compared to the unselected Cycle 0 topcross control. Lodging resistance was not evaluated in either inbred or topcross studies due to limited expression of lodging in the test environments. These results suggest that both direct and indirect selection responses are in the favorable direction, but that additional cycles of recurrent selection are needed to increase favorable allele frequencies for each of the target traits.

Introduction

Fusarium ear rot most commonly affects maize grown in the warm, dry conditions prevalent in the southern United States and lowland tropics, but *Fusarium verticillioides* (Sacc.) Nirenberg (formerly *F. moniliforme* Sheldon; teleomorph *Gibberella moniliformis*) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*), causal agents of the disease, can be found worldwide in grain or maize crop residue (van Egmond *et al*., 2007). Fusarium ear rot can reduce yields and grain quality and no commercial maize hybrids are immune to the disease (Munkvold and Desjardins, 1997). In addition to the direct effect of Fusarium ear rot on yield, *F. verticillioides* and *F. proliferatum* can produce mycotoxins called fumonisins that contaminate corn grain. Fumonisins are suspected carcinogens (Gelderblom *et al.*, 1988; Miller, 1994; Prelusky *et al.*, 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).

Increased resistance to both ear rot and mycotoxin contamination are important objectives in the effort 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 = $\mu g\ g^{-1}$) for many milled corn products used for human consumption (CFSAN, 2001). European Union regulations limit fumonisin concentration to less than 1 $\mu g\ g^{-1}$ for human foods, and to less than 0.2 $\mu g\ g^{-1}$ 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).

Response of fumonisin levels to indirect selection against ear rot is predicted to be less effective than direct selection against fumonisin accumulation because fumonisin concentration is more highly heritable than resistance to Fusarium ear rot (Robertson *et al.*, 2006). Fumonisin assays are likely more highly heritable than ear rot scores because they are more precise than visual scores of percent ear rot. However, ear rot can be scored quickly in the field, whereas assaying fumonisin content requires harvesting the inoculated ears, shelling grain, grinding grain to a precise particle size, weighing samples, performing fumonisin extractions in the laboratory, and finally conducting ELISA or HPLC assays. Considering the additional labor, time, and the price of ELISA or HPLC assays, Robertson *et al*. (2006) proposed that indirect selection against ear rot could be a more economically efficient way to reduce fumonisin content in grain than direct selection against fumonisin contamination. Because ear rot is easier to evaluate than fumonisin, population sizes could be increased, permitting higher selection intensity, or the number of evaluation environments and replicates could be increased, resulting in a greater entry mean heritability for ear rot compared to fumonisin concentration for fixed economic resources.

Breeding for resistance to Fusarium ear rot and fumonisin contamination will rely on identification of germplasm with good resistance to both aspects of the disease. High levels of resistance to fusarium ear rot appear to be rare in U.S. commercial hybrids, so germplasm not closely related to the progenitors of modern U.S. commercial hybrids may be useful

untapped sources of resistance. Two important alternative sources of diverse maize germplasm are older public inbred lines, which are often well adapted to U.S. corn production areas, but can exhibit poor stalk strength, lower inherent yield potential and other undesirable traits, and tropical germplasm which may have higher yield potential, but is poorly adapted to temperate production environments limiting its immediate utility to breeding programs in the United States (Goodman, 1999; Goodman *et al*., 2000).

Clements *et al*. (2004) evaluated Fusarium ear rot and fumonisin accumulation in testcrosses of 1,589 public inbred lines to the relatively highly susceptible tester, FR1064. They identified several older public inbreds with superior resistance to production or accumulation of fumonisin. Resistance in these lines appears to be dominant, as it is evident both in the inbreds and the hybrids between 35 selected resistant lines and FR1064 (Clements *et al*., 2004). Additional sources of resistance have been identified by screening tropical-derived inbred lines for both Fusarium ear rot and fumonisin concentration (Eller *et al*., 2008). Two key public tropical germplasm projects have been included in these screens: the Germplasm Enhancement of Maize (GEM) project (Pollak, 2003) and Dr. Major Goodman's inbred line development breeding program at N.C. State University (Goodman *et al*., 2000; Holland and Nelson, 2009). Both of these programs focus on identifying superior tropical maize germplasm, adapting it to temperate growing conditions, and breeding for maximum hybrid combining ability (Betrán *et al*., 2004). The proportion of tropical germplasm parentage in the inbred lines screened ranged from 25% to 100%.

The objective of this study was to develop a genetically broad-based population that combines alleles from diverse maize germplasm with many desirable agronomic traits of Corn Belt maize with multiple sources of resistance to Fusarium ear rot. We then used this Resistance to Fusarium (ReFus) population to test the hypothesis that selection for reduced Fusarium ear rot results in reduced fumonisin contamination.

Material and Methods

Population Development

The ReFus population was developed from 22 inbred founders. Lines were chosen based on low fumonisin content and ear rot observed under artificial inoculation conditions in previous evaluations of diverse public maize germplasm or based on recommendations by other maize breeders, regardless of adaptation to North Carolina growing environments. Some parents were selected on the basis of a large scale evaluation of older publicly developed inbred lines in topcross trials by Clements *et al*. (2004). Additional parents were chosen from among experimental and released inbreds developed by Dr. Major Goodman at North Carolina State University (Holland and Nelson, 2009), Dr, Javier Betrán at Texas A&M University, or Dr. Michael Blanco of the USDA-ARS Germplasm Enhancement of Maize program at Iowa State University based on data from two years of screening trials at Clayton, NC (Eller *et al*., 2008), or general fungal resistance observed in unreplicated nursery plots.

Consideration was given to superior combining ability for yield and agronomic performance and adaptation to North Carolina growing environments; in addition to general fungal ear rot resistance based on unreplicated nursery observations (M.M. Goodman, personal communication).

Parental lines were divided into two sets and crossed using a Design II factorial mating approach (Comstock and Robinson, 1948), in which each parent in set I was mated to each parent in set II. The following crosses were not made because lines did not flower simultaneously: A131 × NC258, A131 × NC320, A131 × NC346, A131 × NC446, A131 × NC448, and A131 × NC492, Ky21 × NC456, and NC300 ×NC448. The two groups of parents overlap both for resistance levels and for adaptation. Germplasm from the Stiff Stalk heterotic group was excluded from both sets of parents of this population; all parental lines have a tropical or non-stiff stalk pedigree and a better likelihood of combining well with Stiff Stalk inbreds.

F_1s from the factorial mating were grouped by predicted maturity classes, based on parental flowering times. We developed 11 such groups, each represented by a balanced bulk of F_1 seeds from the different crosses in that group. Groups with more crosses were represented by more seeds, so the entire set of F_1s planted represented a balanced bulk of seeds across all paired matings. The groups were planted on different days to minimize differences in their predicted flowering dates. The first generation of intermating was conducted by bulking pollen from the entire population, thoroughly mixing, and applying it to available silks each day. A second generation of intermating was conducted by planting a

balanced bulk of seeds from each ear of the previous intermating. The planting was divided into two sets; pollen from set 1 was applied to silks in set 2 and vice versa to reduce the chances of inadvertent selfing. A minimum of 200 ears was harvested each generation to minimize genetic drift. After the second intermating, the population was self-fertilized to obtain 206 $S_{0:1}$ families, the base population (Cycle 0) to which selection was applied. The 206 $S_{0:1}$ families were also self-fertilized a second generation and harvested in bulk to make $S_{0:2}$ lines.

Field Evaluation of Base Population

The 206 $S_{0:1}$ lines comprising the C0 population were evaluated in 2007 at two North Carolina environments: the Central Crops Research Station at Clayton, and the Peanut Belt Research Station at Lewiston-Woodville. Each replication contained 206 C0 $S_{0:1}$ families, plus GE440 and FR1064, resistant and susceptible inbred standards respectively, which were included twice in each replication, to result in a total of 210 plots per complete replication. Experimental design was a 14 × 15 α-lattice, with two replications at each location.

Plots consisted of single-rows containing individual C0 $S_{0:1}$ families. At both locations, plots were 3.05 m in length with 0.965 m between plots in Clayton, and 0.914 m between plots at Lewiston-Woodville. Plots were overplanted and thinned to a uniform density of 23 plants per row, resulting in population densities of 65,100 plants hectare^{-1} at Clayton and 68,750 plants hectare^{-1} at Lewiston.

Inoculation Technique

Six isolates of *F. verticillioides* (NC-i6, NC-i7, NC-i9, NC-n16, NC-n17, and NC-n22) were cultured separately on PDA (Potato Dextrose Agar, Fisher Scientific Pittsburg, PA). Isolates were submitted to the Fusarium Research Center collection for identity verification and storage (http://frc.cas.psu.edu/). Conidia were collected by washing the cultures with distilled water, loosening conidia by brushing with a paint brush, straining the suspension through cheese cloth, and diluting the conidia suspension of the six isolates to approximately 2×10^{6} mL^{-1} in water. Two inoculations were conducted seven days apart to reduce escapes and simulate common methods of natural infection. A silk channel inoculation ten to fourteen days post mid-silk was followed by a direct ear inoculation seven days later. The primary ear of the first twelve plants in each row was injected with 5 mL of 2×10^{6} conidial suspension using a 5mL Allflex draw-off injection syringe (Allflex Inc, Dallas, TX) fitted with a 16 gauge needle that had the point filed down. One drop of undiluted Tween-20 was added to each liter of inoculum suspension to break the surface tension of the suspension. This inoculation method was used for both selection in 2007 and evaluation in 2008.

Phenotypic Data Collection

Stand count was determined four to six weeks after planting. Silk date and tassel date were recorded at Clayton, NC when half the ears in each plot showed 50% silk

emergence and approximately 50% of the pollen in the plot had been shed. Plant and ear heights were recorded to the nearest 5 cm after inoculations were complete. Plant height was measured to the flag leaf, and ear height to the node from which the ear emerged.

When all plants reached physiological maturity, each plot was rated for the number of plants lodged (leaning more than 30% from vertical, with broken stalks, or with a dropped ear). Percent lodging was calculated as number of plants lodged / stand count. The first 10 primary ears from each plot were harvested by hand and air-dried to approximately 140g kg^{-1} moisture. These ears were visually rated for the percentage of kernels displaying visible symptoms of Fusarium ear rot. Ear rot ratings were estimated to 5% increments. Grain yield (g $plant^{-1}$) was determined by weighing the bulk shelled grain from the sample ears for each plot and dividing by the number of ears.

Statistical Analysis of Cycle 0 Population Evaluation

A model in which genotypes were considered fixed effects and location, replication within location, incomplete block, and location × entry interactions were considered random was fit for each trait using the MIXED procedure of SAS (SAS Institute, 2004). Significance of genotypic variation was tested with F-tests from this model. In addition, least square means for genotypes and their average standard errors of pairwise comparison were estimated from this model.

The long-term goal of this research is to develop germplasm with enhanced resistance to Fusarium ear rot and fumonisin contamination combined with good agronomic

potential. Therefore, based on observation of the material and initial statistical analyses, three traits were chosen as the basis of selection within this population: percent ear rot, grain yield, and percent lodging. Following independent culling for flowering time, an optimal selection index (Baker, 1986; Falconer and Mackay, 1996) was developed to integrate selection on these traits.

The weights applied to the three traits in the selection index were obtained from the equation $\mathbf{b}=\mathbf{P}^{-1}\mathbf{G}\mathbf{a}$; where **b** is the vector of weights applied to the traits, **P** is the phenotypic covariance matrix, **G** is the genotypic covariance matrix, and **a** is the vector of trait value economic weights (Falconer and Mackay, 1996). We chose economic weights of $\frac{-2}{\hat{\sigma}_{\mathrm{Pr}}}$, $\frac{+1}{\hat{\sigma}_{Py}}$, and $\frac{-1}{\hat{\sigma}_{Pl}}$ for ear rot, yield, and lodging, respectively, where $\hat{\sigma}_{Pi} = \sqrt{\hat{\sigma}^2_{Gi} + \frac{\hat{\sigma}^2_{GEi}}{2} + \frac{\hat{\sigma}^2_{\varepsilon i}}{4}}$, the phenotypic standard deviation of $S_{0:1}$ line means for trait *i*. Thus, reductions in two phenotypic standard deviations of ear rot were given equal economic importance to one phenotypic standard deviation reduction in lodging and one phenotypic standard deviation increase in grain yield.

The MANOVA option of the GLM procedure was used to conduct multivariate analysis (excluding checks) of each pair of traits for the purpose of building a selection index. Genotypic, genotype-by-environment, and error variances and covariances for each pair of traits were estimated using the method of moments (Holland, 2006). Elements of the **P** matrix were obtained by dividing the matrix of mean squares and cross products for entry

by the coefficient of the variance component for entry. **G** was estimated by subtracting the matrix of means squares and cross products for genotype × environment from the matrix of mean squares and cross products for genotype and dividing by the coefficient of the variance component for genotype in the expected mean square for genotype (Mode and Robinson, 1959).

Selection index values for each line were obtained by summing the product of each trait's selection index weight by the centered least square mean for the line and trait in the original units used for measurement. Twenty $S_{0:1}$ lines were selected by truncation selection using the selection index values. The effectiveness of selection was then evaluated by comparing phenotypes of $S_{0:3}$ lines derived from selected or unselected $S_{0:1}$ lines. In addition, the selected lines were intermated twice to form a Cycle 1 population to initiate a longer-term recurrent selection population.

The original least squares multivariate analysis to develop the selection index was by necessity performed within a one-week time frame between scoring ear rot on dried, harvested ears and shipping seeds of selected lines to winter nursery. For the purposes of presentation, we later reanalyzed each trait individually to estimate entry means heritability using a completely random model with Proc MIXED, excluding check entries (Holland *et al.*, 2003). Each pair of traits from the selection trial were also reanalyzed using multivariate mixed models, excluding checks and considering all effects random, to estimate phenotypic and genotypic correlations and their standard errors (Holland, 2006).

Evaluation of Selected $S_{0:3}$ Lines

To provide an assessment of the effectiveness of selection, $S_{0:3}$ lines from the 20 selected $S_{0:1}$ families were evaluated in 2008. They were compared to the 22 founder inbreds, a balanced bulk of the entire C0 population at the $S_{0:3}$ generation (repeated 6 times in each replication), one resistant check (GE440) and one susceptible check (FR1064). The lines were evaluated at the same two locations where selections were conducted. Experimental design was a 7 × 7 α-lattice design with two replications per environment.

