ABSTRACT

OTT, OLIVER OTIS. Resistance to Northern Leaf Blight from Teosinte-Maize Introgression Lines and Gray Leaf Spot Resistance Inheritance and Yield Effects. (Under the direction of Matthew Krakowsky and Major Goodman.)

The first part of this thesis involves evaluating the potential of teosinte as a source of novel resistance to northern leaf blight (NLB), caused by *Setosphaeria turcicum.* A set of near isogenic lines (NILs) derived from five *Z. mays* ssp. *parviglumis* teosinte accessions in a background of the commonly-used maize inbred line B73 were screened for resistance to NLB to identify teosinte alleles that confer increased levels of resistance. Teosinte alleles conferring enhanced resistance to NLB were identified, but when the effects of these alleles were compared to the effects of alleles from maize inbred lines Tx303 and Mo17, none of the teosinte alleles conferred significantly more resistance than the maize alleles.

The second part involves the study of Gray Leaf Spot (GLS), an important foliar disease of maize caused by *Cercospora zeae-maydis* and its sister species *Cercospora zeina*. To date there has been little published research on the correlation of GLS resistance between inbred lines and their respective hybrids. For this study forty-seven inbred lines were chosen which spanned the spectrum of GLS resistance, based on the analysis of historical data, with the addition of historically significant lines as reference points. These lines were crossed to two hybrid testers, Pioneer 3394 (GLS susceptible), and Pioneer 33M54 (GLS moderately resistant). Disease screening of inbred lines and hybrids was conducted at three locations in 2010 and 2011. Yield tests were conducted at one location in 2010 and 2011 with two treatment groups, one fungicide sprayed and the other inoculated. Inbred line to hybrid correlation of GLS resistance was found to be high with R^2= 0.87 in crosses with Pioneer 3394 and R^2=0.86 with Pioneer 33M54. In yield trial experiments, hybrids under disease pressure lost an average of 1.04 metric tons per hectare, a 9.9% reduction. Hybrids with a resistant parent had significantly lower losses in yield than hybrids with a susceptible parent, indicating that the use of resistant parents can help maintain yield in the presence of disease.

Resistance to Northern Leaf Blight from Teosinte-Maize Introgression Lines and Gray Leaf Spot Resistance Inheritance and Yield Effects.

by
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BIOGRAPHY

Oliver is from northwest Ohio where he grew up on a small family-run grain farm. He received his undergraduate degree in Plant Sciences at Cornell University, where he worked in the Lab of Rebecca Nelson. From there he moved to North Carolina State University to study maize breeding, with Major Goodman and Matthew Krakowsky. He will continue to study with them for a PhD.

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-CHAPTER I-
Literature Review

Overview of Types of Plant Disease Resistance

Major Gene Mediated Resistance

There are three broad classes of plant disease resistance: major gene, non-host and quantitative. Major effect resistance, conferred by so-called resistance (or "R") genes was the first type of genetic disease resistance mechanism to be closely studied, and to date is the most highly characterized type of disease resistance in plants mainly due to relative ease of study owing to their relatively large effects as compared to other forms of disease resistance. The classical definition of an R gene is a type of gene that confers a high level of plant disease resistance against pathogens. In most cases, upon infection, specific proteins are produced by pathogens and excreted into the host. Some of these proteins, known as a virulence (or AVR) proteins, are recognized by R proteins, which then triggers a defense response leading to resistance. Effectors are secreted pathogen proteins and other molecules that change plant defense reactions and general metabolism circuitry to enable parasitic colonization of plant tissue (Hogenhout et al. 2009, Nimchuk et al., 2003; Ravensdale et al., 2011).

Scientists have been able to collect more comprehensive data on both plant hosts and pathogens, which has led to a deeper understanding of the distribution and evolutionary aspects of R-genes, as well as the genomes and effector gene complements of pathogens (Egan and Talbot, 2008; van de Wouw and Howlett, 2011). R protein-triggered responses usually involve reactive oxygen species (ROS) accumulation and defense gene activation typically leading to a rapid, localized cell death around the point of pathogen penetration called the hypersensitive response (HR;Chisholm et al., 2006; Jones and Dangl, 2006; Maekawa et al., 2011). Most commonly, R proteins are composed of nucleotide-binding domains (NB) followed by series of leucine repeats (LRRs). In contrast to animal NB-LRR proteins, plant R proteins usually have different N-terminal domains. The N-

terminal domain in plants may be a coiled coil (CC) sequence or a Toll/Interleukin-1 receptor (TIR) domain, which shares sequence similarities with the *Drosophila* TOLL and human interleukin-1 receptors (Ronald and Beutler, 2010). Membrane-resident pattern recognition receptors (PRRs) define a second class of immune receptor, which detect microbe-associated molecular patterns (MAMPs). MAMPs are evolutionarily conserved structures that include components of fungal cell walls, such as chitin (N-acetyl-chitooligo-saccharide oligomers), short peptides derived from bacterial flagellin, the elongation factor EF-Tu, and lipopolysaccharides from Gram-negative bacteria, among many others (Zipfel and Felix, 2005; Altenbach and Robatzek, 2007).

The activation of HR by effector recognition has to be tightly regulated (Lukasik and Takken, 2009). This regulation includes upstream interactions of R proteins that may involve conserved chaperone complexes to insure proper folding, accumulation and regulation (Shirasu, 2009). Effector recognition is thought to induce conformational changes in the R proteins that triggers a translation of recognition into signal initiation and frees NB-LRR domains to activate downstream signaling (Collier and Moffett, 2009). Understanding of the downstream signaling events that links NB-LRR activation to HR remains elusive, and several studies suggest that nuclear activity of some NB-LRRs is needed to trigger proper responses.

It has been observed that plants have a finite number of R genes, and that there are many more pathogenic organisms which produce different complements of effectors. This observation has led to proposal of a new model, which contends that R proteins do not interact directly with Avr proteins, but instead interact indirectly with or guard the host proteins, targets of pathogen Avr proteins. R proteins then activate a resistance or defense response once target host proteins are disturbed by the pathogen Avr protein (van der Hoorn et al., 2002; Innes, 2004; Bent and Mackey, 2007). van der Biezen and Jones (1998) proposed that R proteins detect pathogen Avr proteins indirectly; later, Dangl and Jones (2001) coined the term guard hypothesis. Under the guard

hypothesis, Avr protein function, rather than structure, is recognized. One example of this described by Shao et al. (2003) where *Arabidopsis* requires an *R* protein (RPS5) to detect *AvrPhpB*-expressing *Pseudomonas syringae* strains. *AvrPhpB* is a cysteine protease that cleaves the protein kinase, PBS1; this cleavage is then detected by RPS5 (Shao et al., 2003). Many other cases of this type of model have been identified(Mackey et al., 2002, 2003; Axtell and Staskawicz, 2003; Rooney et al., 2005).

Indirect interactions between Avr and R proteins place major limitations on the evolution of pathogens, since avoidance of host detection requires an essential change in the pathogen‘s virulence function (Shao et al., 2003; Dangl and McDowell, 2006; Bent and Mackey, 2007). A limited number of multi-specific *R* proteins could then be employed by host plants to identify and confer resistance to a large number of pathogen Avr proteins converging on the same host target (Dodds et al., 2001; van der Hoorn et al., 2002; Shao et al., 2003; Dangl and McDowell, 2006; McDowell and Simon, 2006). Although the guard model may not explain all R gene Avr interactions, the model is able to explain a significant proportion which aids in the overall understanding of major gene resistance.