Plot dimensions, planting densities, and inoculation techniques were identical to those in 2007. Data collection was identical to that conducted in 2007 with the following exceptions: when scoring percent ear rot, if less than 5% rot was visible, scores of 0, 1, or 3% were assigned. Lodging was minimal and not recorded, and grain yield (as g $plant^{-1}$) was measured at Lewiston-Woodville only.

Fumonisin data were collected on the selected $S_{0:3}$ lines in 2008 using enzyme-linked immunosorbent assay (ELISA, Ono *et al*., 2000). A sub-sample of shelled kernels was ground in a Romer Series II Mill (Romer Labs, Union, MO). Twenty grams tested for fumonisin content (μg g^{-1}) using Diagnostix fumonisin ELISA assay kits (Diagnostix, Mississanga, ON, Canada).

Statistical Analysis of $S_{0:3}$ Line Evaluations

Traits measured at multiple environments were analyzed with a standard model including fixed genotype effects and random location, location-by-genotype interaction,

replication and incomplete block within replication effects. This model was compared to a model with heterogeneous error variances within each environment, and the model with the lowest Akaike's Information Criterion (AIC; Akaike, 1974) was selected. The heterogeneous error model was best for fumonisin content and plant height; the standard model was best for ear height. In addition, ear rot data (collected on multiple ears per plot) were analyzed with a weighted analysis using the number of ears scored per plot as weight, and a weighted analysis with heterogeneous within-environment error variances. Grain yield, days to silk, and days to anthesis were measured at only one location and analyzed with a model containing only replication and incomplete block within replication as random factors and genotype as a fixed factor. The null hypotheses of no significant differences between resistant and susceptible checks (GE440 and FR1064) and between the selected lines and the unselected Cycle 0 population were tested using the ESTIMATE statement in PROC MIXED.

The full dataset was used to estimate differences involving Cycle 0 controls and parental lines, with specific standard errors for genotype group comparisons obtained with the "estimate" statement in PROC MIXED. Checks were then removed from the dataset and the analysis models were re-run to test the null hypothesis of no variation among experimental lines only using F-tests.

Field Evaluation of Selected Topcross $S_{0:2}$ Lines

To determine if selection among $S_{0:1}$ lines resulted in correlated responses to selection in a related topcross hybrid generation, $S_{0:2}$ lines from the selected $S_{0:1}$ families were crossed to the susceptible commercial stiff-stalk tester inbred, FR1064 (Robertson *et al*., 2006). The resulting topcrosses were evaluated in four North Carolina locations in 2008: Central Crops Research Station in Clayton, the Tidewater Research Station in Plymouth, the Peanut Belt Research Station in Lewiston-Woodville, and the Sandhills Research Station near Jackson Springs, NC. A total of 30 entries were tested: the top 20 ReFus C0 lines topcrossed to FR1064, three commercial checks (Pioneer hybrids 31G98, 31G66, and DeKalb hybrid 697), and a bulk of randomly chosen Cycle 0 lines topcrossed to FR1064. This random Cycle 0 bulk was included seven times in each replication to represent the unselected population. Experimental design was a split-plot design with two replications at each location. Genotype was the whole plot factor and inoculation treatment was the sub plot treatment. Whole plots were arranged following a 5×6 α-lattice design.

Each whole plot contained six rows of a common genotype. Inoculation treatment factor levels (inoculation with *Fusarium verticillioides* or no inoculation) were assigned to two subplots within each whole plot. The subplots consisted of three rows, each measuring 3.66 m in length, with a 1.22-m alley between ranges of plots. Inter-row spacing was 0.914 m in Lewiston, NC and 0.9652 m in Clayton, Plymouth, and Jackson Springs, NC. Plots were over seeded and thinned to target population densities (62,288 plants ha^{-1} in Clayton, Plymouth, and Jackson Springs, NC or 65,750 plants ha^{-1} in Lewiston, NC).

10 ears from the outer rows of each whole plot (one row per subplot) were hand harvested, dried, and scored for percent ear rot while the inner four rows (two rows per subplot) were mechanically harvested to measure grain yield and grain percent moisture on each sub plot. Inoculated and noninoculated plots were not separated by border rows, because *F. verticillioides* spreads very little from plant to plant during the growing season (Yates and Sparks, 2008).

Data collection was identical to the $S_{0:3}$ lines with the following modifications. Plots were thinned to the target population density when stand was recorded, four to six weeks after planting. Silk and anthesis date for each plot were recorded at Clayton, NC as described above. Two rows of each subplot, comprising the center four rows of each whole plot, were mechanically harvested to collect grain moisture and yield data. Lodging data were collected just before mechanical harvest; number of root lodged and stalk lodged plants were recorded separately and later merged together. Percent erect plants was calculated as (stand – lodged plants) / stand. Hand harvested ears from the outside rows of each whole plot were air dried to approximately 140g kg^{-1} moisture, rated for Fusarium ear rot, shelled, ground, and tested for fumonisin content ($\mu g\ g^{-1}$) as described for the $S_{0:3}$ lines.

Statistical Analysis of Topcrossed $S_{0:2}$ Lines

Each trait was analyzed using both traditional analyses of variance and spatial analyses with the MIXED procedure in SAS. A standard model with fixed treatment, genotype, and treatment-by-genotype interaction effects and random location, location-by-

treatment location, location-by-genotype interaction, location-by-treatment-by-genotype interaction, replication, and incomplete block effects was compared to up to three alternative models for each trait. Alternative models incorporated heterogeneous error variances across locations or weighted error weighted by the square root of the inverse of the number of ears scored (where appropriate), or included both modifications together. The model which minimized the AIC was chosen. Because experimental design was a split plot, the Satterthwaite option of Proc MIXED (SAS Institute, 2004) was used to estimate denominator degrees of freedom for tests of fixed effects and treatment comparisons for all traits except fumonisin.

A correlation between residual and predicted values was observed in the original ear rot data. Square root and natural log transformations were compared and the natural log transformation was used because it most effectively eliminated the dependency of residual values on predicted values. Error variances for the natural log of percent ear rot were consistent across locations, so a heterogeneous error model was not used; the ANOVA was weighted by the number of ears scored because this improved the AIC score of the analysis.

Grain yield for each plot was adjusted to 155g kg^{-1} grain moisture. Spatial analyses were investigated for each location to estimate genotypic least squared means for grain yield within each environment. The final model accounted for heterogeneous error variances across locations. Unweighted, homogeneous error variance models were selected as best for plant and ear heights, and percent erect plants.

The full dataset was used to estimate differences involving check hybrids, with specific standard errors for comparison obtained with the "estimate" statement in PROC MIXED. Checks were then removed from the dataset and ANOVA estimated using PROC MIXED to determine the overall F-test significance of effects for genotype, inoculation, and genotype by inoculation interaction effects for the experimental hybrids only.

Results and Discussion

Evaluation of Base Population for Selection

$S_{0:1}$ lines varied significantly ($p \leq 0.0001$) for ear rot, grain yield, and lodging, with family mean heritabilities for those traits estimated to be 74% (SE = 0.04), 57% (SE = 0.06), and 64% (SE = 0.05), respectively, in the evaluation of the base population in 2007. Significant ($p \leq 0.0001$) differences among entries were also observed for days to silking and anthesis (Table 4.2).

In the ReFus population percent ear rot had negative phenotypic correlations with both grain yield (r= -0.21, SE=0.04) and lodging (r= -0.13, SE = 0.04; Table 4.3). Grain yield also exhibited a slight negative phenotypic correlation with lodging (r = -0.09, SE=0.04), perhaps because heavy ears contribute to lodging under the high wind conditions experienced in 2007 (Table 4.3). Genotypic correlations for percent ear rot and lodging were higher than phenotypic correlations (r = -0.26, SE = 0.10), but those for percent ear rot and grain yield, as well as grain yield and lodging were not significant (Table 4.3).

Following selection among $S_{0:1}$ lines based on the multiple trait index, the selection differential, or "average superiority of the selected parents" (Falconer and Mackay, 1996), was estimated for each trait. Selection differentials were -2.12 percentage points for ear rot, 22.2 g plant $^{-1}$ for grain yield, and -0.6% for lodging, indicating that the direction of selection was favorable for each trait, although the selection differential on any one trait was not as large as it would have been under single trait selection.

Comparison of Selected $S_{0:3}$ Lines and Cycle 0 Population

In the 2008 evaluation of $S_{0:3}$ lines *per se*, genotype-by-environment interactions were not significant for ear rot; however error variation was different between the two locations, so a heterogeneous errors model was used to analyze percent ear rot. A significant ($p = 0.003$) difference of 29 percentage points in ear rot was observed between the resistant control GE440 and the susceptible control, FR1064 (Table 4.4), indicating that enough ear rot was observed in this population to distinguish resistant and susceptible lines. The 22 founder lines of the population varied widely for grain yield, Fusarium ear rot, and fumonisin contamination; several of the founders appeared to have very poor resistance to Fusarium ear rot, possibly due to their poor adaptation (Supplemental Table B.1).

A key test of the direct response to selection against ear rot susceptibility was the comparison of the mean of the 20 selected lines and the bulk of the unselected lines from the ReFus population. Selected lines exhibited lower ear rot than the unselected control $S_{0:3}$

lines in this study, but those differences were not statistically significant ($p = 0.3$) (Table 4.5).

Grain yield was measured in Lewiston-Woodville only in 2008. Analysis of variance revealed significant ($p < 0.0001$) differences among entries (Table 4.4). The selected lines yielded 11 g plant^{-1} ($p = 0.002$) more than the control $S_{0:3}$s, on average, indicating that grain yield *per se* was improved through this selection (Table 4.5). Since lodging was extremely rare in 2008 when the selected lines were tested, we were not able to estimate the effect of selection on this trait.

These results demonstrate that selection among $S_{0:1}$ lines for a multi-trait index resulted in a direct response for grain yield only, as measured in the descendant $S_{0:3}$ generation. The testing environment in 2008 was not appropriate to evaluate response to selection for lodging. The primary trait of interest, Fusarium ear rot, exhibited a non-significant response to selection in the favorable direction. To compare observed selection responses to expectations, we computed expected gains for each trait as $R = h^2(X_S - X_O)$, where R is genetic gain, h^2 is entry mean heritability estimated among $S_{0:1}$ lines in 2007, X_S is the mean of the selected $S_{0:1}$ lines in 2007, and X_O the mean of all $S_{0:1}$ lines composing the C0 population in 2007. Realized genetic gain was estimated as the difference between the mean values of the selected $S_{0:3}$ lines and the mean values of the unselected control bulk of $S_{0:3}$ lines from the evaluation experiment conducted in 2008. We expected a mean reduction of 1.6 percentage points in Fusarium ear rot and achieved a reduction of 5.0 percentage points, which represented 14% of the population mean value for ear rot. For

yield, we expected a gain of 14.0 g plant^{-1} and realized a gain of 10.8 g plant^{-1} (Table 4.6). This gain represented 24% of the population mean value for grain yield. We expected a reduction in lodging of 0.4%, but were unable to determine actual gain because lodging was very low in the 2008 season (Table 4.6). Thus, for Fusarium ear rot, we observed a greater gain from selection than was predicted, however the amount of gain was not statistically significant, because our experiment lacked power to distinguish relatively small differences in ear rot. In contrast, we observed less gain for yield than predicted, but this gain was statistically significant.

Effect of Selection on Fumonisin Content

Robertson *et al.* (2006) proposed that indirect selection against ear rot could be a more economically efficient method than direct selection to improve resistance to fumonisin contamination in maize. Plot values for fumonisin content and Fusarium ear rot from the 2008 evaluation experiment were moderately positively correlated ($r = 0.39$, $p < 0.0001$), congruent with previously reported estimates of the phenotypic correlation (Roberson *et al.*, 2006, Clements *et al*., 2004). Genetic correlations between these two disease traits have been consistently measured as greater than phenotypic correlations (Robertson *et al*., 2006; Eller *et al*., 2008) however we did not have a random set of lines in 2008 on which to estimate a genotypic correlation. Analysis of variance revealed significant differences among entries in 2008 ($p = 0.05$) for fumonisin content. A comparison of GE440, the resistant check, and FR1064, the susceptible check, revealed that the lines vary by 19 μg g^{-1},

but this difference was not significant (p = 0.19), suggesting that fumonisin content data from these two locations were insufficient to detect differences previously observed between lines with quite divergent levels of fumonisin contamination resistance (Robertson *et al*., 2006). Thus, it is not surprising that, although the selected $S_{0:3}$ ReFus lines had lower mean fumonisin contents than the control $S_{0:3}$ lines, this difference was not significant.

Evaluation of Topcrosses of Selected and Unselected $S_{0:2}$ Lines

Combined analysis across environments excluding commercial checks indicated that experimental genotypes varied significantly for percent ear rot ($p \leq 0.0001$), fumonisin content (p = 0.03), and silking date ($p \leq 0.0001$), but not for grain yield or erect plants (Table 4.7). Inoculation treatment significantly affected percent ear rot (p = 0.01). Grain yield was reduced on average 0.75 Mg ha^{-1} due to inoculation, and this difference was nearly significant (p = 0.056). Genotype-by-treatment interactions were not significant for any trait (Table 4.7).

Selected topcross lines differed significantly from the Cycle 0 unselected population control. Across treatments, selected line topcrosses exhibited 1.3 percentage points less ear rot than the Cycle 0 unselected population control (p = 0.018). Under inoculated conditions, the selected topcross lines exhibited a significant reduction in mean ear rot (by 1.5 percentage points, p = 0.009) compared to the population control (Table 4.5). The ReFus selected line topcrosses had significantly (p = 0.0008) lower fumonisin content under inoculation than the unselected ReFus topcrosses by 15.2 µg g^{-1} (Table 4.5).

When compared to commercial hybrid checks, ReFus selected topcross lines did not differ significantly for percent ear rot under either inoculated or noninoculated conditions. The selected line topcrosses had greater fumonisin content (24.3 μg g^{-1}, $p = 0.0002$) under inoculation than the hybrid checks, however.