Non-Host Resistance

The majority of disease resistance mechanisms are extraordinarily robust as any given plant species is resistant to almost all of the plant pathogens to which it is exposed. This type of species-wide disease resistance, termed non-host resistance, operates using less-understood mechanisms and provides resistance against pathogens throughout all members of a plant species. Non-host resistance is the most common and durable form of plant resistance to disease-causing organisms. Understanding non-host responses on a molecular level is attractive, as its implementation in susceptible species could lead to more durable or broad-spectrum resistance to all strains/races of a pathogen species compared to the limited resistance of specific *R*-genes. A handful of studies have provided new insights in this area. Schulze-Lefert and Panstruga (2011) hypothesized that the relative contribution of R protein and PRR-triggered immunity to non-host resistance changes as a

function of phylogenetic divergence time between host and non-host plants. Likewise, expansion of a pathogen's host range could be driven by variation in its effector repertoire, leading to its reproductive isolation and subsequent speciation. Recent progress in functional genomic technologies has made available tools such as gene expression profiling and virus-induced gene silencing that can be used to dissect the complex phenomenon of non-host resistance. In-depth study of the mechanisms of non-host resistance will bring us a much better view of the dynamics of plant disease resistance in general and the potential to genetically engineer plants for resistance against a broad range of pathogens (Mysore and Ryu, 2004).

Quantitative Disease Resistance

Quantitative Disease Resistance (QDR) lies on a continuous scale from no disease to complete infection that may contribute to premature death of a plant (Swiezyński et al., 2000; Gebhardt and Valkonen, 2001). The genetic bases and mechanisms underlying quantitative disease resistance in plants are still largely unknown (Richardson et al., 2006; Johal et al., 2008). The uniqueness of the host-pathogen interaction in each pathosystem may contribute to the gap in our knowledge of quantitative resistance mechanisms. The large effects and easily ascertainable Mendelian segregation of many qualitative resistance genes allow for their relatively efficient cloning and characterization (Dangl and Jones, 2001; Gebhardt and Valkonen, 2001; Hammond-Kosack and Parker, 2003). Very little of the characteristics and functions of the genes responsible for disease resistance quantitative trait loci (QTL) in any given plant species is understood (Johal et al., 2008). QDR is considered to be an outcome of many genes with small and varying levels of effects on the disease phenotype. These smaller effects cause great difficulty in determining which genetic segments contribute to the given phenotype, and how they are able to confer defense against pathogen entrance, growth, attack, and multiplication. An additional problem is generation of adequate

phenotypic estimates of this type of resistance. Many more replications of much larger experiments are needed to reliably estimate QDR trait values, in contrast to what is needed for characterizing R gene- mediated resistance. Furthermore, understanding the genetic and physiological processes underlying quantitative traits has been limited due to the intricate, incomplete expression of these genes, as well as inconsistent screening methodologies, and extensive testing needs (Geiger and Heun, 1989; Kelly et al., 2006).

Though QDR is often thought of as being non-race-specific, and possibly more durable than qualitative disease resistance, there are examples of race-sensitive disease resistance QTL. Fifteen of eighteen QTL identified by Talukder et al. (2004) in rice for resistance to the rice blast pathogen were deemed race-specific. Another example of this race-specificity can be found in maize. Chung et al. (2010) highlighted the effect of a QTL in maize bin 8.06 on resistance to Northern Leaf Blight. When NILs carrying a resistance allele from the tropical DeKalb hybrid DK888 were tested with the four different race sets of the disease (0, 1, 23, and 23N), they found that the region conferred quantitative resistance to races 0 and 1, but not to 23 or 23N. The two examples listed above are not isolated reports, but examples of a large body of evidence for isolate- and race-specific disease resistance QTL (Avila et al., 2004; Calenge et al., 2004; Denby et al., 2004; ; Marcel et al., 2008). There is also much additional evidence that genes conferring partial resistance may be defeated qualitative resistance genes with residual levels of effectiveness, but without race specificity against the pathogen (Li et al., 1999; Quint et al., 2003; Stewart et al., 2003; Talukder et al., 2004). Results from two experiments by Stewart et al. (2003) suggested that defeated *R* genes, or genes closely linked to the defeated R genes, provided small, but significant race-nonspecific increases in resistance to *Phytophthora infestans* in potato.

The numerous reports of coincident genetic mapping of qualitative resistance genes, or *R* gene analogs (RGAs), and QTLs further link quantitative and qualitative resistance (Gebhardt and

Valkonen, 2001; Quint et al., 2003). Discovery of major resistance genes and RGAs located in *R* gene hotspots suggests that genes conditioning qualitative or quantitative resistance to multiple diseases may have evolved from common ancestors by local gene duplications (Swiezyński et al., 2000). Clustering of qualitative and quantitative resistance genes may occur because some QTL for QDR may be related structurally to R genes conferring resistance to the same or to different pathogens. In addition, QTL associated with resistance to multiple pathogens may be structurally related to *R* gene loci, or may be similar at the molecular level. Regions of synteny for resistance to specific pathogens were found when potato-relative genomes (tomato, tobacco, and pepper) were compared (Gebhardt and Valkonen 2001).

An increasing number of QTLs for traits have been cloned in plants, due to extensive characterization of the diversity of resistance alleles in germplasm. Though there are a large number of publications outlining the discovery of disease resistance QTL, relatively few studies have reported the cloning and functional validation of causal genes (Gururani et al., 2012). When a causal gene is identified and cloned, a functional marker can be developed for marker assisted selection (MAS) for use in a more diverse range of germplasm, especially when MAS replaces more expensive and time-consuming phenotypic assays (Bernardo, 2008). With the use of functional markers MAS has been used to transfer and pyramid major-effect qualitative genes for traits with high heritability into elite breeding lines or cultivars. An example of this in rice involved the use of the pi21 allele which conditions resistance to Rice Blast, *Magnaporthe grisea* (Fukuoka et al., 2009); other examples include resistance to soybean cyst nematode (Cregan et al., 1999), in wheat resistance to leaf rust using resistance genes *Lr10, Lr21*, powdery mildew using *Pm3b,* and stem rust *Sr2* (Varshney et al., 2006).

Traditional Breeding for Disease Resistance

Breeders for hundreds if not thousands of years have been able to improve the disease resistance of varieties without understanding the exact mechanisms of QTL, or with the assistance of MAS. As the development of new varieties is a slow process, it has been important for breeders to not only think on the current growing conditions but also the potential environments a variety could come into contact with in the future. When breeders are developing more disease resistant varieties, an important consideration is the time during which that variety will remain resistant in the face of continued pathogen challenge and evolution. This may be conceptually less important in crops for which variety turnover is very high, as in maize or soybeans, but the breeder must still carefully choose the pools of genetic resistance from which they draw to insure durable resistance. Durable resistance is resistance that has remained effective over many years under wide deployment a characteristic which is very hard to predict in the breeding nursery. QDR is usually believed to confer durable resistance, but this is not always the case. The definition of durability should not assume a particular genetic basis of the host resistance (Palloix et al., 2009; Pariaud et al., 2009). An example of this premise is cabbage resistance to cabbage yellows (caused by *Fusarium oxysporum* f. sp. *conglutinans*), which is conferred by a single gene. Although first implemented in the early 1920's, it has remained durable for more than 90 years, and continues to be effective in most cabbage-growing regions (Bosland et al., 1988). Other examples of a durable single genes are wheat leaf rust resistance genes *Lr13* and *Lr34*, effective for 30 years with proven low-level, broad-spectrum resistance (Kolmer, 1996). *Lr13* and *Lr34* are present, either singly or together, in nearly all hard red spring wheat bred for leaf rust resistance in North America. As there is no single resistance phenotype, and no single genetic basis for durable resistance, it is evident that longevity of resistance to a pathogen is not due solely to the genetic basis of host plant resistance, but also to the population genetics of the pathogen, the degree of host specialization of the pathogen, cultural practices of the cropping system,

and the deployment of resistance genes into cultivars. Breeding for durable resistance may, in fact, mean breeding for diverse mechanisms of resistance, creating difficulty for pathogens to develop multiple mutations that enable them to overcome a large amount of defense or resistance types. These plant defense mechanisms may evolve to alter regulation of morphological and developmental traits, basal defense gene expression, catabolism of secondary metabolites or toxins, defense signal transduction, or cell signaling. No single mechanism accounts for the entire spectrum of QDR; many remain to be discovered, and undoubtedly, some will be unique to single plant species.