Conclusions

One generation of selection followed by self-fertilization resulted in significantly improved grain yield *per se* in selected lines compared to unselected controls of the ReFus population. The selected lines, however, did not have significantly reduced mean ear rot percentage. Given the lack of statistical power to detect a significant direct response to selection for ear rot resistance, it is not surprising, that no significant reduction was made in the amount of fumonisin present in the grain.

Although significant reductions in ear rot were not observed in $S_{0:3}$ lines when compared to the ReFus Cycle 0 population mean, realized gain in $S_{0:3}$ lines was greater than expected gain, and significant reductions in ear rot were demonstrated in $S_{0:2}$ topcrosses. Gain from selection for ear rot resistance is expected to be greater when selections are based solely on percent ear rot. However, germplasm with polygenic resistance to ear rot will be more useful as breeding material if it also possesses acceptable combining ability for yield, lodging resistance, and other agronomic traits. The diverse range of parental materials used to develop this population necessitated selection on agronomic traits as well as ear rot resistance to provide a reasonable chance of obtaining useful breeding lines. Evaluation of

a complete cycle of selection will require comparison of lines from C1 and C0 populations. Substantial progress for a single trait under index selection may not be observed until several full cycles of selection are completed.

Despite the lack of indirect response in fumonisin content observed in the $S_{0:3}$ *per se* evaluation, the topcrosses of those lines demonstrated a significant reduction in fumonisin contamination. The evaluation of topcrosses across four environments instead of only the two environments used for $S_{0:3}$ evaluations likely contributed to the greater power to detect significant differences for this trait, significant differences are observed between the selected lines and the population control for both percent ear rot and fumonisin content. Here we were able to demonstrate that lines selected for ear rot resistance resulted in hybrids with greater ear rot resistance and greater resistance to fumonisin accumulation than the population from which these lines were selected. Conversely, we were able to demonstrate improvement for grain yield *per se* in the selected lines over the population as $S_{0:3}$s, but this difference was not significant in the $S_{0:2}$ topcross hybrids.

Because ear rot is a highly quantitative trait that cannot be dramatically altered with a single generation of selection, and because we employed multiple trait selection, which reduced the potential selection differential on ear rot, several cycles of selection will likely be required to demonstrate clear responses to selection for this trait. The same is true for the other quantitative traits of interest measured in this experiment, fumonisin content, grain yield, and lodging resistance. In addition, although we demonstrated improvement for Fusarium ear rot and fumonisin contamination resistance in topcrosses resulting from

selection among $S_{0:1}$ lines, our selected lines were significantly poorer performing than the commercial check hybrids for grain yield, ear rot, and fumonisin content. The commercial checks were selected specifically because they have exhibited relatively high levels of resistance to Fusarium ear rot in previous trials (unpublished observations). Nevertheless, these results demonstrate that multiple cycles of multiple trait selection in the ReFus population will be required to develop lines that have sufficiently high levels of ear rot and fumonisin contamination resistance united with combining ability for yield to represent a useful source of germplasm for improvement of commercial U.S. maize.

Acknowledgements

This research was supported by a USDA-IFAFS multidisciplinary training grant to North Carolina State University (award number 2001-52101-11507). Many thanks to Teagen Gray, Kathleen Hamby, Heather Hunt, J. Bradsher Wilkins, Harry Wilson III and others for assistance performing inoculations, preparing samples, and processing ELISA.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Cont. AC-19: 716-723.

Baker, R.J. 1986. Selection Indices in Plant Breeding. CRC Press, Boca Raton, FL.

Betrán, J., Menz, M., and Bänziger, M. 2004. *Corn breeding*. In: Smith, C. W., Betrán, J., Runge, E. C. A., eds. Corn: Origin, History, Technology and Production. Hoboken, NJ: John Wiley & Sons, pp. 305–395.

CFSAN. 2001. Guidance for Industry: Fumonisin levels in human foods and animal feeds. Final Guidance. Available online at http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm109231.htm (verified June 10, 2009). USFDA Center for Food Safety and Applied Nutrition and the Center for Veterinary Medicine.

Clements, M. J., C.M. Maragos, J.K. Pataky, D.G. White. 2004. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopathology. 94: 251-260.

Colvin, B.M., and L.R. Harrison, 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia. 117: 79-82.

Commission of the European Communities. 2007. Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. Official J. Europ. Union. 255:14-17.

Comstock, R.E., and H. F. Robinson. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics. 4:254-266.

Eller, M.S., G.A. Payne, and J.B. Holland. 2008. Breeding for improved resistance to fumonisin contamination in maize. Toxin Reviews. 27:371-389.

Falconer, D. S. & Mackay TFC. 1996. *Introduction to Quantitative Genetics*. Fourth edition. Addison Wesley Longman, Harlow, Essex, UK.

Fusarium Research Center, http://frc.cas.psu.edu/. Verified June 10, 2009.

Gelderblom, W. C. A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar, N.P.J. Kriek. 1988. Fumonisins- novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microb. 54:1806-1811.

Goodman, M.M. 1999. Broadening the genetic diversity in Maize breeding by use of exotic germplasm. In J. G. Coors and S. Pandey, Eds. *The Genetics and Exploitation of Heterosis in Crops.* pp. 139 -148.

Goodman, M. M., Moreno, J., Castillo-Gonzalez, F., Holley, R., and Carson, M. L. 2000. Using tropical maize germplasm for temperate breeding. Maydica. 45:221–234.

Hendricks, K. 1999. Fumonisins and neural tube defects in south Texas. Epidemiology. 10:198–200.

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breeding Reviews. 22:9-111.

Holland, J.B. 2006. Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation in SAS Proc MIXED. Crop Science. 46:642-654.

Holland, J.B., and P.T. Nelson. 2009. Dedication: Major M. Goodman, maize geneticist and breeder. Plant Breeding Reviews. (accepted).

Miller, J.D. 1994. Epidemiology of *Fusarium* ear diseases of cereals. p. 19-36. *In*: J.D. Miller and H.L. Trenholm (ed.) Mycotoxins in grain. Eagan Press, St. Paul, MN.

Missmer, S.A., Suarez, L., Felkner, M., Wang, E., Merrill, A.H., Rothman, K.J., and Hendricks, K.A. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environ. Health Perspect. 114:237–241.

Mode, C.J., and H.F. Robinson. 1959. Pleiotropism and the genetic variance and covariance. Biometrics. 15:518–537.

Morgavi, D.P., and R.T. Riley. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. Animal Feed Science and Technology. 137:201–212.

Munkvold, G.P., and Desjardins, A. 1997. Fumonisins in maize: can we reduce their occurrence? Plant Disease. 81:556–565.

Ono, E.Y.S., O. Kawamura, M.A. Ono, Y. Ueno, E.Y. Hirooka. 2000. A Comparative Study of Indirect Competitive ELISA and HPLC for Fumonisin Detection in Corn of the State of Parana, Brazil. Food and Agricultural Immunology. 12:5-14.

Prelusky, D., B. Rotter, R. Rotter. 1994. Toxicology of mycotoxins. In *Mycotoxins in Grain: Compounds other than Aflatoxin*, pp. 359-403. Edited by J. Miller & H. Trenholm. St. Paul: The American Phytopathological Society.

Pollak, L.M. 2003. The history and success of the public-private project on germplasm enhancement of maize (GEM). Advances in Agronomy. 78: 45-87.

Robertson, L.A., C.E. Kleinschmidt, D.G. White, G.A. Payne, C.M. Maragos, J.B. Holland, 2006. Heritabilities and correlations of Fusarium ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Science. 46: 353-361.

Ross, P.F., L.G. Rice, G.D. Osweiler, P.E. Nelson, J.L. Richard, T.M. Wilson. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia. 117:109-114.

SAS Institute Inc. 2004. *SAS/IML 9.1 User's Guide*. Cary, NC: SAS Institute Inc.

Van Egmond, H.P., R.C. Schothorst, M.A. Jonker. 2007. Regulations relating to mycotoxins in food; Perspectives in a global and European context. Anal Bioanal Chem. 389:147–157.

Yates, I.E., Sparks, D. 2008. *Fusarium verticillioides* dissemination among maize ears of field-grown plants. Crop Protection Journal. 27:606-613.

Table 4.1. Pedigrees of 22 parent lines used to create the ReFus population.

Inbred	Pedigree
Set I	
B116	(B97 × B99)-047-1-1-1-1-1-1
B97	BSCB1(C9)
NC258	TZ(2) × [(NC248 × 246) × C103]
NC320	SC76-type (non-SS) B52
NC346	Pioneer X105A × H5
NC446	KU2301 × NC296 BC3
NC448	Pioneer X105A × H5
NC450	101-12-2-1 × NC296
NC452	NC296 × NC304
NC456	Pioneer IJ100 × PioneerX304C
NC492	NC258 × NC296
Set II	
A131	A12 × A39
GE440	Hasting's Prolific
UR13085:N0215-21-1-B-B-B	UR13085 × N02
Ki21	Pacific 9-S8-45
Ky21	Boone County White
Mo17	CI187-2 × C103
NC300	Pioneer X306B × (Pioneer X105A × H5)
NC356	TROPHY low moist. rec. selection
NC458	KU2301 × PM703
T236	T115×(I205 × L289)/×T115-S11
NC300/CML288-B-4-B-B-B-B	NC300 × CML288

Table 4.2. Family mean heritabilities and their standard errors, overall population means, and ANOVA F- tests on the ReFus Cycle 0 base population before selection was applied, conducted over two environments in 2007.

	Ear rot (%)	Grain Yield (g plant^{-1})	Lodging (%)	Tassel (DAP)	Silk (DAP)
Family Mean h^2	0.74	0.63	0.72	0.88	0.54
ReFus C0 mean	12.73	78.93	16.50	74.24†	75.52†
ANOVA F-test of base population	3.80***	2.60***	3.81***	8.13***	2.61***

†DAP = Days after planting.

Table 4.3. Phenotypic and genotypic covariance matrix estimates of Cycle 0 ReFus population estimated from the evaluation of 206 $S_{0:1}$ lines at two locations in 2007. The phenotypic and genotypic covariances are shown on the upper half of the matrices, phenotypic and genotypic correlations (r) and standard errors (SE) of correlations are shown on the lower half.

	Phenotypic covariance Matrix			Genotypic covariance matrix		
	Ear rot (%)	Grain Yield (g plant^{-1})	Lodging (%)	Ear rot (%)	Grain Yield (g plant^{-1})	Lodging (%)
Ear rot	9.13	-4.05	-4.07	5.42	-1.91	-4.05
Grain Yield	*r* = -0.21 SE = 0.04	172.95	-15.47	*r* = -0.15 SE = 0.11	97.47	-8.81
Lodging	*r* = -0.13 SE = 0.04	*r* = -0.09 SE= 0.04	35.32	*r* = -0.26 SE = 0.10	*r* = -0.1 SE = .13	25.67

Table 4.4. Analysis of variances on selected ReFus $S_{0:3}$ lines over two environments in 2008. ANOVA (Overall F-test) was conducted on the complete data set, and contrasts estimated between resistant and susceptible checks (GE440 –FR1064). Checks and parents were then excluded from the dataset and ANOVA conducted on selected ReFus $S_{0:3}$ entries (significance of genotype F-test) to determine the overall F-test significance of effects for genotype.

	Overall F-test	GE440 – Fr1064	significance of genotype F-test
Ear rot (%)	9.4***	-28.97**	16.91***
Weight (g plant^{-1})†	7.01***	-10.16^{ns}	2.62^{ns}
Fumonisin (µg g^{-1})	1.8*	-19.24^{ns}	2.24*

† Weight is based on data from one location only.

ns = not significant at $p = 0.05$.

*, **, *** = significant at $p = 0.05$, 0.01, and 0.001, respectively.

Table 4.5. Entry means from $S_{0:1}$ selected lines in the selection study (conducted in 2007 at two locations), selected lines advanced to the $S_{0:3}$ generation in the testing study (conducted in two locations in 2008), and advanced as topcrossed $S_{0:2}$ to the early generation testing study (four locations in 2008). Differences between the selected lines and checks or controls used in each study are reported.

	Selection Study (2007)			$S_{0:3}$ line Testing Study (2008)		
	Ear rot (%)	Yield (g plant^{-1})	Lodging (%)	Ear rot (%)	Yield[†] (g plant^{-1})	Fumonisin (µg g^{-1})
ReFusC0-010	9.32	100.12	08.46	37.89	41.24	39.98
ReFusC0-016	8.92	102.06	39.41	26.29	46.41	32.25
ReFusC0-020	12.67	96.74	15.08	24.15	72.42	43.08
ReFusC0-032	7.04	89.78	34.10	8.33	55.86	13.51
ReFusC0-046	11.24	112.04	13.53	33.73	53.77	24.42
ReFusC0-051	14.91	100.18	10.41	39.01	64.03	20.66
ReFusC0-064	12.12	111.52	19.78	17.73	45.09	16.87
ReFusC0-089	23.95	110.91	14.40	82.38	56.55	55.45
ReFusC0-108	8.93	94.15	12.41	11.67	39.68	14.98
ReFusC0-124	7.66	97.07	22.82	20.29	37.50	20.23
ReFusC0-129	13.60	106.53	08.33	18.70	58.98	17.00
ReFusC0-131	5.94	90.03	18.47	26.29	71.93	40.25
ReFusC0-142	9.44	96.53	10.52	24.35	48.09	17.27
ReFusC0-143	7.72	110.44	12.47	16.30	66.96	22.94
ReFusC0-147	6.36	99.33	35.19	12.75	88.37	14.06
ReFusC0-154	10.35	94.24	30.79	21.27	48.56	26.41
ReFusC0-157	9.66	93.26	0.00	52.69	60.13	17.09
ReFusC0-161	6.19	93.78	11.52	25.67	64.15	26.97
ReFusC0-173	17.23	113.61	10.05	37.06	76.41	54.12
ReFusC0-177	8.99	92.04	1.01	64.45	28.35	61.68
Mean of Selected lines	10.61	100.22	16.39	30.05	56.22	28.96
Population mean	12.74	78.01	17.1	-	-	-
C0 Control mean	-	-	-	35.01	45.44	32.43
Selected Mean- C0 control mean	-	-	-	-4.96^{ns}	10.78**	-2.68^{ns}
LSD[§]	8.51	24.97	21.95	19.14	20.27	29.37

[†] $S_{0:3}$ grain yield was measured at only 1 location in 2008.
[‡] Back transformed from the natural log.
[§] LSDs are appropriate for pair wise comparisons.
ns = not significant at $p = 0.05$
$^{*, **, ***}$ = significant at $p = 0.05$, 0.01, and 0.001, respectively.