Breeding Efforts for Increased Resistance in Maize

Enhanced disease resistance in maize has been accomplished in many ways. One of the most common breeding methodologies used in disease resistance work is screening germplasm for identification of resistant lines or accessions and then utilizing superior lines in a backcross breeding scheme to introgress resistance from the donor germplasm into an agronomically superior, adapted inbred. This system works best for traits that are controlled by a relatively few number of genes, which is not the case for many maize traits. The genetic architecture of the underlying disease resistance is very important to understand, in order to better inform breeding efforts. Population improvement over several cycles, or recurrent selection, is generally thought to be effective for dealing with polygenic traits, and this method has significantly improved performance of the germplasm both for agronomic traits and disease resistance (Pratt and Gordon, 2010). Resistance to foliar diseases is effectively obtained through conventional breeding, where susceptible genotypes under disease pressure can be eliminated before harvest (Ali and Yan, 2012). The reason that relative progress can be made with these diseases may be due to their easy inoculation, phenotyping and relatively high heritability of resistance.

There are several diseases where less progress has been achieved including diseases that are more difficult to quickly and accurately phenotype. Because of this, resistance to some diseases may be better improved with the use of molecular markers, either with MAS or with the use of genome wide prediction (Technow et al., 2013). With all of the tools that are available to the maize breeder today increased disease resistance can be achieved more quickly than ever before. This is facilitated by the use both of genotypic methods to locate and understand the genetic architecture of disease resistance, and traditional breeding to develop lines that have application in growers' fields.

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-CHAPTER II-

Identifying Northern Leaf Blight Resistance in Maize-Teosinte Introgression Lines

by

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Abstract

The potential of teosinte as a source of novel resistance to northern leaf blight (NLB), caused by *Setosphaeria turcicum,* has not been extensively studied. For this study, a set of near isogenic lines (NILs) derived from five *Z. mays* ssp. *parviglumis* teosinte accessions in a background of the commonly-used maize inbred line B73 were screened for resistance to NLB in order to identify teosinte alleles that confer increased levels of disease resistance. Teosinte alleles conferring enhanced resistance to NLB were identified, but when the effects of these alleles were compared to the effects of alleles from maize inbred lines Tx303 and Mo17, none of the teosinte alleles conferred significantly more resistance than the maize alleles.

Northern Leaf Blight Literature Review

Fungal Biology and Infection

Northern leaf blight (NLB), caused by the fungi *Setosphaeria turcica (*anamorph *Exserohilum turcicum)*, has been an economically damaging foliar disease of maize throughout the world. *S. turcica* is a hemibiotrophic Ascomycete, which has a feeding pattern characterized by three stages; initial feeding which occurs on live plant tissue, subsequent inducement of necrosis, and finally necrotrophic feeding. Infection begins when an *S. turcica* spore lands on a susceptible plant leaf; successful colonization is dependent on specific climate conditions, and usually begins after a morning dew period when moisture levels are high, the temperature is moderate, and light levels are low. As the spore begins to germinate, hyphae grow from the spore in a bipolar manner. Germination is dependent on light, with constant light inhibiting germination by up to 85% when compared to a twelve hour light dark cycle (Levy and Cohen, 1983). The hyphae protruding from the spore grow along the leaf surface and produce appresoria. An infection peg develops from the appresorium, which penetrates directly through the cuticle and epidermis of the leaf, but rarely through the stomata (Knox-Davies, 1974). The mycelia grow through the leaf tissue and into the vascular system, spreading systemically throughout the plant (Muiru et al., 2009). In later stages xylem are blocked by mycelial growth causing wilting from water deficiency (Thakur et al., 1989). The typical disease symptoms observed are cigar-shaped lesions that are gray to tan in color (White, 1999). The infection is generally limited to leaves.

The fungus produces asexual conidia on conidiophores that protrude directly out of lesions on the leaf surface (Knox-Davies and Dickson, 1960). Conidial dispersal is triggered by a reduction in humidity and light intensity. A minimum of two hours of darkness is needed, as constant light hampers the formation of conidia in culture (Flaherty and Dunkle, 2005). Short-range dispersal can occur through rain splash, whereas longer range dispersal can occur via an electrostatic force that

volleys spores into the air (Leach et al., 1977). The conidial spread can cause secondary infections on the same plant or spread the infection to neighboring plants or fields. In temperate climates, the fungus overwinters as mycelia, conidia, or chlamydospores (resting spores) on stubble and decaying plant material, allowing for re-infection when conditions are favorable (Leach et al., 1977). Reproduction is almost entirely asexual, via production of conidia. Like many Ascomycete plant pathogens, *S. turcica* is thought to spend the majority of its life as a haploid organism, only becoming diploid for a brief stage during sexual recombination before undergoing meiosis to produce haploid ascospores (Moghaddam and Pataky, 1994). To date, the sexual stage has only been observed under laboratory conditions and has not been reported on field material (Borchardt et al., 1998; Ramathani et al., 2011).

The dissection of the molecular mechanisms underlying *S. turcica* infection has been critical to gaining a full understanding of the disease complex. Many up-regulated genes associated with asexual conidial development under constant darkness have been identified, such as transcription factors and receptor proteins (Flaherty and Dunkle, 2005). In addition, germination of conidia and formation of appressoria are regulated by mitogen-activated protein kinase signal transduction pathways (Fan et al., 2007). The pathogen facilitates penetration and colonization via the production of a range of secondary metabolites and toxins. The *S. turcica* genome contains two genes encoding xylanase enzymes that degrade arabinoxylan in the plant cell wall, causing loss of integrity and aiding in penetration (Degefu et al., 2004). Two particular isoforms of catalases identified from artificial cultures are implicated in spore germination and early infection processes (Keissar et al., 2002). A multitude of toxins have been isolated from *S. turcica* (Robeson and Strobel, 1982; Zhang et al., 2006); one toxin, *Helminthosporium turcicum* (HT) toxin, has been identified as a lipophilic phytotoxin called Monocerin. Monocerin causes an inhibition in chlorophyll synthesis, a reduction in root growth, and formations of lesions and necrotic areas on susceptible genotypes of maize and

sorghum (Cuq et al., 1993; Bashan et al., 1995; Zhang et al., 2006). Furthermore, leaves punctured and treated with Monocerin develop necrosis that spreads throughout the vascular system; thus Monocerin HT-toxin is not only involved in penetration but also in later stages of infection (Cuq et al., 1993).

Northern Leaf Blight Resistance in Maize

As early as the 1950's, resistance to NLB was a target of maize breeding programs (Welz and Geiger, 2000, Robert and Findley, 1952). NLB resistance became a major concern after a series of heavy NLB epidemics occurred in the USA in the 1940's and 50's (Ferguson and Carson, 2007). During the early 1940's, double cross hybrids were being used, and the majority of the hybrids were highly susceptible to NLB. Farmers, who for the first time had significant amounts of money invested in quality seeds, became more aware of the presence of disease in the newly developed maize varieties. Thus breeding programs were initiated with polygenic resistance being the first type of resistance utilized (Jenkins and Robert, 1961).

It was in 1959 that qualitative resistance to NLB was first described in detail. Resistance was categorized into six dominant genes, *Ht1* to *Ht3*, *Htm*1, *Htn1* and *NN*, which were shown to control resistance to specific races of *S. turcicum*. The *Ht1, Ht2,* and *Ht3* genes confer a "chlorotic lesion" type of reaction to the pathogen whereas the *Htn1* gene helps delay of symptoms until after anthesis (Carson, 1995). In the 1960s and 1970s these genes were utilized in US germplasm; the *Ht1* gene was used extensively until a race of *E. turcicum* with corresponding virulence to it was found in Hawaii in 1972 and subsequently spread to the continental USA (Smith and Kinsey, 1980). Pratt and Gordon (2010) state that deployment of major gene resistance to NLB in the US had been over-done, which had left US maize germplasm vulnerable to race shifts, and thus further breeding efforts would require a combination of the Ht genes and quantitative resistance. Breeders in tropical environments

have placed more emphasis on quantitative resistance, partly due to different race types than in the USA, and have been able to develop lines with high levels of resistance (Paliwal et al., 2000).

Many sources of qualitative and quantitative resistance have been identified within the maize primary gene pool. Promising resistant public inbred lines from the US and Canada have been identified, including C.I.66, CH.586, H60, H95, H99, Mo42, Oh509A and W570 (Darrah, 1986). Resistant tropical germplasm and temperate inbreds adapted to tropical conditions identified by Brewbaker et al. (1989). These include CM118 from India, Fla2AT115 from Florida, Hi39 from Hawaii, and H55 from Indiana. As well, many CIMMYT maize lines have been screened and been shown be potential sources of resistance, including CMLs 437, 438, and 439. A full list would be too extensive to present here but a good summary is provided by Pratt and Gordon (2010). While the identification of resistant maize lines with NLB resistance has been relatively straight forward, there is a continued search for new sources of disease resistance.

Teosinte Biology

Wild teosintes (*Zea* spp.) are the putative ancestors of domesticated maize (*Zea mays* ssp. *mays*) and are native to Mexico, Guatemala and Nicaragua. As early as the nineteenth century, botanists realized that there was a close relationship between the teosintes and maize. The subspecies *Z. mays* ssp. *parviglumis* is now considered the most likely direct ancestor of modern cultivated maize. Currently four species and three subspecies of *Z. mays* are collectively referred to as teosinte: *Z. diploperennis*, *Z. nicaraguensis*, *Z. luxurians,* and *Z. perennis,* and the subspecies, *Z. mays* spp. *huehuetenangensis*, *Z. mays* ssp. *mexicana,* and *Z. mays* ssp. *parviglumis* (Funkunaga et al., 2005).

Cytologically, all of the teosintes have chromosome lengths and centromere positions similar to those of modern maize, facilitating hybridization and cross-species study, with only *Z.* perennis being tetraploid. Teosinte morphology is also similar to that of maize, but teosinte produces tillers

more profusely than maize, with tillers often as tall as the main stalk. Teosinte also has a tendency to produce branches at the upper nodes of the plant. The largest differences between teosinte and maize occur in the female inflorescence. In teosinte, the female flower develops as a spike and is very brittle, shattering at maturity for seed dispersal. The teosinte seeds are enclosed in hard, mostly triangular or trapezoidal fruit cases that are, for humans, indigestible while the maize ear produces seeds without hard fruit cases. Maize has also lost the trait of seed shattering, so kernels remain attached to the ear at maturity (Mangelsdorf, 1974).

Despite the differences in ear and seed morphology between teosinte and maize, all diploid species of teosinte can form hybrids with maize under natural conditions. Crosses of maize with *Z. mays* ssp. *mexicana* and *parviglumis* are the most common and fertile. George Beadle observed that about one in five hundred F_2 progeny from a maize by teosinte cross were either completely maize-like or teosinte-like, and noted that this ratio corresponds to four or five major genes controlling the modern maize growth habit (Doebley, 2004).

Teosinte germplasm may be a potential source of valuable alleles that are capable of improving disease resistance (Desjardins et al., 2000). Through the use of modern genetic tools, it is possible to identify, map and study the effect of individual loci that control quantitatively inherited traits or quantitative trait loci (QTL) with much more precision than in previous years. This will allow researchers to better identify favorable alleles contained in wild species that are obfuscated by heterozygosity or the deleterious effects of other genes. Termed as “hidden genetic variation” by some researchers these “hidden genes” once detected can be utilized for breeding and crop improvement (Tanksley and McCouch, 1997).

Experimental Rationale

There has been little work done to date evaluating the resistance of wild teosinte populations to fungal pathogens. Teosintes are reportedly susceptible to some fungi that are pathogenic to maize,

but the potential of teosinte species as sources of genes for improved resistance to northern leaf blight is unknown (White, 1999). Because subspecies of teosinte grow in tropical environments with no winter freeze to keep pathogen populations under control, teosintes must tolerate significant disease pressure. Estimates of the percentage of diversity retained during maize domestication range greatly, and there some indications that teosinte is a potential genetic resource (Warburton et al., 2011). Teosinte alleles for disease resistance could be incorporated into elite maize lines, as the use of genetic resistance is currently the most effective strategy for reducing yield-limiting diseases in maize production (Tripp, 2006).

Methods and Materials

For this study, a set of near isogenic lines (NILs) derived from five teosinte (Z. mays ssp. parviglumis) accessions by Dr. Sherry Flint-Garcia (USDA-ARS, Columbia, Missouri), was evaluated in a preliminary study. The five donor teosinte accessions are documented by the North Central Plant Introduction Station, Ames, Iowa (http://www.ars-grin.gov). Four accessions (Ames 21889, Ames 21785, Ames 21786, and Ames 21789) were used to develop populations of BC4S2 (backcrossed four times to B73, selfed twice) lines, with fifty lines generated per accession (S. Flint-Garcia, personal communication). The fifth population is a BC4 population derived from teosinte accession PI 384071; these backcrosses went through a doubled haploid (DH) system, with fifty lines produced. These populations were genotyped by S. Flint-Garcia with 1536 single nucleotide polymorphism (SNPs) using the Golden Gate Genotyping Assay developed by Illumina (San Diego, CA, USA).

The teosinte introgression lines were first evaluated in 2008 at the Cornell University Musgrave Research Farm at Aurora, NY, planted on May 14th laid out in an augmented incomplete block design (AIBD) with two replications, five blocks per replication, with each block containing ten experimental lines and augmented with two rows of B73. The maize/teosinte lines were inoculated

with the NLB pathogen *Setosphaeria turcica*, isolate EtNY001, which had been collected in 1983 in Freeville, New York; the inoculation methodology can be found in Chung et al. (2010). Plants were inoculated on the 27th of June 2008 and data was collected on incubation period (IP; number of days after inoculation until 50% of plants in a row showed disease symptoms) and diseased leaf area (DLA is measured on a 0-100% scale with one percent increments). Diseased leaf area measurements were taken at three time points in the season, on August 5th, 15th and 25th. From these three DLA measurements the standardized area under disease progress curve (sAUDPC) was calculated as in Campbell et al., (1990).

Data were analyzed using a mixed linear model in the statistical software package ASReml 3.0 (Gilmour et al., 2009). Based on the field design used, replications were nested within population, and blocks were nested within replications and populations. sAUDPC least square mean estimates of the fixed source effects were then compared to B73, and introgression lines that were significantly more resistant than B73 were selected as lines of interest. Putative QTL were identified by comparing the location of teosinte introgressions present in the top performing lines to the known locations of QTL that have been identified in the maize Nested Association Mapping Population (NAM; Poland et al., 2011) and/or a summary of prior QTL studies on disease resistance in maize (Wisser et al., 2006). From the initial screening of the two hundred and fifty NILs, three lines derived from two accessions were used for further study: 07PR172301A (developed from Ames 21789), with introgressions of interest in maize chromosomal bins 8.06, 1.06, and 7.02; 07PR140101A (Ames 21889), with introgressions of interest in bins 1.06 and 1.08; and 07PR141901A (also Ames 21889) with introgressions of interest in bins 2.02 and 5.05 (Table 2.1).