Table 4.5 continued.

	Early generation $S_{0:2}$ Topcross Testing Study					
	Inoculated Ear rot (%)‡	Noninoculated Ear rot (%)‡	Inoculated Yield (Mg ha^{-1})	Noninoculated Yield (Mg ha^{-1})	Fumonisin (µg g^{-1})	Erect Plants (%)
ReFusC0-010	15.30	3.97	5.34	6.74	50.23	0.97
ReFusC0-016	11.04	4.48	6.25	6.76	44.42	0.97
ReFusC0-020	13.40	1.94	6.48	7.59	73.31	0.95
ReFusC0-032	7.90	2.33	6.25	7.46	45.85	0.94
ReFusC0-046	12.17	3.42	6.40	7.08	51.34	0.93
ReFusC0-051	18.28	4.33	6.43	7.63	65.83	0.97
ReFusC0-064	23.89	5.93	6.85	7.92	59.69	0.95
ReFusC0-089	45.47	10.15	6.01	7.29	75.43	0.97
ReFusC0-108	17.15	4.55	5.94	7.23	45.58	0.99
ReFusC0-124	12.29	2.24	6.37	7.28	59.52	0.95
ReFusC0-129	14.07	3.55	6.42	7.46	45.37	0.98
ReFusC0-131	18.02	3.90	6.46	7.56	62.87	0.94
ReFusC0-142	18.46	4.80	6.46	7.41	57.55	0.93
ReFusC0-143	10.53	3.24	6.66	7.45	40.38	0.98
ReFusC0-147	11.09	4.37	5.99	6.91	48.40	0.95
ReFusC0-154	17.57	4.95	6.35	8.04	75.98	0.97
ReFusC0-157	21.37	2.53	6.05	7.22	63.30	0.99
ReFusC0-161	12.44	4.99	6.78	7.87	50.54	0.97
ReFusC0-173	26.44	4.42	6.70	7.55	55.33	0.97
ReFusC0-177	15.67	3.35	6.47	7.23	38.13	0.95
Selected lines mean	17.13	4.17	6.33	7.38	55.45	0.96
C0 control mean	24.07	4.90	6.15	7.26	70.63	0.97
Selected line mean- C0 control mean	-1.37*		0.15ns		-	-0.66*
Selected line mean- C0 control treatment mean	-1.55**	-1.24ns	0.18ns	0.12ns	-15.18***	-
Commercial check mean	16.63	6.42	7.60	8.67	31.13	0.97
Commercial check – Selected line mean	-1.02^{ns}		1.33***		-	1.1*
Commercial check - Selected line treatment mean	-1.12^{ns}	-1.15^{ns}	1.33***	1.33***	-24.31**	-
LSD§	1.88‡	2.03‡	0.91	0.81	27.31	

† $S_{0:3}$ grain yield was measured at only 1 location in 2008.
‡ Back transformed from the natural log.
§ LSDs are appropriate for pair wise comparisons.
ns = not significant at $p = 0.05$
*, **, *** = significant at $p = 0.05$, 0.01, and 0.001, respectively.

Table 4.6. Gain from selection estimated from heritabilities over two locations in 2007 in the selection environment, and from means of selected line $S_{0:3}$s and random control unselected $S_{0:3}$ lines in two locations in 2008 in the testing environment. Selected line averages across selection environments, and testing environments. Expected and realized gains from selection, and the percentage of the mean population value those gains account for.

	Ear Rot (%)	Yield (g plant^{-1})	Lodging (%)[†]
Selected $S_{0:2}$ average (2007)	10.6	100.2	16.4
Selected $S_{0:3}$ average (2008)	30.1	56.2	-
Expected gain (R)	-1.57	13.99	-0.4
R as a percent of population mean	-0.12	.24	-2.6
Observed gain (R_{obs})	-4.96	10.78	-
R_{obs} as a percent of the population control mean	-0.14	.18	-

[†]Lodging was not measured in the selected $S_{0:3}$s in 2008, minimal levels occurred.

Table 4.7. ANOVA F-tests on early generation ReFus C0 selected $S_{0:2}$ topcrosses over four environments in 2008 with commercial checks excluded from the dataset. Genotype, inoculation treatment, and genotype-by-treatment interaction effects are reported.

Source of variation	Ear rot[†] (%)	Fumonisin (μg g^{-1}) [‡]	Grain Yield (Mg ha^{-1})	Erect Plants (% of row)	Silking Date[§] (DAP)
Genotype	4.05***	1.88*	1.18^{ns}	1.4^{ns}	3.8***
Treatment	14.07**	-	9.03^{ns}	1.2^{ns}	5.4*
Genotype × treatment	1.13^{ns}	-	0.6^{ns}	$.78^{ns}$	$.58^{ns}$

*, **, *** = significant at p = 0.05, 0.01, and 0.001, respectively.

ns not significant at p =.05.

† The natural log of percent ear rot was used to calculate F-tests.

‡ Fumonisin analysis was only conducted on inoculated sub plots.

§Flowering time data was recorded at Clayton only.

CHAPTER 5: Increased Yield Conferred by a Tropical Maize-Derived QTL Allele Identified in a Mapping Population Derived from Highly Related Lines

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Abstract

Tropical maize germplasm represents an important source of rare or unique alleles that could contribute to improved productivity of temperate maize. Previous breeding work identified highly related "sister" lines derived from a common F_4 parent that differed substantially for grain yield potential in topcrosses. The F_4 parent was obtained from a cross between a tropical hybrid and a U.S. commercial inbred as part of the Germplasm Enhancement of Maize project. To identify the genome region(s) conferring this yield difference in a nearly-isogenic genetic background and to determine if the tropical hybrid parent was the source of the favorable allele(s), the phenotypically distinct sister lines were crossed to develop a segregating $F_{2:3}$ population. SSR markers were screened on the F_5 lines, the original tropical parent, and a segregating F_2 bulk of the tropical by temperate cross to identify genomic regions that were segregating in the $F_{2:3}$ population and to identify the founder origin of the alleles carried by each sister line. A sample of 39 $F_{2:3}$ lines was

genotyped at polymorphic markers, and topcrossed to a common tester line. Topcrosses were evaluated for grain yield and other agronomic traits in replicated trials in two environments in 2005. QTL mapping revealed a grain yield QTL in chromosomal bin 3.06, at which the alleles derived from the tropical hybrid founder were associated with increased yield. These results directly demonstrate the advantage conferred by an allele of tropical origin over an elite commercial U.S. inbred line for yield under U.S. growing environments.

Introduction

QTL mapping populations range from widely segregating parental crosses to near-isogenic line pairs that typically segregate only for small genomic regions. Traditionally, backcross- or F_2- derived populations, including recombinant inbred lines, are developed from genetically distinct parental lines to increase the frequency of segregating QTL and markers (Lynch and Walsh, 1997). Large samples sizes are required to obtain accurate estimates of the effects of numerous QTL if they are segregating simultaneously in a population (Beavis, 1994; Melchinger *et al.*, 1998; Schon *et al.*, 2004; Vales *et al.*, 2005). Alternatively, near isogenic lines (NILs) limit the genome differences between each line and its recurrent parent, improving reliability of QTL effect estimation in the tested regions (Eshed and Zamir, 1995; Szalma *et al.*, 2007). NILs, however, require substantial effort to backcross specific genome regions into the recurrent parent, limiting their widespread use. Heterogeneous inbred families (HIFs, Tuinstra *et al.*, 1997) represent a similar approach that is used to fine-map QTL previously mapped in a single genomic region segregating within an RIL. As an intermediate alternative to the extremes of widely segregating crosses used for whole-genome scans and NIL-type populations used for fine mapping selected regions, we demonstrate here QTL mapping in a cross between related inbred lines derived from a common partially inbred parent. This approach has some of the advantages of NIL mapping (e.g., reduced background genetic segregation) but rather than targeting a previously identified QTL region (e.g., Yamamoto *et al.*, 1998) or randomly sampling genome segments (e.g., Monforte and Tanksley, 2000; Szalma *et al.*, 2007), this method

begins with the identification of a phenotypic difference segregating between highly related "sister" lines descended from a common partially inbred parent plant.

Because the two parental lines are closely related, genetic variation is limited to only a fraction of the genome. More extensive initial parental surveys for marker polymorphisms are required to identify polymorphic genome regions. However, with a smaller population sample and thorough parental screens, it is possible to accurately identify and estimate the effects of one or a few segregating QTL in an otherwise highly homogeneous genetic background. Although the proportion of polymorphic markers will be smaller in related line crosses, the chance that a polymorphic marker is associated with a phenotypic difference increases because the sample of polymorphic loci is enriched with those linked to QTL. This approach should decrease the time and expense of genetic mapping by reducing the population size and the number of markers tested on the progeny.

We tested this method of mapping QTL in crosses between highly related lines by examining a population derived from related parents developed through the USDA Germplasm Enhancement of Maize (GEM). The GEM program (Pollak, 2003) is a collaborative effort between universities, private companies, and the USDA which focuses on enhancing the genetic diversity of maize grown in the United States. Crosses are made between tropical and U.S. germplasm by cooperating companies, segregating F_2 lines are distributed to cooperating universities, and new inbred lines are selected and released to participating companies and public institutions. A number of lines with good agronomic potential and disease resistance have been publicly released from the GEM program (Abel

et al., 2001; Pratt, Pollak, and Montgomery, 2005; Balint-Kurti *et al.*, 2006; Carson *et al.*, 2006) but direct demonstration that tropical germplasm contributed to improvements in these lines has been hindered because the proprietary nature of one parent line prevents genetic screening by public sector researchers.

In one population created through the GEM program, topcross grain yields were observed to differ substantially between a pair of F_5 lines descended from a common F_4 ancestor across a large number of environments. To identify the genome region(s) controlling this difference, we developed a segregating $F_{2:3}$ population from a cross of the two F_5 sister lines. Here we demonstrate the effectiveness of QTL analysis in a highly related population derived from a cross between a tropical commercial hybrid and a U.S. inbred line and identify the origin of the allele contributing to increased grain yield.

Materials and Methods

Population Development

A population was developed by a GEM collaborator between a Brazilian tropical single cross hybrid, Dekalb XL212, and a proprietary non-stiffstalk inbred coded N11a. Seed of this cross was selfed to the F_5 generation by ear to row method and evaluated in topcross trials for yield. Yield trials were conducted in 17 environments using two different testers. A 2.01 Mg ha^{-1} difference ($LSD_{.05}$ =0.66 Mg ha^{-1}, $p = 0.05$) was observed between two lines with a common F_4 ancestor when using a B73 type tester. A significant yield difference (0.87 Mg ha^{-1}, $LSD_{.05}$ =0. 70 Mg ha^{-1}, $p = 0.05$) was also observed when an

LH198 tester was used (Hawk, unpublished). The higher-yielding line was released by the University of Delaware as DE4 (Hawk and Weldekidan, 2005). Its lower-yielding "sister" line, 365-1-1-1, was not released. Since these lines share a common F_4 progenitor they are expected to be 92% genetically similar. To identify the genome region(s) underlying this large difference in topcross yield, DE4 was crossed to 365-1-1-1 to form a mapping population comprised of 39 random $F_{2:3}$ lines.

PCR Methods

Leaf tissue was collected from eight plants of each parent and mapping line, and from 50 plants of the XL212 × N11a F_2 bulk. DNA was extracted from ground tissue using a standard C-TAB extraction with three ethanol washes or using a Qiagen MaxiPrep DNA Easy kit (Qiagen, Inc., Valencia, CA) and samples were bulked by line. SSR (Simple Sequence Repeat) markers were chosen based on Intermated B73 × Mo17 (IBM) map position in the Maize Genetic and Genomic Database (maizegdb.org, verified June 10, 2009) and assayed using the PCR (Polymerase Chain Reaction) method (Senior, 1998). PCR amplification products were separated by electrophoresis on 4% (v/w) SFR agarose gels (Amresco, Solon, OH). Gels were stained with 0.05% (v/w) ethidium bromide and exposed to ultra-violet light to fluoresce DNA.

Parental Screens

SSR markers were used to genotype DE4, 365-1-1-1, XL212 (the tropical parent of DE4), and a bulk of F_2 seed made by intermating the original F_1s of the XL212 × N11a

cross to identify polymorphic genome regions between the sister lines and to identify the founder sources of the alleles carried by the two sister lines. With representatives of two parental generations, markers were scored as follows: non-informative markers produced a single amplicon of the same size in XL212 and the N11a × XL212 F_2 bulk, and therefore did not allow classification of the amplicon fluoresced in DE4 and 365-1-1-1. Informative markers produced at least one unique amplicon in N11a × XL212 F_2 bulk compared to XL212, making it possible to determine which parent contributed alleles to DE4 and 365-1-1-1. Informative markers were then divided into two classes: monomorphic when the same amplicon appeared in DE4 and 365-1-1-1, and polymorphic when DE4 and 365-1-1-1 contained different amplicons (Figure 1.) With this method it was possible to determine identity by descent relationships between each sister line and XL212 and N11a for each informative marker locus.

The maize genome is divided into 100 bins, representing approximately even genetic distances over each of the 10 chromosomes (Davis *et al.*, 1999). A region was determined to be monomorphic if 1) four markers in that bin were non-informative, 2) if two markers were informative but monomorphic in that bin or 3) if one informative monomorphic, and four non-informative markers were mapped to a bin.

Progeny Screens

Progeny screens were conducted on the 39 $F_{2:3}$ lines derived from the DE4 × 365-1-1-1 F_1 for informative polymorphic markers identified with the parental screens. Loci were

ordered based on their IBM consensus order, and, where multiple markers in a region were polymorphic by linkage analysis, using MapmakerEXP (Whitehead Institute, Cambridge, MA).