As the three lines found in the initial study carried teosinte introgressions at more than one locus of interest, lines homozygous for only one introgression of interest were then developed during the summer of 2009. This was done by using a single marker (the marker closest to the QTL peak

based on the NAM analysis) (Table 2.1) within each teosinte introgression to fix single loci of interest and eliminate lines which had teosinte introgressions at any of the other five loci. This created seven classes of lines in total, corresponding to the seven individual allele classes of interest. This process resulted in varying numbers of lines in each class ranging from one to twenty eight. The scheme of line development and testing is presented in Table 2.2. An evaluation trial of these lines was planted on April 20, 2010, in Clayton, North Carolina, at the Central Crops Research Station. This experiment used 80 entries total, and was laid out in an AIBD with three replications. There were twenty blocks in each replication, and each block consisted of four experimental lines augmented with one B73 from the same source used to develop the NILs. Plant material was inoculated on the 3rd of June 2010 with isolate 52B race 2,3 N (collected in Union City TN), which was cultured using the methodology outlined in Chung (2010). Data were collected on stand count and diseased leaf area (DLA). DLA ratings were taken on a 0 to 100% scale in increments of one on July 7th, 17th, 27th and August 6th, and used to calculate sAUDPC. Only entries which were significantly better than B73 in 2010 were used in 2011 and 2012 trials with varying numbers of teosinte introgression lines in each class ranging from one to ten, with 25 in total.

Further testing was conducted to investigate how the teosinte-sourced introgressions compared to resistance in publically available maize inbred lines. Two other comparable NIL sets which have B73 as the recurrent parent were available. The first of these, a Mo17 x B73 NIL set at the BC_3S_4 stage (Eichten et al., 2011), was provided by Nathan Springer of the University of Minnesota.. The other NIL population is a set of 89 lines of Tx303 by B73 at the $BC_3F_{2:3}$ stage (Szalma et al., 2007). As there was no unified genotyping platform across the three NIL sets, each of the six markers used to develop the lines homozygous for only one introgression of interest was localized on the physical map of the reference B73 sequence (Schnable et al., 2009). Mo17 or Tx303 lines which had homozygous single introgressions that spanned the position of these markers were

included in the study. Lines which were fixed for more than one of the six regions of interest were also included. Two other regions that underlie QTL which had been identified as conferring resistance to NLB in another study (in bin 4.08, and a combination of bins 4.08 and 9.02; Balint-Kurti et al., 2010) were also added. In total 51 maize introgression lines were chosen for evaluation. In all, 76 experimental lines were used which comprised 28 different allele or combinations of allele classes (Table 2.3 and 2.4).

The comparison of lines homozygous for only one teosinte introgression of interest to the maize-maize NILs occurred in 2011 and 2012 in NC and in 2012 at a DuPont Pioneer (Wilmington, DE) research station in Windfall, Indiana. In 2011, the entries were planted on April 5th in North Carolina in an AIBD with three replications and 15 blocks per replication, with each block consisting of six experimental lines and a B73 check. In 2012, trials were planted on March 29 in NC, and on May 10 in Indiana using the same experimental design as 2011, with three replications at each location. In 2011 and 2012, plant material grown in NC was inoculated on the 27th and 17th of May, respectively, with isolate 52B race 2,3 N (collected in Union City TN), using the methodology outlined in Chung (2010). In Indiana, race one inoculum was provided by DuPont Pioneer and was applied on the 19th of June.

At all three locations, data were collected on stand count and diseased leaf area (DLA). DLA in the NC environments was taken on a 0 to 100 scale in increments of one, while Indiana was rated on a 1 to 9 scale in one unit increments with 9 being least diseased. This scale was then reversed and multiplied by ten to place the ratings in better alignment with those taken in the two NC environments. In 2011, NC ratings were taken on June 28th and the 8th and 18th of July, while in 2012 NC ratings were taken twice, on June 20th and 30th. The Indiana location was rated once due to quick plant senescence, on the 7th of September. Because there was just one rating in Indiana the

standardized area under disease progress curve (sAUDPC) was only calculated for the two NC environments.

Each of the four environments was analyzed separately using ASReml v. 3.0 (Gilmour et al., 2009). Genotypes were designated by the chromosomal bin of the fixed chromosomal segment of interest and the source of that introgression (e.g. 1.08_{A21789} is a NIL in which the introgression of interest derives from accession Ames 21789 and is in bin 1.08). Genotype was modeled as a fixed factor with replication, block within replication, and the interaction of these with genotypes modeled as random factors. Also included in the random effects was the spatial designation of row and column for each of the plots within the field experiment. The spatial trend effects were modeled as first through fourth degree polynomial effects for rows and columns in the trend analyses, and included if $p < .01$ (Tamura et al., 1988).

A combined analysis was also done with the NC 2010 – 2012 and 2012 IN data. This included data on lines which were also tested in 2010, that were tested again in 2011 and 2012. The combined analysis used ASReml v. 3.0. to assess whether any of the loci tested provided resistance across years and race types. A heterogeneous error matrix was used to account for the different rating scale used at the Indiana location. Replication within each environment, blocking within reps within environments, and the interaction of these with the genotypes were modeled as random variables. Also included in the random effects was the spatial designation of row and column for each of the plots within environment within the field experiment.

Results

In 2008 NY, lines which had sAUDPC scores less than 13.45 were significantly different than B73 at α=0.05 (Table 2.5). The first line of interest was 07PR172301A from the B73×Ames 21789 BC_4S_2 population. 07PR172301A had a sAUDPC score of 11.5 and an introgression of particular interest which covers bin 8.06, the same location as the QTL with the largest effect on

sAUDPC found in the NAM population. Other introgressions in the line include a single introgression that covers two QTL identified in the NAM population in bin 1.06 (the marker with the larger effect under the QTL in 1.06 was used to develop lines isolated for a single region of interest) and an introgression that ends about 0.6 cM from a QTL that had been identified on chromosome 7 in the NAM. The second line selected, with a sAUDPC of 11.9, was 07PR140101A (B73×Ames 21889 BC_4S_2). This line contained two introgressions, the first of which covers the region that includes larger effect QTL found in 1.06. The second introgression lies in the same region which contains the QTL found in the NAM population in bin 1.08. The third line selected was 07PR141901A (derived from B73×Ames 21889 BC_4S_2), with a sAUDPC of 10.5. This line had two introgressions, one each on chromosomes 2 and 5. The introgression on chromosome 2 corresponds to bin 2.02, where a QTL has been identified in the NAM, and the introgression on chromosome 5, located in bin 5.05, corresponds to a NLB resistance locus that was identified through a synthesis of previous studies (Wisser et al., 2006). A summary of the seven teosinte allele classes under investigation can be seen in Table 2.6.

In the 2010 experiment testing lines homozygous for only one teosinte introgression of interest, all seven of the introgression classes produced AUDPC estimates which were significantly different than that of B73 (Tables 2.7 and 2.8). The trials in 2011 and 2012 compared the seven lines homozygous for only one teosinte introgression of interest to maize-sourced introgressions in the same regions (Table 2.3). In 2011 NC, genotypes were significant at $p < .001$ (Table 2.9), and six of the allele classes had sAUDPC scores significantly better than B73. Those which were better include 8.06_{A21789}, 2.02_{A21889}, 2.02_{Mo17}, $1.06 + 2.02_{Mo17}$, $1.06 + 7.02_{Mo17}$ and the $1.08 + 2.02_{Mo17}$ (Table 2.10). In the 2012 NC ANOVA, genotypes were significant at $p < .001$ (Table 2.11), and four of the lines had sAUDPC scores significantly better that B73; these include 7.02_{TX303}, 1.08_{TX303}, $1.08+2.02_{Mo17}$, and $4.08+9.02_{Mo17}$. A full summary of the results can be seen in Table 2.12. In 2012 IN,

genotypes were significant at p <.001 (Table 2.13) with two of the introgression classes having sAUDPC scores significantly better that B73; these include 1.08_{TX303} and $1.08+2.02_{Mo17}$ (Table 2.14).

In the combined analysis of 2010-2012 NC and 2012 IN, genotypes overall were not significant (Table 2.15) and none of the lines containing teosinte or maize introgressions had estimates significantly different than B73 (Table 2.16). This may be due to the fact that different race types were used in Indiana and North Carolina and/or that the change in rating scales increased the experiment wise error, making the lines less directly comparable to one another. More testing would be needed in other environments to provide getter estimates of the true amount of disease resistance each of these introgressions may confer.

Discussion

In this study, teosinte-sourced resistance introgressions have been tested for the first time, and initially were found to offer enhanced disease resistance when compared to a susceptible elite maize inbred. However, after testing in multiple environments, it was found that none of the lines containing tesosinte introgressions (or comparable maize introgressions) outperformed the susceptible check. This may be due to the use of only one marker in each region to isolate each teosinte introgression, which may have resulted in segregation between the marker used and the targeted gene underlying the QTL descried in the NAM population. Teosinte may be a useful resource of maize breeding for other traits, but in the case of NLB resistance these accessions may not have been the best resource. *Zea mays ssp. parveglumis* is predominately found in the Balsas River valley of Mexico (Wilkes, 1988). This region has a tropical arid climate, which is not conducive to the development of NLB, as the pathogen is not tolerant of high temperatures and low humidity, and thus there may not have been selection for increased NLB resistance over time in these teosinte populations. It may be better, in the context of finding superior alleles for NLB resistance, to sample teosinte from cooler and more humid regions were NLB is more likely to be prevalent, and phenotype the accessions themselves before

producing backcross lines. This would be followed by QTL mapping in the attempt to locate loci that can contribute to disease resistance and which have not yet been described in maize.

Maize may be a better source of NLB resistance for other reasons; humans have taken the domesticated form of maize to many more environments than teosinte is currently found. Due to the large number of environments there may have been more chances for a maize line to have come into contact with the NLB pathogen in an NLB-favorable environment, and been selected for NLB resistance. Though it is true that teosinte may have been exposed to an equally large number of NLB-favorable environments throughout evolutionary time, if the climate or these environments only rarely produced an NLB-favorable environment then the development of resistance may not have occurred.

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Table 2.1 Teosinte loci of interest and markers used to isolate introgressions

INTROGRESSION REGION WITH NOTATION OF GERMPLASM SOURCE	MARKER USED TO PRODUCE FIXED INTROGRESSOIN LINE
1.06 $_{A21889\ and}$ *1.06* $_{A21789}$	PZA02191_1
1.08 $_{A21889}$	PZA03301_2
2.02 $_{A21889}$	PZB00901_4
5.05 $_{A21889}$	PZA02356_7
7.02 $_{A221789}$	PZA03344_2
8.06$_{A221789}$	PHM4757_14

Markers were developed by the Maize Diversity Project and Illumina, and are now used in the Golden Gate Assay, more information on each marker is available at www.panzea.org

Table 2.2. Timeline for the derivation of lines for testing of individual QTL

Season	Method	Location
Summer 08	BC_4S_2 arrived from Missouri, BCs made from eight individuals in three BC_4S_2 lines to make ($BC_4S_2BC_1$)	Aurora, NY
Winter 08	BC all ($BC_4S_2BC_1$)	Homestead, FL
Summer 09	Development of lines fixed at a single teosinte introgression $BC_4S_2BC_2S_1$ or $BC_4S_2BC_2S_2$	Clayton, NC
Winter 09	Increase of seed and fixation of favorable recombinants Made some $BC_4S_2BC_2S_2$	Homestead, FL
Summer 10	Phenotypic observation	Clayton, NC
Summer 11 and 12	Comparison of Teosinte NILs to Maize NILs	Clayton, NC Windfall, IN

Table 2.3. Allele classes of experimental lines tested

1.06	1.08	2.02	5.05	7.02	8.06	Other	Mixed
1.06_{A21789}	1.08_{A21889}	2.02_{A21889}	$5.04+5.06_{TX303}$	7.02_{A21789}	8.05_{TX303}	$4.08+9.02_{Mo17}$	$1.06+1.08+7.02_{TX303}$
1.06_{A21889}	1.08_{Mo17}	2.02_{Mo17}	$5.04-5.06_{TX303}$	7.02_{Mo17}	8.06_{A21789}	4.08_{Mo17}	$1.06+2.02_{Mo17}$
1.06_{Mo17}	1.08_{TX303}		5.04_{TX303}	7.02_{TX303}	8.06_{Mo17}		$1.06+7.02_{Mo17}$
1.06_{TX303}			5.05_{A21889}				$1.08+2.02_{Mo17}$
			5.05_{Mo17}				$1.08+7.02_{TX303}$
							$1.08+8.06_{Mo17}$

Lines are listed by the bin in the maize genome of the introgression of interest, then by the donor of that introgression denoted by the subscript. A plus (+) between the loci means the line carried both of those loci, a dash (-) indicates a large introgression across several bins.

Table 2.4. Allele classes of experimental lines tested and the number of entries making up each class

Allele Class	Number of Lines in Each Class
1.06_{A21789}	10
1.06_{A21889}	1
1.06_{Mo17}	3
1.06_{TX303}	1
1.08_{A21889}	1
1.08_{Mo17}	7
1.08_{TX303}	1
2.02_{A21889}	7
2.02_{Mo17}	3
$5.04+5.06_{TX303}$	1
$5.04-5.06_{TX303}$	1
5.04_{TX303}	1
5.05_{A21889}	1
5.05_{Mo17}	3
7.02_{A21789}	3
7.02_{Mo17}	1
7.02_{TX303}	9
8.05_{TX303}	1
8.06_{A21789}	1
8.06_{Mo17}	11
$4.08+9.02_{Mo17}$	1
4.08_{Mo17}	2
$1.06+1.08+7.02_{TX303}$	1
$1.06+2.02_{Mo17}$	1
$1.06+7.02_{Mo17}$	1
$1.08+2.02_{Mo17}$	1
$1.08+7.02_{TX303}$	1
$1.08+8.06_{Mo17}$	1

Table 2.5. Summary of the sAUDPC and IP estimates of top performing lines NY 2008

TRAIT	GENOTYPE	LS MEAN	STANDARD ERROR
Incubation period (days after inoculation)	B73	15.9	0.5
	07PR146701A	17.4	1.7
	07PR170601A	17.4	1.5
	AR70744-1	17.4	1.3
Overall Standard Error of Difference 2.36	07PR170901A	17.5	1.5
	07PR172301A	18.0	1.5
	07PR140101A	18.0	1.4
	07PR141901A	18.0	1.5
	07PR141701A	18.1	4.3
sAUDPC	B73	20.8	0.7
	07PR144901A	10.5	3.7
	07PR141701A	11.4	6.8
Overall Standard Error of Difference 3.73	07PR172301A	11.5	2.3
	07PR140101A	11.9	2.2
	AR70760-1	12.1	3.3
	07PR170601A	13.5	2.4
	07PR169101A	13.6	2.2
	07PR143701A	14.0	2.4

Table 2.6. Summary of teosinte introgressions under investigation

INTROGRESSION REGION WITH NOTATION OF GERMPLASM SOURCE	RANK IN EFFECT IN NAM (POLAND ET. AL., 2011)
8.06_{A21789}	1
$1.06_{A21889\ and}\ 1.06_{A21789}$	3
2.02_{A21889}	8
1.08_{A21889}	9
7.02_{A21789}	23
5.05_{A21889}	Not indicated by NAM

Table 2.7. ANOVA table of 2010 NC analysis of sAUDPC

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Bin donor	7	19.3	16.15	<.001
Random Effects				
Source	Component			
Replication	0.21			
Column	2.82			
Block(replication)	0.42			
Error variance	5.05			

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3. NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 2.8. Least squared means estimates of sAUDPC in 2010 of lines fixed for introgressions of interest.

Bin Donor	Estimate	Standard Error
8.06_{A21789}	17.05	0.97
1.06_{A21789}	21.29	0.34
2.02_{A21889}	20.77	0.43
1.08_{A21889}	21.55	0.75
1.06_{A21889}	22.07	0.97
5.05_{A21889}	21.61	1.69
7.02_{A21789}	21.92	0.84
B73	24.85	0.38
Overall SE of Difference	0.8	
LSD α=0.05	1.6	

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3.

Table 2.9. ANOVA table of 2011 NC analysis of sAUDPC

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Bin donor	27	83.4	5.33	<.001

Random Effects	
Source	Component
Replication	3.16
Row	0.11
Column	15
Block(replication)	2.65
Bin donor*rep	0.04
Error Variance	6.35

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3. NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 2.10. LSM estimates of sAUDPC of lines 2011 NC.