Phenotypic Data Collection

The $F_{2:3}$ lines were topcrossed to the public tester B73*Ht* and evaluated at two Delaware locations (Magnolia and Smyrna) in 2005. A randomized complete block design with two replications was grown at each location. In addition to the 39 $F_{2:3}$ mapping lines, entries included three commercial hybrid checks, the topcross of each parent (DE4 and 365-1-1-1), and the topcross of the DE4 × 365-1-1-1 F_1. Each plot was comprised of four rows 4.57 m in length, with a 0.71 m ally between ranges of plots. Inter-row spacing was 0.76 m. Planting density was about 74,000 plants per hectare.

Stand counts were taken in the center two rows of each plot. Plant height was measured to the height of the node from which the tassel emerges. Ear height was measured to the node from which the topmost ear emerges. Plant and ear heights were measured to the nearest 5 cm on six different plants from the two center rows of each plot.

Days to anthesis were recorded as the number of days since planting on which 50% of the plants in the plot have shed some pollen. Days to mid silk were recorded as the number of days since planting on which 50% of the plants in the plot have visible silk emergence. Measurements of flowering time were taken at both locations. Ears per plant

were counted on the two center rows of each plot. An ear was considered any cob with one or more kernels.

The two center rows of each plot were mechanically harvested to measure grain yield and grain moisture. Grain yield for each plot was adjusted to 155 g kg^{-1} grain moisture before analysis. Two primary ears were pulled from the outside rows of each plot by hand for measurements on ear traits. Ear length was measured to the nearest cm and the number of rows per ear was counted. Ears were shelled and total grain was weighed. A sample of 100 randomly-chosen kernels was weighed to estimate 100-kernel weight. The number of kernels per ear was then estimated according to the following formula: Estimated number of kernels per ear = [(total grain weight/ weight of 100 kernels) * 100]. Percent grain fill was estimated visually. Individual ear measurements were averaged over ears within each plot before further analysis.

Statistical Analysis

Traditional analyses of variance for the randomized complete block design were compared to spatial analyses of grain yield data from each environment separately using Proc MIXED in SAS version 9.1 (SAS Institute, 2004). Spatial models tested included field row and column trend analyses, isotropic and anisotropic correlated errors models (Brownie *et al.*, 1993). Spatial analyses did not improve the efficiency of the analyses, so were not used further. Therefore, all traits were analyzed with a combined mixed model for data from both environments where stand within location and entry were considered fixed, and

location, replication within location, and location × entry were considered random variables. A model which fit stand as a covariate, and accounted for heterogeneous error variance among the two environments was used to for all traits. The null hypothesis of no difference between the two F_4 derived F_5 lines was tested with estimate statements in Proc MIXED for each trait. Parents and checks were then removed from the dataset, and the same model was used to test the null hypothesis of no variation among $F_{2:3}$ topcrosses with the F-test for entry. Finally, a completely random model was applied to the data set containing only $F_{2:3}$ topcrosses to obtain genotypic variance component and heritability estimates.

The CORR procedure in SAS was used to estimate Pearson correlation coefficients among traits using the least squared means of each trait over both environments.

QTL Mapping

Genotype least square means were obtained for each trait from the combined analysis model including heterogeneous within-environment error variances. One-way analyses of variance were conducted for each informative polymorphic SSR marker to test the null hypothesis of no difference between genotypic class means. A simple model where the marker was fixed, and all other factors were considered random was run in proc GLM of SAS. For markers detected with significant effects, regression of marker scores onto phenotypic trait variation was performed to determine the percent of the variation which each marker explained. For regions with more than one polymorphic marker, linkage group maps were established using Mapmaker/EXP. Those regions were then analyzed with

Windows QTL Cartographer version 2.5 (Wang *et al.*, 2005) using single marker and interval mapping methods.

Results and Discussion

Marker Assay

A total of 702 markers were assayed in parental screens, 477 of which were reliably scorable. This resulted in a minimum of two markers per bin, with an average bin density of 4.8 markers. Of the scorable markers, 79 (17%) were informative but monomorphic and 26 (5%) showed polymorphism between DE4 and 365-1-1-1. The remaining 78% of markers were non-informative.

Pedigree relationship suggests that about 8% of the genomes of DE4 and 365-1-1-1 are expected to be not identical by descent. The polymorphic markers identified in this study account for 6% of the IBM total map length, in good agreement with the expectation. Regions polymorphic between DE4 and 365-1-1-1 were found on all chromosomes. Chromosomes 1, 2,4, 5, 7, 8 and 10 each only showed 1 polymorphic region. Chromosome 6 had 3 segregating regions and chromosomes 3 and 9 both had 4 segregating regions.

Phenotypic Variation Among Topcrosses

The DE4 topcross had 1.93 Mg ha^{-1} greater grain yield than its sister line's topcross (Table 5.1), congruent with previous observations of the yield potential difference between the two sister lines, which prompted this study. In addition, DE4 had significantly higher

grain percent moisture (3.6%), ear height (16.7cm), and plant height (17.5 cm) than 365-1-1-1 topcross lines. Silk emergence occurred 1.6 days earlier in the DE4 topcrosses than in the 365-1-1-1 topcross, but anthesis was not significantly different between DE4 and 365-1-1-1.

Analysis of variance on the ear traits that were taken to elucidate a mechanism for the observed difference in combining ability between the two parents revealed the DE4 topcross had significantly higher ear weight (47.3 g), number of kernels per ear (132.4), and ear length (2.3 cm), but was not significantly different than the 365-1-1-1 topcross for 100 kernel weight, average ear fill, number of ears per plant or number of rows per ear (Table 5.1).

To determine if genotypic variation among the segregating F2:3 line topcrosses was significant, analysis of variance for grain yield was performed across locations with checks and parental topcrosses removed. Grain yield varied significantly among $F_{2:3}$ entries ($p=0.03$) (Table 5.1). Significant differences among $F_{2:3}$ entries were also observed for grain percent moisture ($p=0.0006$), ear height ($p=<0.0001$), plant height ($p=0.0003$), but not for ears per plant, days to anthesis or days to mid silk.

A relatively low heritability was estimated for days to anthesis (0.29), moderate heritabilities were estimated for grain yield (0.50), grain percent moisture (0.52), and days to mid silk (0.50), while plant and ear height both had high heritabilities (0.74 and 0.85 respectively). Ear weight heritability was estimated at 0.24 and heritability for number of kernels at 0.66. Heritability estimates for ear length, 100 k weight, ear fill, and 100 kernel

weight were zero because estimates of the entry variance component were zero for these traits. Grain yield was positively correlated with plant height (r = 0.42) and ear height (r = 0.56) but not with flowering time, or any of the ear traits measured (Table 5.2).

QTL Mapping

Four small linkage groups were identified: an 18.2 cM region which includes regions of IBM bins 9.03 and 9.04, a 14.1 cM region the covers parts of bins 4.06 and 4.07, a 33.5 cM region in bins 3.06 and 3.07, and a group that has two markers in bin 3.05 (Figure 2).

Four polymorphic markers with significant effects for grain yield were identified in the linkage group around bin 3.06. Umc2050, bnlg1160, umc2169, and umc2076, with estimated additive effects of between 0.30, and 0.46 Mg ha^{-1}, individually explained 25, 21, 15, and 18% of the total variation, respectively (Table 5.1). These four markers were identified as linked to a grain yield QTL based on analyses in both Proc GLM in SAS and in Windows QTL Cartographer.

Interval mapping identified the maximum likelihood peak position of the grain yield QTL in this region as position 29.7 cM of linkage group, flanked by markers umc2169 and umc2050. At this position, the LOD score for grain yield is 2.66 and the estimated additive effect is 0.53 Mg ha^{-1}. The 2-LOD support interval for this QTL includes the entire linkage group, about 30 cM (Figure 3). The high LOD support for this QTL suggests that our sample of 39 lines was sufficient for detecting this QTL of large effect on yield. The broad

support interval, however, reveals a limitation of QTL mapping with a relatively small population, which is low precision on the QTL position estimate.

Since the goal of the GEM program is to introduce diverse germplasm that positively impacts U.S. maize diversity, it is of interest which founder parent contributed the favorable allele at the chromosome 3 grain yield QTL. Without access to seed of the proprietary inbred parent N11a, it was still possible to determine the parental origin of alleles in the mapping population by comparing XL212 to a bulk F_2 population derived from intermating several F_1s from the original cross. At the umc2050 and umc2076 loci, the DE4 amplicon is identical to that of XL212, indicating that XL212 contributes the favorable allele. At the bnlg1160 and umc2169 loci, XL212 is heterozygous and the DE4 amplicon matches one of the XL212 amplicons, but is not present in the XL212:N11a DNA. The absence of these bands in the XL212:N11a bulk DNA is likely due to allelic sampling variation in the formation of the XL212:N11a bulk combined with allelic competition for PCR amplification (Wattier *et al*., 1998). These results indicate that a linkage block encompassing all four marker loci in the region of this QTL was inherited from the tropical parent, XL212, and that the XL212 allele at the QTL in this region confers greater topcross yield.

Markers that were associated with overall differences in grain yield (linkage group in bins 3.05-3.06) were also associated with ear height (umc2076 and umc2169) and moisture (umc2076; Table 5.1). When interval mapping was performed in Windows QTL Cartographer, the ear height QTL's maximum likelihood position is directly at marker

umc2076 (position 0 cM) and is associated with a LOD score of 1.98. The grain moisture QTL covers the first 2 cM of the linkage group, with its highest point at 0 cM, directly over umc 2076, where it has a maximum LOD score of 2.21, and an additive effect of 0.83 percentage points. Although other markers have main effects significant at $p < 0.05$, none have LOD scores greater than 2.0 (Table 5.1). These results suggest that the allele at the grain yield QTL also causes greater grain moisture at harvest or is linked to an allele that increases grain moisture and probably also ear height. These pleiotropic or linked correlated effects are unfavorable, but do not eliminate the overall positive contribution to crop value provided by the favorable grain yield QTL allele.

This study provides direct evidence that incorporating a specific allele from tropical germplasm can add value to the genetic base of U.S. maize. Previous studies have shown that tropical germplasm is a valuable breeding resource (Goodman,1999; Goodman *et al.*, 2000) and that tropical-derived alleles have been recovered in superior progeny derived from crosses between tropical and temperate parents (Lewis and Goodman, 2003; Tarter *et al.*, 2004).

This study is the first to our knowledge to demonstrate that an allele of tropical origin contributed to higher grain yield compared to an elite commercial inbred maize line. It must be noted however, that exotic commercial lines often contain U.S. germplasm. In this case the final population used by Jim Hawk was probably 87.5% US germplasm even though XL212 is an exotic line (Walter Trevisan, personal communication). The additive genetic effect of this allele in topcrosses was 0.53 Mg ha^{-1}. Thus, topcrosses of lines

homozygous for this allele had an average of 1.06 Mg ha^{-1} yield compared to topcrosses of lines homozygous for the temperate parent allele.

This allelic difference for yield potential was measured in lines with approximately 94% identity by descent, suggesting that the estimated genetic effect was measured reliably, and was not confounded by segregation of many yield QTL throughout the genome. The large effect conferred by this QTL and the high genetic homogeneity of the mapping population permitted efficient use of a relatively small population sample size to identify the QTL. A much larger population sample will be required to map this QTL to a higher level of resolution, however.

References

Abel, C.A., L.M. Pollak, W. Salhuana, M.P. Widrlechner and R.L. Wilson. 2001. Registration of GEMS-0001 maize germplasm resistant to leaf blade, leaf sheath, and collar feeding by European corn borer. Crop Science. 41:1651-1652.

Balint-Kurti, P.J., M. Blanco, M. Millard, S. Duvick, J. Holland, M. Clements, R. Holley, M.L. Carson and M.M. Goodman. 2006. Registration of 20 GEM maize breeding germplasm lines adapted to the Southern USA. Crop Science. 46:996-998.

Beavis, W.D. 1994. The power and deceit of QTL experiments: Lessons from comparative QTL studies. *In:* D. B. Wilkinson (ed.) 49th Ann Corn Sorghum Res Conf. Am Seed Trade Assoc., Chicago, IL. pp. 250-266.

Brownie, C., D.T. Bowman, J.W. Burton, 1993. Estimating spatial variation in analysis of data from yield trials: a comparison of methods. Agron. J. 85: 1244-1253.

Carson, M.L., P.J. Balint-Kurti, M. Blanco, M. Millard, S. Duvick, R. Holley, J. Hudyncia, and M.M. Goodman. 2006. Registration of nine high-yielding tropical by temperate maize germplasm lines adapted for the Southern USA. Crop Science. 46:1825–1826.

Davis, G.L., M.D. McMullen, C. Baysdorfer, T. Musket, D. Grant, M. Staebell, G. Xu, M. Polacco, L. Koster, S. Melia-Hancock, K. Houchins, S. Chao, and E.H. Coe, Jr. 1999. A maize map standard with sequenced core markers, grass genome reference points and 932 Expressed Sequence Tagged sites (ESTs) in a 1736-Locus Map. Genetics. 152:1137-1172.

Eshed, Y., and D. Zamir. 1995. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics. 141: 1147-1 162.

Goodman, M.M. 1999. Broadening the genetic diversity in Maize breeding by use of exotic germplasm. In J. G. Coors and S. Pandey, Eds. *The Genetics and Exploitation of Heterosis in Crops.* pp. 139 -148.

Goodman, M. M., J. Moreno, F. Castillo-Gonzalez, R. Holley, and M.L. Carson. 2000. Using tropical maize germplasm for temperate breeding. *Maydica.* 45:221–234.

Hawk, JA, and T. Weldekidan. 2005. Registration of DE3, DE4, and DE5 parental inbred lines of maize. Crop Science. 45:2269-2270.

Lewis, R.S. and M.M. Goodman. 2003. Incorporation of tropical maize germplasm into inbred lines derived from temperate × temperate-adapted tropical line crosses: agronomic and molecular assessment. Theoretical and Applied Genetics. 107: 798-805.

Lynch, M., B. Walsh, Genetics and Analysis of Quantitative Traits (Sinauer, Sunderland, MA, 1998).

Melchinger, A.E., H.F. Utz and C.C. Schon. 1998. Quantitative Trait Locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics. 149: 383–403.

Monforte, A.J. and S.D. Tanksley. 2000. Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: A tool for gene mapping and gene discovery. Genome. 43: 803–813.

Pollak, L.M. 2003. The history and success of the public-private project on germplasm enhancement of maize (GEM). Advances in Agronomy. 78: 45-87.

Pratt, R.C., L.M. Pollak and K.T. Montgomery. 2005. Registration of maize germplasm line GEMS-0002. Crop Science. 45:2130-2131.

SAS Institute Inc. 2004. *SAS/IML 9.1 User's Guide*. Cary, NC: SAS Institute Inc.

Schön, C.C., H.F. Utz, S. Groh, B. Truberg, S. Openshaw, and A.E. Melchinger. 2004. Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics. 167:485-498.

Senior, M.L., J.P. Murphy, M.M. Goodman, and C.W. Stuber. 1998. Utility of SSRs for determining similarities and relationships in maize using an agarose gel system. Crop Science. 38:1088–1098.

Szalma, S.J., B.M. Hostert, J.R. LeDeaux, C.W. Stuber and J. B. Holland. 2007. QTL mapping with near-isogenic lines in maize. Theoretical and Applied Genetics. 114:1211–1228.

Tarter, J.A., M.M. Goodman, and J.B. Holland. 2004. Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperate line. Theoretical and Applied Genetics. 109:609-617.

Tuinstra, M.R., G. Ejeta and P.B. Goldsbrough. 1997. Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. Theoretical and Applied Genetics. 95:1005-1011.

Vales, M.I. , C.C. Schön, F. Capettini, X.M. Chen, A.E. Corey, D.E. Mather, C.C. Mundt, K.L. Richardson, J.S. Sandoval-Islas, H.F. Utz, P.M. Hayes. 2005. Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. Theoretical and Applied Genetics. 111: 1260–1270.

Wang S., C.J. Basten, P. Gaffney, and Z.-B. Zeng. 2005. Windows QTL Cartographer 2.5 User Manual. Available online at: http://statgen.ncsu.edu /qtlcart/WQTLCart.htm (verified June 10, 2009). NC State University Bioinformatics Research Center, Raleigh, NC.

Wattier R., C.R. Engel, P. Saumitou-Laprade, and M. Valero. 1998. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). Mol. Ecol. 7: 1569–1573

Yamamoto, T., Y. Kuboki, S. Y. Lin, T. Sasaki and M. Yano.1998. Fine mapping of quantitative trait loci Hd-1, Hd-2 and Hd-3, controlling heading date of rice, as single Mendelian factors. Theoretical and Applied Genetics. 97:34-37.

Table 5.1. ANOVA, Contrasts, and estimates of marker effects for grain yield, grain percent moisture, number of ears per plant, 100 kernel weight, ear weight, ear fill, number of kernels per year, ear length, ear fill, number of kernels per row, ear height, plant height, silk date and anthesis. Analysis of variance was conducted on $F_{2:3}$ topcross lines with the commercial hybrid checks and parental topcrosses removed. Contrasts between parental topcrosses are estimated using the complete data set.

	Grain yield (Mg ha^{-1})	Grain Moisture (%)	Ears per Plant	100 kernel weight (g)	Ear weight (g)	Kernels per Ear	Ear length (cm)	Ear fill (%)	Rows per Ear	Ear height (cm)	Plant height (cm)	Silk Date (DAP)	Anthesis Date (DAP)
F-test	1.9*	2.1**	0.8 ^{ns}	1.1 ^{ns}	1.5 ^{ns}	1.9*	0.8 ^{ns}	0.9 ^{ns}	0.8 ^{ns}	4.1***	3.2***	1.6 ^{ns}	1.4 ^{ns}
Difference between parental topcrosses	1.93*	3.6***	-0.001 ^{ns}	1.4 ^{ns}	47.3**	132.4*	2.3***	0.06 ^{ns}	0.79^{ns}	16.69***	17.5**	1.6 **	1.1 ^{ns}
Bnlg1233											-3.93**		
IBM 509.2; bin 2.08											$r^2 = 0.29$		
Umc1027						17.7*							
IBM 401.2; bin 3.06						$r^2 = 0.05$							
Umc2076	0.46**	0.61**								2.1*			
IBM 461.1; bin 3.06	$r^2 = 0.18$	$r^2 = 0.22$								$r^2 = 0.17$			
Bnlg1160	0.43**												
IBM 491.4; bin 3.06	$r^2 = 0.21$												
Umc2169	0.30*									1.8*			
IBM 491.4; bin 3.06	$r^2 = 0.15$									$r^2 = 0.12$			
Umc2050	0.44**												
IBM 538.3; bin 3.07	$r^2 = 0.25$												
Dupssr34						23.3**							
IBM 409.4; bin 4.07						$r^2 = 0.25$							
Bnlg 1444				-0.93*							3.3*		
IBM462.5; bin 4.08				$r^2 = 0.14$							$r^2 = 0.05$		
Bnlg1131		0.44*											
IBM628.2; bin 8.09		$r^2 = 0.16$											
Umc1589										2.2*			
IBM 259.7; bin 10.04)										$r^2 = 0.12$			

*, **, *** p-values of 0.05, 0.01. and 0.001 respectively.
ns = not significant at P=0.05
DAP days after planting

Table 5.2. Correlations among $F_{2:3}$ topcross least squared means with checks and parents removed.

	Grain Yield (Mg ha^{-1})	Weight of 100 kernels (g)	Average Ear Weight (g)	Average kernels per Ear	Average Ear Length (cm)	Average number of rows per ear	Grain Moisture (%)	Plant Height (cm)	Ear Height (cm)	Anthesis Date (DAP)	Silk Date (DAP)
Grain Yield (Mg ha^{-1})	1	0.13^{ns}	0.06^{ns}	-0.05^{ns}	0.24^{ns}	-0.02^{ns}	0.30^{ns}	0.42**	0.56***	-0.06^{ns}	-0.08^{ns}
Weight of 100 kernels (g)		1	0.64***	-0.13^{ns}	0.47**	-0.18^{ns}	0.27^{ns}	-0.28^{ns}	-0.01^{ns}	0.08^{ns}	-0.21^{ns}
Average Ear Weight (g)			1	0.67***	0.60***	0.37*	0.26^{ns}	-0.05^{ns}	0.03^{ns}	0.08^{ns}	-0.07^{ns}
Average kernels per Ear				1	0.33*	0.66***	0.05^{ns}	0.20^{ns}	0.08^{ns}	0.02^{ns}	0.10^{ns}
Average Ear Length (cm)					1	0.03^{ns}	0.30^{ns}	0.07^{ns}	0.17^{ns}	0.23^{ns}	0.06^{ns}
Average Rows per Ear						1	-0.17^{ns}	0.18^{ns}	0.07^{ns}	-0.07^{ns}	-0.07^{ns}
Grain Moisture (%)							1	0.36*	0.25^{ns}	0.19^{ns}	0.23^{ns}
Plant Height (cm)								1	0.57***	0.02^{ns}	0.13^{ns}
Ear Height (cm)									1	0.03^{ns}	-0.09^{ns}
Anthesis Date (DAP)										1	0.79***
Silk Date (DAP)											1

ns = not significant at p = 0.05

*,**,*** significant at 0.05, 0.01, and 0.001

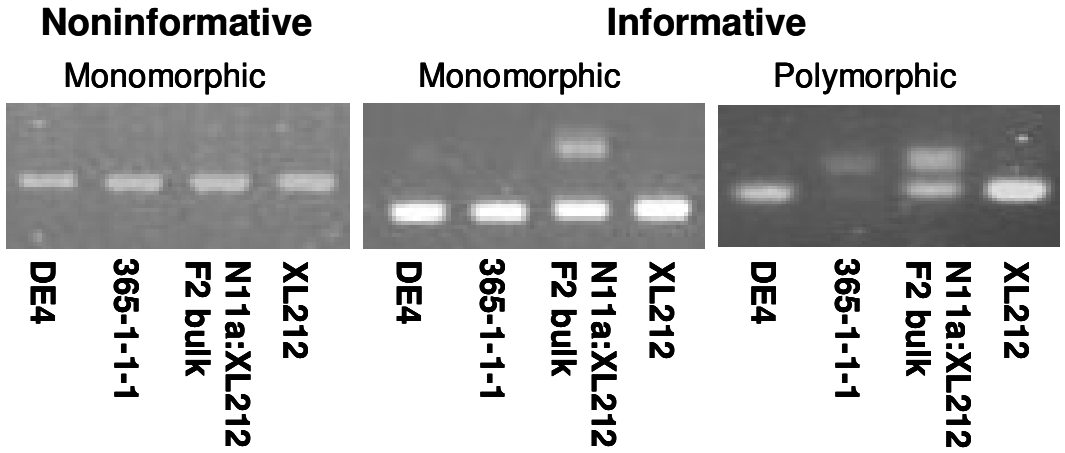

Figure 5.1. Parental screening of SSR markers. Noninformative markers do not distinguish which parent (N11a or XL212) contributed an allele to the sister lines. Informative markers differentiate between N11a and XL212. Informative polymorphic markers differentiate between DE4 and 365-1-1-1.

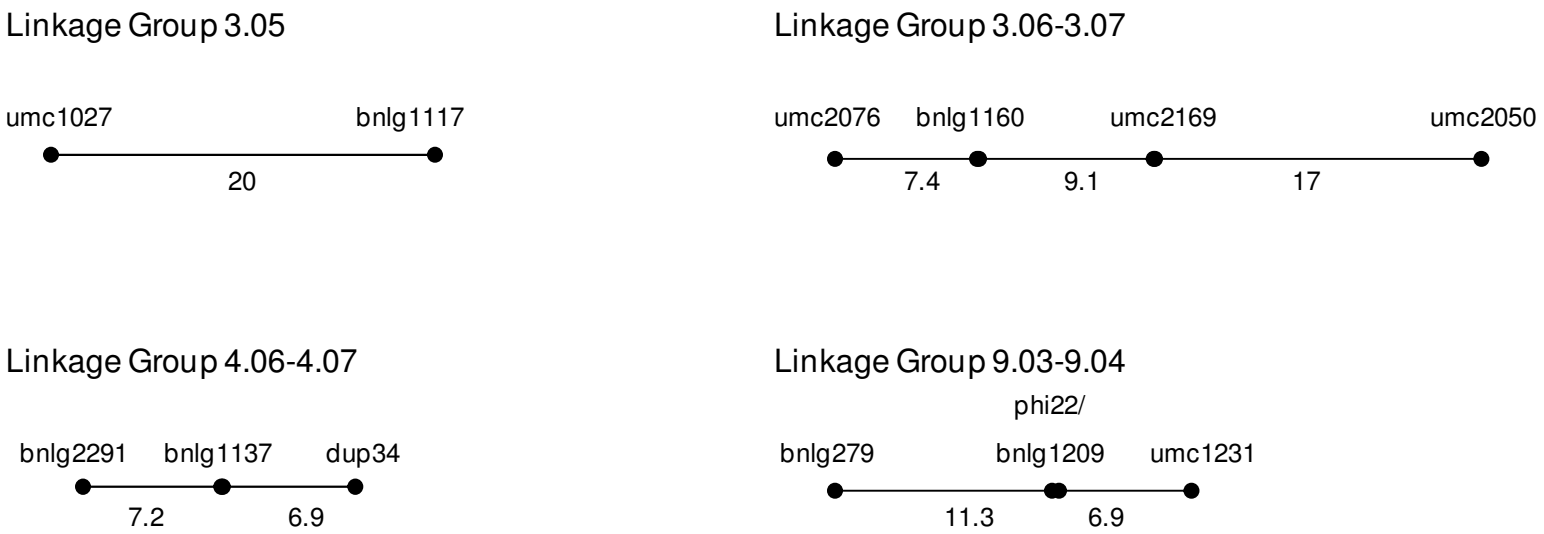

Figure 5.2. Map distances between linked polymorphic markers. Other tested markers did not associate with any linkage groups.

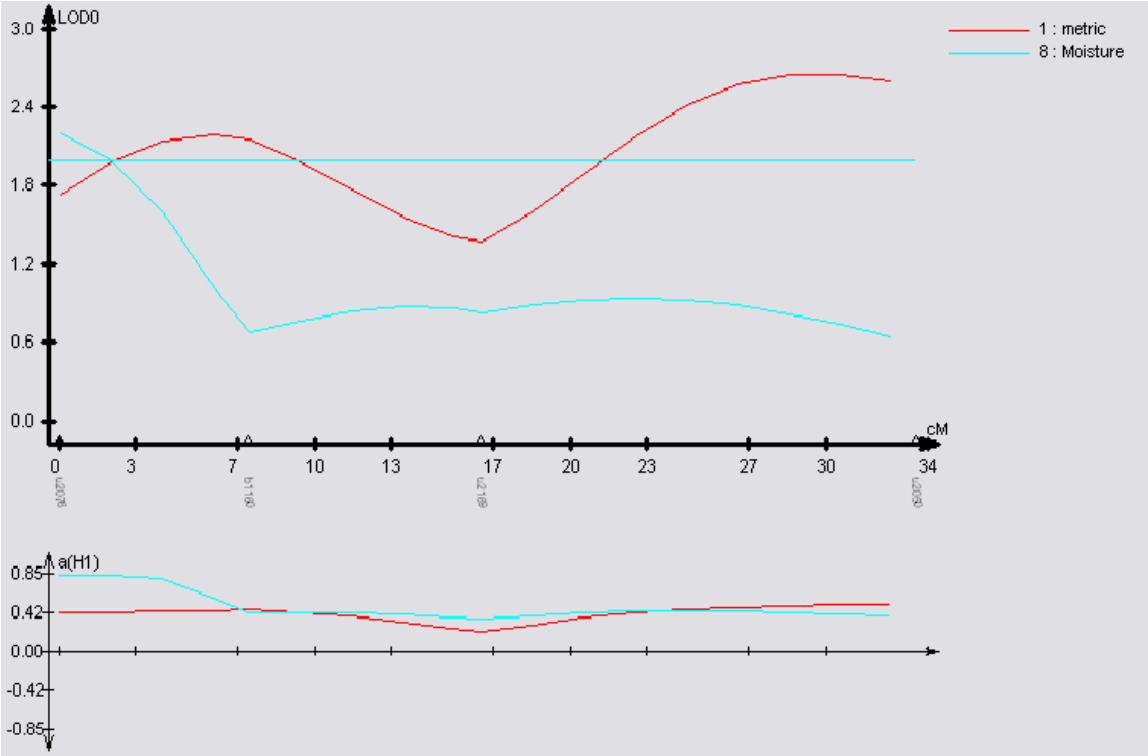

Figure 5.3. Interval mapping QTL peaks for grain yield and moisture in region 3.06 - 3.07.