Bin Donor	Estimate	Standard Error
$1.08+2.02_{Mo17}$	15.58	1.94
8.06_{A21789}	16.69	1.94
2.02_{Mo17}	17.51	1.59
2.02_{A21889}	17.77	1.27
$1.06+7.02_{Mo17}$	18.13	1.94
$1.06+2.02_{Mo17}$	18.44	1.95
1.08_{Mo17}	18.53	1.25
8.06_{Mo17}	18.74	1.19
1.06_{A21789}	18.98	1.20
7.02_{Mo17}	19.27	1.94
$1.08+7.02_{TX30}$	19.36	1.94
1.06_{Mo17}	19.48	1.43
1.06_{A21889}	19.64	3.00
$1.08+8.06_{Mo17}$	19.74	1.95
4.08_{Mo17}	19.85	1.56
7.02_{A21789}	19.97	1.42
8.05_{TX303}	20.04	1.95
1.08_{TX303}	20.53	1.94
1.06_{TX303}	21.70	1.98
$1.06+1.08+7.02_{TX30}$	21.99	1.94
B73	22.24	1.16
1.08_{A21889}	22.41	1.94
5.05_{Mo17}	22.56	1.42
5.04_{TX303}	22.82	1.94
$4.08+9.02_{Mo17}$	22.94	1.94
$5.04+5.06_{TX303}$	23.76	1.94
7.02_{TX303}	23.79	1.21
$5.04\text{-}5.06_{TX303}$	23.84	1.94
Overall Std Err of Difference	1.94	
LSD α=0.05	3.80	

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3.

Table 2.11. ANOVA table of 2012 NC analysis of sAUDPC

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Bin donor	27	217.3	3.71	<.001

Random Effects	
Source	Component
Row	2.81
Column	0.59
Block(replication)	1.09
Error Variance	12.04

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3. NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 2.12. LSM estimates of sAUDPC of lines 2012 NC

Bin Donor	Estimate	Standard Error
1.08_{TX303}	10.44	2.19
$4.08+9.02_{Mo17}$	11.07	2.65
$1.08+2.02_{Mo17}$	11.13	2.19
7.02_{TX303}	11.40	0.85
$1.08+7.02_{TX303}$	11.72	2.17
2.02_{Mo17}	12.03	1.32
4.08_{Mo17}	12.36	1.58
5.05_{Mo17}	12.53	1.32
2.02_{A21889}	12.83	0.93
1.08_{A21889}	13.05	2.19
1.08_{Mo17}	13.08	0.92
1.06_{Mo17}	13.29	1.31
7.02_{A21789}	13.29	1.31
$1.08+8.06_{Mo17}$	13.36	2.20
5.04_{TX303}	13.61	2.19
$5.04\text{-}5.06_{TX303}$	13.84	2.18
$1.06+7.02_{Mo17}$	14.14	2.17
8.06_{Mo17}	14.27	0.80
1.06_{A21789}	14.77	0.81
$1.06+1.08+7.02_{TX303}$	14.82	2.17
8.06_{A21789}	15.57	2.21
1.06_{TX303}	15.59	2.18
$5.04+5.06_{TX302}$	16.36	2.17
B73	16.42	0.71
8.05_{TX303}	16.68	2.17
7.02_{Mo17}	18.94	2.18
$1.06+2.02_{Mo17}$	26.63	2.18
Overall Std Err of Difference		2.53
LSD α=0.05		4.96

Table 2.13. ANOVA table of 2012 IN analysis of sAUDPC

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Bin donor	27	229.9	2.33	<.001

Random Effects	
Source	Component
Replication	1.34
Block(replication)	27.06
Error Variance	232.75

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3. NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Note: Error Variance in this environment many be much larger do to the greater range of DLA due to the adjustment from a 1to 9 rating scale, also this environment only received one rating.

Table 2.14. LSM Estimates of sAUDPC of Lines 2012 IN

Bin Donor	Estimate	Standard Error
$1.08+2.02_{Mo17}$	37.19	9.18
1.08_{TX303}	37.60	9.17
5.04_{TX303}	49.96	9.17
1.06_{Mo17}	50.19	5.34
4.08_{Mo17}	51.02	6.51
7.02_{TX303}	51.68	3.21
$1.06+1.08+7.02_{TX303}$	56.15	9.18
2.02_{Mo17}	56.63	5.34
1.06_{TX303}	58.45	9.17
$1.08+8.06_{Mo17}$	60.25	9.17
$1.06+7.02_{Mo17}$	62.12	9.18
8.06_{A21789}	62.28	9.17
1.08_{A21889}	62.80	9.17
$1.08+7.02_{TX303}$	62.82	9.17
1.08_{Mo17}	62.86	3.56
B73	63.67	2.50
5.05_{Mo17}	64.12	5.38
8.05_{TX303}	64.23	9.17
2.02_{A21889}	65.80	3.60
8.06_{Mo17}	65.89	2.92
7.02_{A21789}	66.58	5.38
1.06_{A21789}	66.75	2.93
$5.04\text{-}5.06_{TX303}$	67.48	9.17
7.02_{Mo17}	71.82	9.17
$4.08+9.02_{Mo17}$	72.83	11.22
$5.04+5.06_{TX303}$	79.68	9.17
$1.06+2.02_{Mo17}$	90.25	9.17
Std Err of Difference		10.89
LSD α=0.05		21.34

Table 2.15. ANOVA table of 2010-2012 combined analysis of sAUDPC

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Bin donor	27	31.6	1.28	0.247

Random Effects	
Source	Component
Environment	476.745
Replication(Environment)	1.2367
Row(Environment)	0.125184
Environment*Bindon	4.65502
Column(Environment)	1.44839
Block(Replication(Environment))	1.88186
Error Variance IN12	267.213
Error Variance NC10	4.90704
Error Variance NC11	6.56482
Error Variance NC12	12.48

Bindon stands for maize bin of the introgression and donor line of that introgression as noted in table 2.3. NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 2.16 Estimated values of sAUDPC scores of introgression lines tested in 2010-2012 grouped by introgression of interest

Bin Donor	Estimated Value	Standard Error
B73	31.9	11.0
Bin Donor	Estimated Value	Standard Error
1.06_{A21789}	29.7	11.0
1.06_{A21889}	29.5	11.1
1.06_{Mo17}	27.6	11.1
1.06_{TX303}	30.5	11.1
Bin Donor	Estimated Value	Standard Error
1.08_{A21889}	29.8	11.0
1.08_{Mo17}	28.3	11.0
1.08_{TX303}	27.0	11.1
Bin Donor	Estimated Value	Standard Error
2.02_{A21889}	28.5	11.0
2.02_{Mo17}	26.8	11.1
Bin Donor	Estimated Value	Standard Error
5.05_{A21889}	30.8	11.3
5.05_{Mo17}	29.9	11.1
5.04_{TX303}	30.0	11.1
$5.04+5.06_{TX303}$	33.0	11.1
$5.04-5.06_{TX303}$	31.2	11.1

Bin Donor	Estimated Value	Standard Error
7.02_{A21789}	29.5	11.0
7.02_{Mo17}	31.3	11.1
7.02_{TX303}	28.2	11.0
Bin Donor	Estimated Value	Standard Error
8.06_{A21789}	26.8	11.0
8.06_{Mo17}	29.4	11.0
8.05_{TX303}	30.8	11.1
Mixed Bin Donor	Estimated Value	Standard Error
$1.06+2.02_{Mo17}$	34.9	11.1
$1.06+7.02_{Mo17}$	28.1	11.1
$1.08+2.02_{Mo17}$	24.8	11.1
$1.08+8.06_{Mo17}$	28.4	11.1
$1.06+1.08+7.02_{TX303}$	30.6	11.1
$1.08+7.02_{TX303}$	28.3	11.1
Regions Identified in Balint-Kurti et al. 2010	Estimated Value	Standard Error
$4.08+9.02_{Mo17}$	29.9	11.1
4.08_{Mo17}	27.7	11.1
Overall Standard Error of the Difference		11.1
Least Significant Difference .05		21.8