APPENDICES

APPENDIX A
Supplemental Material for Chapter 3

Table A.1 SSR scores on BC_4F_1 GEFR families segregating for ear rot and fumonisin accumulation resistance. SSR markers evaluated were identified as flanking major QTL for ear rot or fumonisin accumulation resistance in ancestral BC1F1:2 lines (Robertson-Hoyt *et al.*, 2006).

	Gefr397-8-2-2-1	Gefr397-8-2-2-2	Gefr397-8-2-2-3	Gefr397-8-2-2-4	Gefr397-8-10-6-1	Gefr397-8-10-6-2	Gefr397-8-10-6-3	Gefr397-8-10-6-4	Gefr397-8-10-8-1	Gefr397-8-10-8-2	Gefr397-8-10-8-3	Gefr397-8-10-8-4	Gefr397-10-2-3-1	Gefr397-10-2-3-2	Gefr397-10-2-3-3	Gefr397-10-2-3-4	Gefr397-10-5-2-1	Gefr397-10-5-2-2	Gefr397-10-5-2-3	Gefr399-1-5-1-1	Gefr399-1-5-1-2	Gefr399-1-5-1-3	Gefr399-1-5-1-4
07CL row #	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395
well position	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
bnlg1017	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1270	1	1	1	1	1	H	H	1	H	1	.	H	1	1	1	1	1	1	1	1	1	1	1
bnlg1347	1	1	1	1	1	1	1	1	1	1	2	2	1	2	1	2	1	1	1	1	1	1	1
bnlg1520	H	H	1	2	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	1	2
bnlg1606	1	2	1	H	1	1	.	1	.	.	1	1	1	1	1	1	1	1	1	2	1	1	.
bnlg1662	1	1	1	1	H	1	1	1	1	1	H	H	1	1	1	1	1	1	1	H	1	1	H
bnlg1811	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1953	2	H	H	H	1	H	1	H	1	1	H	1	1	1	1	1	1	1	1	2	2	2	2
bnlg2144	1	1	1	1	1	1	1	1	.	1	1	1	.	1	.	.	1	1	.	.	.	1	.
bnlg2244	1	1	1	1	1	1	1	1	1	1	1	1	H	1	H	H	1	H	1	1	1	1	1
bnlg2331	1	1	1	1	1	1	1	1	.	1	.	1	1	H	1	H	.	H	.	1	1	1	1
dupssr06	H	1	H	1	H	.	1	1	1	1	1	H	2	1	1	1	1	H	H	.	1	.	.
dupssr34	1	1	1	1	1	1	1	1	1	1	1	1	H	1	.	H	H	H	1	H	1	1	1
mmc271	1	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1	.	1	1	H	1	1	H
phi001	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table A.1 continued.

	Gefr397-8-2-2-1	Gefr397-8-2-2-2	Gefr397-8-2-2-3	Gefr397-8-2-2-4	Gefr397-8-10-6-1	Gefr397-8-10-6-2	Gefr397-8-10-6-3	Gefr397-8-10-6-4	Gefr397-8-10-8-1	Gefr397-8-10-8-2	Gefr397-8-10-8-3	Gefr397-8-10-8-4	Gefr397-10-2-3-1	Gefr397-10-2-3-2	Gefr397-10-2-3-3	Gefr397-10-2-3-4	Gefr397-10-5-2-1	Gefr397-10-5-2-2	Gefr397-10-5-2-3	Gefr399-1-5-1-1	Gefr399-1-5-1-2	Gefr399-1-5-1-3	Gefr399-1-5-1-4
umc1078	1	1	1	1	1	1	H	.	H	1	1	H	1	1	1	1	1	1	1	1	1	1	1
umc1085	2	H	2	H	1	1	1	1	1	1	H	H	1	1	1	H	H	1	1	1	1	1	1
umc1086	1	1	1	1	1	1	1	1	1	1	1	1	2	1	H	H	1	H	1	1	1	1	1
umc1193	1	1	1	1	1	1	1	1	1	1	1	1	2	H	1	1	.	1	1	1	1	1	1
umc1355	1	1	1	1	1	1	1	1	1	1	1	1	H	1	1	H	1	1	1	1	1	1	1
umc1360	1	1	1	1	1	H	1	1	1	H	.	1	1	2	1	H	1	1	1	1	.	1	1
umc1489	.	H	H	2	1	1	1	1	1	H	1	1	1	1	1	1	.	1	1	1	1	1	H
umc1594	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1778	H	H	H	1	1	1	1	1	H	H	1	1	1	H	1	H	1	1	1	1	H	1	1
umc2047	H	1	2	1	1	1	1	1	1	1	H	H	1	1	1	1	1	1	1	1	1	H	1
umc2061	1	1	1	1	1	2	1	1	1	H	.	1	H	1	H	1	1	H	1	H	1	H	H
umc2098	1	1	1	1	1	1	1	1	1	1	1	1	2	H	H	1	.	1	1	1	1	H	1
umc2111	2	H	.	H	1	1	1	1	.	H	2	1	1	1	1	1	1	1	H	1	1	1	1
umc2150	1	1	1	1	1	.	.	.	.	.	.	1	1	1	1	1	.	1	1	1	1	1	.
umc2280	1	2	1	1	1	1	1	H	1	H	1	H	H	1	1	1	1	H	1	H	2	1	2
umc2281	1	1	1	1	1	1	1	1	1	H	1	H	1	1	H	1	1	H	1	H	H	1	H

Table A.1 continued.

	Gefr399-2-10-4-1	Gefr399-2-10-4-2	Gefr399-2-10-4-3	Gefr399-2-10-4-4	Gefr399-2-10-6-1	Gefr399-2-10-6-2	Gefr399-2-10-6-3	Gefr399-2-10-6-4	Gefr399-2-3-1-1	Gefr399-2-3-1-2	Gefr399-2-3-1-3	Gefr399-2-3-1-4	Gefr399-2-5-1-1	Gefr399-2-5-1-2	Gefr399-2-5-1-3	Gefr399-2-5-1-4	Gefr399-3-7-4-1	Gefr399-3-7-4-2	Gefr399-3-7-4-3	Gefr399-3-7-4-4	Gefr400-9-5-2-1	Gefr400-9-5-2-2	Gefr400-9-5-2-3	Gefr400-9-5-2-4
07CL row #	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419
well position	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
bnlg1017	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	H	H	1	H	1	H	H
bnlg1270	1	1	1	1	1	1	1	1	1	H	H	H	H	1	H	H	1	1	1	1	1	1	1	.
bnlg1347	H	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	H	2	1	1	1	1	1
bnlg1520	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	1	H	1	1	1	1
bnlg1606	2	H	1	.	.	1	1	1	H	H	1	1	1	H	1	.	.	1	1	.	1	1	1	1
bnlg1662	2	H	1	H	1	1	1	1	.	1	1	1	1	H	1	1	1	1	1	1	1	1	1	1
bnlg1811	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1953	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg2144	H	1	1	H	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1	.	1		1	1
bnlg2244	H	H	2	2	1	2	H	2	1	2	1	1	1	1	1	1	1	1	1	1	H	1	H	H
bnlg2331	H	H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	H	1	.	1	1	1
dupssr06	1	H	1	1	1	1	1	1	1	2	H	1	H	H	2	2	.	.	1	1	1	1	1	1
dupssr34	H	1	H	1	1	H	H	H	2	H	1	1	1	1	1	1	1	H	1	H	H	1	.	1
mmc271	2	H	1	H	1	1	1	1	.	1	1	1	1	1	H	1	.	1	1	.	1	1	1	1
phi001	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
umc1078	1	1	1	1	1	1	1	1	.	H	1	H	1	1	H	1	1	1	1	1	1	1	1	1
umc1085	1	1	1	1	1	1	1	.	1	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1086	.	.	.	.	.	.	.	.	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1101	1	1	1	1	.	H	.	H	.	H	.	.	.	1	.	.	.	.	1	.	.	H	.	.
umc1134	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1193	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	H	1	1	1	1	1
umc1355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	1

Table A.1 continued.

	Gefr399-2-10-4-1	Gefr399-2-10-4-2	Gefr399-2-10-4-3	Gefr399-2-10-4-4	Gefr399-2-10-6-1	Gefr399-2-10-6-2	Gefr399-2-10-6-3	Gefr399-2-10-6-4	Gefr399-2-3-1-1	Gefr399-2-3-1-2	Gefr399-2-3-1-3	Gefr399-2-3-1-4	Gefr399-2-5-1-1	Gefr399-2-5-1-2	Gefr399-2-5-1-3	Gefr399-2-5-1-4	Gefr399-3-7-4-1	Gefr399-3-7-4-2	Gefr399-3-7-4-3	Gefr399-3-7-4-4	Gefr400-9-5-2-1	Gefr400-9-5-2-2	Gefr400-9-5-2-3	Gefr400-9-5-2-4
umc1360	1	1	.	1	1	1	1	1	1	.	1	1	1	1	.	1	1	1	1	.	1	.	1	1
umc1489	1	1	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1	1	1	.	1	1	1	1
umc1594	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1778	1	1	.	1	1	1	1	1	1	.	1	1	1	1	1	1	.	1	1	1	1	1	.	1
umc2047	H	1	1	2	1	1	1	1	H	1	H	1	1	1	H	1	1	1	.	1	1	1	1	1
umc2061	1	1	H	1	1	1	1	1	H	1	1	1	H	H	H	H	1	1	1	1	H	1	1	.
umc2098	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1
umc2111	1	H	1	1	1	1	H	1	1	H	1	H	H	1	1	H	1	.	1	1	2	.	.	1
umc2150	1	1	1	1	1	1	1	.	1	1	1	1	1	1	1	1	1	1	1	1	2	1	.	2
umc2280	2	2	H	H	H	1	1	H	1	1	1	H	H	2	1	H	2	H	1	H	H	H	2	H
umc2281	H	1	1	1	1	1	1	H	1	1	1	1	1	1	1	1	1	1	1	1	H	H	.	.

Table A.1 continued.

	Gefr400-9-9-3-1	Gefr400-9-9-3-2	Gefr400-9-9-3-3	Gefr400-9-9-3-4	Gefr402-3-5-1-1	Gefr402-3-5-1-2	Gefr402-3-5-1-3	Gefr402-3-5-1-4	Gefr402-3-6-2-1	Gefr402-3-6-2-2	Gefr402-3-6-2-3	Gefr402-3-6-2-4	Gefr402-3-7-5-1	Gefr402-3-7-5-2	Gefr402-3-7-5-3	Gefr402-3-7-5-4	Gefr402-3-7-6-1	Gefr402-3-7-6-2	Gefr402-3-7-6-3	Gefr402-3-7-6-4	Gefr411-4-6-3-1	Gefr411-4-6-3-2	Gefr411-4-6-3-3	Gefr411-4-6-3-4
07CL row #	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443
well position	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
bnlg1017	1	1	1	1	1	1	1	1	1	1	1	1	H	H	H	H	1	H	H	1	1	1	1	1
bnlg1270	1	1	1	1	1	1	1	H	1	1	1	.	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1347	1	1	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1	1	1	1	1	H	H	1
bnlg1520	1	1	1	1	1	1	1	1	2	.	2	1	1	H	1	1	1	H	1	H	1	.	1	.
bnlg1606	1	.	1	.	.	1	1	1	H	H	H	1	1	1	1	1	H	.	1	1	1	1	1	1
bnlg1662	.	1	.	.	1	2	H	.	H	.	.	.	H	1	1	1	1	1	1	1	1	1	1	1
bnlg1811	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1953	1	2	1	1	1	1	1	1	1	1	1	1	H	1	2	2	1	1	1	1	1	1	1	1
bnlg2144	1	1	1	1	1	1	1	1	H	H			1	1	1		1	H		1	1	1	1	1
bnlg2244	1	1	1	1	1	1	1	1	1	1	1	1	2	1	H	1	H	2	1	1	H	1	H	H
bnlg2331	1	1	1	1	1	1	1	1	1	1	1	H	1	1	1	1	1	1	1	1	1	H	H	H
dupssr06	1	1	1	1	1	H	1	1	H	H	1	1	H	1	2	1	1	1	1	1	1	.	1	1
dupssr34	1	H	H	1	1	.	1	1	1	1	1	1	1	1	.	.	1	1	1	.	1	1	1	1
mmc271	1	1	.	.	1	H	H	.	H	.	.	.	1	1	1	1	1	2	H	1	1	1	1	1
phi001	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
umc1078	1	1	.	.	1	1	1	.	1	.	.	.	1	1	1	1	1	1	1	1	1	1	1	1
umc1085	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H	H	1
umc1086	1	1	1	1	1	1	1	1	1	1	1	1	2	H	2	1	1	.	1	1	H	H	H	H
umc1101	1	1	1	1	1	.	1	1	1	1	1	1	.	1	1	1	1	1	1	1	1	1	1	1
umc1134	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table A.1 continued.

	Gefr400-9-9-3-1	Gefr400-9-9-3-2	Gefr400-9-9-3-3	Gefr400-9-9-3-4	Gefr402-3-5-1-1	Gefr402-3-5-1-2	Gefr402-3-5-1-3	Gefr402-3-5-1-4	Gefr402-3-6-2-1	Gefr402-3-6-2-2	Gefr402-3-6-2-3	Gefr402-3-6-2-4	Gefr402-3-7-5-1	Gefr402-3-7-5-2	Gefr402-3-7-5-3	Gefr402-3-7-5-4	Gefr402-3-7-6-1	Gefr402-3-7-6-2	Gefr402-3-7-6-3	Gefr402-3-7-6-4	Gefr411-4-6-3-1	Gefr411-4-6-3-2	Gefr411-4-6-3-3	Gefr411-4-6-3-4
umc1193	1	1	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	H	1	1	1	1	2
umc1355	1	1	1	1	1	1	1	1	1	1	.	1	1	1	1	1	1	1	.	.	1	1	1	1
umc1360	1	1	1	1	1	1	1	1	1	.	H	1	1	H	1	1	1	.	1	1	1	.	1	1
umc1489	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	.	1	1	1	1
umc1594	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1778	1	H	H	1	1	1	1	1	1	1	1	1	1	1	.	1	1	1	1	1	1	1	1	1
umc2047	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	H	H	1
umc2061	1	1	1	.	1	1	1	.	1	.	.	1	1	1	1	.	1	.	.	.	1	.	1	1
umc2098	1	1	1	1	1	1	1	1	1	H	H	H	1	1	1	1	1	1	1	1	1	1	H	H
umc2111	1	1	1	1	1	H	H	1	H	H	H	1	H	H	1	1	1	.	1	1	1	.	1	.
umc2150	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc2280	1	H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	.	1	1	1	1	1	1
umc2281	1	1	1	.	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table A.1 continued.

	Gefr411-4-6-5-1	Gefr411-4-6-5-2	Gefr411-4-6-5-3	Gefr411-4-6-5-4	Gefr412-13-6-1-1	Gefr412-13-6-1-2	Gefr412-13-6-1-3	Gefr412-13-6-1-4	GE440	FR1064
07CL row #	444	445	446	447	448	449	450	451	-	-
well position	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
bnlg1017	1	1	1	1	1	1	1	1	2	1
bnlg1270	H	1	1	1	1	1	1	1	2	1
bnlg1347	H	1	H	1	1	H	H	1	2	H
bnlg1520	1	1	1	1	1	1	1	1	2	1
bnlg1606	.	1	1	1	1	1	1	1	2	1
bnlg1662	1	1	1	1	H	1	H	H	2	1
bnlg1811	1	1	1	1	1	1	1	1	2	1
bnlg1953	H	1	1	1	1	1	1	1	2	1
bnlg2144	1	1	1	1	1	1	1	1	2	1
bnlg2244	H	H	H	H	1	1	1	1	2	1
bnlg2331	1	1	H	1	H	H	1	1	2	1
dupssr06	2	2	2	H	H	1	1	1	2	1
dupssr34	1	.	1	1	1	1	1	1	2	1
mmc271	.	1	1	1	1	1	1	1	2	1
phi001	.	.	.	.	.	.	.	.	.	.
umc1078	2	1	H	1	1	1	1	1	2	1
umc1085	1	1	1	1	H	1	H	H	2	1
umc1086	H	2	2	1	1	1	1	1	2	1
umc1101	1	.	1	1	H	H	H	H	2	1
umc1134	1	1	1	1	1	1	1	1	2	1
umc1193	1	1	1	1	1	1	1	1	2	1
umc1355	1	1	1	1	1	1	1	1	2	1
umc1360	.	1	1	1	1	1	1	1	2	1
umc1489	2	1	1	1	1	1	1	1	.	1
umc1594	.	1	1	1	1	1	1	1	.	1
umc1778	1	1	1	1	1	1	1	1	2	1
umc2047	2	1	H	1	1	1	1	H	2	1
umc2061	.	1	1	1	1	H	1	H	2	1
umc2098	1	1	H	1	1	1	1	1	2	1
umc2111	1	1	1	1	1	1	1	1	2	1
umc2150	1	1	1	1	1	1	1	1	2	1
umc2280	1	.	1	1	1	1	1	1	2	1
umc2281	1	1	1	1	1	1	1	1	2	1

Protocol for Fusarium Isolation from Field Grown Kernels

1. While shelling grain, collect kernels near infected areas of the ear which appear to be uninfected.
2. Wash kernels for 5 minutes in running water.
3. Sit for 5 minutes in 200 mL of 10% 95% Ethanol, 20% household bleach (5%), and 70% distilled water.
4. Using sterile technique, cut open the kernels with a razor blade. Remove the embryo and aleurone layer. Plate each kernel on a separate plate of PDA (Potato Dextrose Agar). (I use 60 × 15 mm plates.) Keep plates in approximately 12 hours light, 12 hours dark for Fusarium growth.
5. Allow a few days for cultures to grow large enough to identify Fusarium from other fungi. Using sterile technique and a thin blade, transfer a single hypheal tip from each plate to a new plate.
6. Allow a few days for growth, then scrape mycelium and conidia from surface into microfuge tube with 1 mL of water. Vortex. Dilute 1:99, and plate 100µl onto the center of a PDA plate.
7. The following day scrape 1 colony from this plate and repeat the procedure.
8. The following day transfer 1 colony to a new plate and grow this plate for conidia collection.

9. After 3-5 days of growth (no more than 2 weeks) plates can be washed of conidia, and stored in glycerol or used to inoculate.

Protocol for Glycerol Storage Stocks.

1. Wash spores of plate. Harvest each isolate separately into 1 mL tube. Bring volume to 1mL. (follow Robertson-Hoyt Protocol for harvesting spores).
2. Spin down tube, for 1 minute at low rpm. Pipette off water.
3. For 1mL of s Add 127.5 µl distilled water, and 22.5 µL glycerol.
4. Vortex to resuspend and freeze at -80C.

Protocol for Inoculating Cracked Corn in the Laboratory (adapted from Charles Woloshuk).

Useful for testing efficacy or fumonisin production of an isolate.

1. Place intact kernels in a beaker of water and autoclave for 10 minutes. This helps the kernels imbibe water. They can sit overnight in the water in a refrigerator.
2. Crack the kernels with pliers. Aeration is important so do not pulverize kernels, just crack them.
3. Place 5 to 10 g of cracked corn in a 50 ml beaker with 1 mL of water and autoclave for 15 min. (You will need a beaker for each isolate that you are testing.)

4. Inoculate with 100 µl of 10^6 spore suspension. Incubate for 5 days (no more than 10), covered loosely. Shake the flask each day, and add a few drops of water if the kernels look dry.
5. Kernels can be frozen before HPLC or ELISA analysis at -80C.

Table A.2. Fusarium strains were isolated from inoculated or noninoculated fields by single hypheal transfer. The 4 isolates with lowest spore counts were not tested for fumonisin content. Fumonisin levels were determined from HPLC analysis. Comments on appearance were noted when isolates were grown on PDA. Control isolates 19, 37-2, 310, ISU94040, ISU 94445, and ISU95082 are stocks previously used for inoculation.

Isolate	Ear treatment	Spore count	Fumonisin Levels (ng g^{-1})				Appearance
			B2a	B2b	B1	Total	
I1	Inoculated	185	13,920	33,863	92,216	139,999	Lavender mycelial growth and agar stain
I2	Inoculated	268	2,672	606,073	10,361	619,105	White mycelial growth, purple agar stain
I3	Inoculated	57	-	-	-	-	-
I4	Inoculated	216	284	130,132	1,675	132,091	White mycelial growth, purple agar stain
I5	Inoculated	195	890	243,581	4,271	248,742	White mycelial growth, purple agar stain
I6	Inoculated	153	3,428	817,447	16,159	837,034	White mycelial growth, purple agar stain
I7	Inoculated	204	89,111	271,951	510,279	871,341	White mycelial growth, purple agar stain
I8	Inoculated	108	-	-	-	-	-
I9	Inoculated	244	16,594	1254,389	112,119	1,383,102	White mycelial growth, lavender agar stain
I10	Inoculated	119	-	-	-	-	-
I11	Inoculated	244	14,848	31,081	101,432	147,360	White mycelial growth, purple agar stain
N12	Noninoculated	392	63,754	157,443	360,443	581,640	White mycelial growth, purple agar stain
N13	Noninoculated	255	120,320	74,835	166,811	361,966	White mycelial growth, lavender agar stain
N14	Noninoculated	130	39,880	62,202	170,757	272,840	White and lavender mycelial growth, purple agar stain
N15	Noninoculated	191	93,452	219,308	512,489	825,249	White mycelial growth, purple agar stain
N16	Noninoculated	207	220,030	219,004	607,452	1,046,485	White and lavender mycelial growth, lavender agar stain
N17	Noninoculated	161	223,976	434,991	943,695	1,602,662	White mycelial growth, purple agar stain
N18	Noninoculated	219	46,198	65,779	215,180	327,157	White mycelial growth, purple agar stain
N19	Noninoculated	159	78,843	174,475	439,367	692,685	White mycelial growth, purple agar stain
N20	Noninoculated	194	6,109	6,683	27,300	40,093	White mycelial growth, purple agar stain
N21	Noninoculated	68	-	-	-	-	White mycelial growth, purple agar stain
N22	Noninoculated	120	172,464	324,873	730,936	1,228,273	White mycelial growth, purple agar stain

Table A.2 continued.

Isolate	Ear treatment	Spore count	Fumonisin Levels (ng g^{-1})				Appearance
			B2a	B2b	B1	Total	
F. proliferatum 19	Freezer stock check	37	11,955	26,224	165,031	203,210	White mycelial growth
F. proliferatum 310	Freezer stock check	46	1,605	1,387	14,918	17,910	White mycelial growth
F. proliferatum 37-2	Freezer stock check	42	40,027	81,698	145,741	267,466	White mycelial growth
F. verticillioides ISU94040	Freezer stock check	31	6,999	18,752	79,679	105,679	Lavender mycelial growth, pink agar stain
F. verticillioides ISU94445	Freezer stock check	181	12,099	3,375	24,292	39,766	White mycelial growth, purple agar stain
F. verticillioides ISU95082	Freezer stock check	43	4,158	11,406	29,514	45,078	White mycelial growth, lavender agar stain

APPENDIX B

Supplemental Material for Chapter 4

Table B.1. Least squared means for 22 founder lines of the ReFus Population evaluated at two environments in 2008.

	Ear rot (%)	Weight (g plant^{-1})	Plant Height (cm)	Ear Height (cm)	Days to Silk (DAP)	Days to Anthesis (DAP)	Fumonisin (µg g^{-1})
Set I Parents							
B116	45.85	53.84	155	67	82	77	32.84
B97	24.74	42.99	123	61	81	76	20.36
NC258	24.92	44.14	145	71	89	85	43.55
NC320	8.25	83.10	146	78	87	83	25.06
NC346	19.69	15.77	115	44	87	82	25.73
NC446	11.93	52.91	110	57	88	87	21.52
NC448	24.97	34.23	118	47	88	81	14.58
NC450	5.25	47.06	122	62	84	83	13.70
NC452	19.91	29.30	117	42	84	81	18.93
NC456	9.76	11.75	145	65	91	90	47.42
NC492	22.58	32.46	136	73	86	83	34.33
Set I Parent Mean	19.80	40.69	130	61	86	82	27.09
Set II Parents							
A131	82.49	5.37	78	19	65	64	16.70
GE440	3.00	23.63	203	120	89	84	9.64
Ki21	52.81	52.79	136	82	86	85	16.41
Ky21	56.86	21.74	140	73	89	84	24.19
Mo17	31.86	36.91	127	49	84	80	18.37
NC300	17.23	31.64	156	62	89	88	18.47

Table B.1 continued.

	Ear rot (%)	Weight (g plant^{-1})	Plant Height (cm)	Ear Height (cm)	Days to Silk (DAP)	Days to Anthesis (DAP)	Fumonisin (μg g^{-1})
NC300/CML288-B-4-B-B-B-B	96.00	3.56	144	79	92	87	65.87
NC356	12.43	38.33	128	58	85	82	25.14
NC458	14.84	47.45	133	61	87	86	13.03
T236	19.52	32.92	157	79	85	83	10.19
UR13085:N0215-21	48.33	48.47	118	50	81	76	26.31
Set II Parent Mean	38.09	31.16	138	67	85	82	22.45
Overall Parental Mean	28.95	35.26	134	64	85	82	24.77
Pairwise LSD	19.14	20.27	17	14	3	3	29.37

Table B.2. Least squared means for Cycle 0 of the ReFus Population evaluated at two environments in 2008.

	Ear rot (%)	Weight (g plant^{-1})	Plant Height (cm)	Ear Height (cm)	Days to Silk (DAP)	Days to Anthesis (DAP)	Fumonisin (μg g^{-1})
Control S0:3s	35.01	45.44	151.96	79.46	85.88	81.74	32.43
ReFusC0-010	37.89	41.24	156.79	79.79	86.34	82.87	39.98
ReFusC0-016	26.29	46.41	159.99	82.77	83.84	82.99	32.25
ReFusC0-020	24.15	72.42	139.48	58.73	84.34	82.21	43.08
ReFusC0-032	8.33	55.86	141.39	60.18	81.38	78.29	13.51
ReFusC0-046	33.73	53.77	122.80	61.06	87.80	83.46	24.42
ReFusC0-051	39.01	64.03	117.76	52.36	81.55	77.53	20.66
ReFusC0-064	17.73	45.10	137.13	64.70	84.24	82.13	16.87
ReFusC0-089	82.38	56.55	160.45	82.77	83.99	79.33	55.44
ReFusC0-108	11.67	39.68	138.8	70.26	86.86	83.39	14.94
ReFusC0-124	20.30	37.50	144.82	74.97	84.94	78.93	20.23
ReFusC0-129	18.70	58.98	126.23	63.60	82.20	79.91	16.99
ReFusC0-131	26.30	71.93	155.31	75.70	80.96	77.69	40.25
ReFusC0-142	24.35	48.09	147.58	71.41	87.01	83.27	17.27
ReFusC0-143	16.30	66.96	126.21	65.92	84.12	81.47	22.94
ReFusC0-147	12.75	88.37	166.92	85.85	80.25	76.43	14.06
ReFusC0-154	21.27	48.56	166.05	76.84	85.74	85.17	26.41
ReFusC0-157	52.70	60.13	146.27	70.99	85.56	80.13	17.10
ReFusC0-161	25.67	64.15	128.85	60.13	86.22	81.55	26.97
ReFusC0-173	37.06	76.41	186.25	113.12	86.70	85.03	54.12
ReFusC0-177	64.45	28.35	126.72	56.59	87.96	82.24	61.68
Cycle 0 mean	30.05	56.22	144.79	71.39	81.20	84.60	28.96
Pairwise LSD	19.14	20.27	17	14	3	3	29.37