-CHAPTER III-

The Correlation of Gray Leaf Spot Resistance of Inbreds to their Topcross Hybrids and the Yield of Those Hybrids With and Without GLS Disease Pressure

by

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Abstract

Gray leaf spot (GLS) is an important foliar disease of maize caused by *Cercospora zeae-maydis* and its sister species *Cercospora zeina*. To date there has been little published research on the correlation of GLS resistance between inbred lines and their respective topcross hybrids. Experiments have been designed to examine this correlation, as well as to assess the yield of such hybrids in both the presence and absence of the disease. For this study a set of forty-seven inbred lines were chosen which spanned the spectrum of GLS resistance, based on an analysis of historical data, and historically significant lines were included as reference points. These lines were crossed to two hybrid testers, Pioneer 3394 (GLS susceptible), and Pioneer 33M54 (GLS moderately resistant). Disease screening of inbred lines and hybrids was conducted at three locations in 2010 and 2011. Yield tests were conducted at one location in 2010 and 2011 with two treatment groups, one fungicide sprayed and the other inoculated. Inbred line to hybrid correlation of GLS resistance was found to be high with R^2= 0.87 in crosses with Pioneer 3394 and R^2=0 .86 with Pioneer 33M54. In yield trial experiments, hybrids under disease pressure lost an average of 1.04 metric tons per hectare or a 9.9% reduction. Hybrids with a resistant parent had significantly lower yield losses than hybrids with a susceptible parent, indicating that the use of resistant parents can maintain yield in the presence of disease.

Introduction

Gray leaf spot (GLS) is an important foliar disease of maize that is caused by *Cercospora zeae-maydis* and its sister species *Cercospora zeina* (Wang et al., 1998; Dunkle and Levy, 2000; Crous et al., 2006a). *Cercospora zeae-maydis* and *Cercospora zeina* are morphologically similar, but can be distinguished by physiological and phylogenetic criteria. First identified from specimens collected in Illinois in 1924 (Tehon and Daniels, 1925), *C. zeae-maydis* remained the main pathogen responsible for GLS in the United States until relatively recently. Now, *C. zeina*, considered to originate in Africa, is also known to contribute to disease. Both species are widespread in the United States (Crous et al., 2006a).

GLS became a major problem in the U.S. only in the 1970's due to widespread adoption of conservation tillage and continuous maize growing systems, increasing the amount of maize debris left in the field from previous growing seasons. GLS infection of maize acreage increased from 7.2 to 14.9 million hectares during the 1980's and 1990's (Sparks, 1997). As of today, GLS has spread to maize-growing areas worldwide, limiting yields to a moderate extent in the U.S. Corn Belt and considerably in Africa. In 1995, reported yield losses due to GLS were as high as 50% in some US maize fields (Ward et al., 1999). Disease severity is unpredictable, and varies from year to year. This unpredictability may become more severe as weather patterns change across the world, and the geographic range of both *Cercospora* species continues to expand (Ward et al., 1999). GLS occurrence will also increase in frequency as more growers adopt corn on corn management and no-tillage practices to increase yield and profitability.

The fungus survives the winter on infected maize residue from the previous growing season; this forms the source of inoculum for the next growing season. As temperatures increase in the spring, the overwintered mycelia produce spores that splash onto young maize leaves, causing the first

observable symptoms on the lower leaves of the plant. Wind may transport spores within a field and to neighboring fields. Symptoms are commonly observed following long periods of heavy dew and overcast days in bottomlands or fields adjacent to woods, where humidity will be higher and dew will persist longer into the morning (Ward et al., 1999). Reproduction and formation of secondary inoculum occurs in colonized tissue through the production of asexual spores called conidia. The sexual mating cycle of *Cercospora* species is not well known as no teleomorphs have been discovered. Phylogenetic analyses using the internal transcribed spacer sequences of a variety of *Cercospora* species have resolved *Cercospora* as a group of species belonging to the genus *Mycosphaerella* though it has yet to be placed due to the lack of teleomorphs (Crous et al., 2006a). To better understand the possible sexual nature of *Cercospora zeae-maydis,* the distribution of the mating type locus alleles (*MAT1* and *MAT2*) has been analyzed. The frequencies of these alleles, which define the two mating types in this phylum, have been estimated for some U.S. populations. For the populations studied, there is an even distribution of both *C. zeae-maydis* mating types, suggesting a lack of strict asexuality. A completely asexual organism would have an imbalance of mating type alleles due to founder effects, genetic bottlenecks, or drift. Further studies are needed to determine whether recombination occurs amongst race types (Crous et al., 2006b).

The onset of noticeable GLS symptoms typically occurs after plants have flowered (Hilty et al., 1979; Rupe, 1982). The relationship between maturity and GLS was demonstrated in these studies by planting of the same hybrid at three-week intervals and assessment of GLS development throughout the season. There was a positive correlation between disease progression and plant maturity, i.e., delayed planting resulted in delayed symptom development. Although GLS results mainly in gray-colored leaf blighting, other initial symptoms include the presence of tan, longitudinal, necrotic lesions, and leaf spots range from small, discrete, round or irregular lesions to large, oblong, rectangular-looking necrotic areas. The formation of lesions causes loss in photosynthetic leaf area,

limiting the production of carbohydrate that can be diverted to grain fill, which can cause a reduction in yield. There is also evidence that a toxin plays a role in disease development; C. *zeae-maydis* produces cercosporin (Dunkle and Levy, 2000), a photosensitizing perylenquinone that disrupts cell membranes in the presence of light. Carrera (1996) observed a negative correlation between plant population density and disease severity, and hypothesized that the increased light intensity at lower population densities enhances activation of cercosporin. Later, Daub and Ehrenshaft (2000) demonstrated that light intensity and cercosporin production are required for disease development by some cercosporin-producing fungi. Other environmental factors affecting levels of GLS infection are plant maturity, humidity, leaf wetness, temperature, initial inoculum concentration, and proximity to the inoculum source.

GLS is managed through use of moderately resistant hybrids, foliar fungicides and tillage practices which remove debris (Payne and Waldron, 1983; Lipps et al., 1996; Ward et al., 1997). Of these methods, resistant hybrids offer the best control since crop residue management cancels out benefits gained from conservation tillage, and fungicide application is often not profitable (de Nazareno et al., 1993 Munkvold et al., 2001). In commercial hybrids, resistance to GLS is derived most often from the Lancaster side of the pedigree (Ulrich et al., 1990; Carson et al., 2002). Some commercial hybrids with increased resistance to GLS have been developed; however, all genotypes eventually show some symptoms given adequate levels of inoculum and appropriate environmental conditions (Bubeck et al., 1993; Holland and Goodman, 1995; Dudley et al., 2000). The efficacy and efficiency of resistance breeding efforts must be improved as this is the most cost-effective and sustainable approach to GLS management.

The genetics underlying GLS resistance have been investigated to inform breeding efforts and two main conclusions have arisen from published studies: first, GLS resistance phenotypes are quantitatively inherited, and secondly, resistance QTL act in an additive manner (Gevers et al., 1994;

Elwinger et al., 1990; Ulrich et al., 1990; Bubeck et al., 1993). Ulrich et al. (1990) chose a set of nine inbred lines spanning the breadth of GLS resistance, crossed them in a diallel mating pattern, and evaluated them for GLS resistance over two years. They concluded that general combining ability (GCA), or additive gene action, was significant, whereas specific combining ability (SCA), or dominance gene action, was not. They discovered that the two most resistant inbreds in their study, T222 and Mo18W, performed worse than expected in hybrids combinations as they had negative GCA effect estimates for GLS resistance. This would suggest that hybrid performance cannot be extrapolated from the inbreds, but their sample size was rather small. Gevers et al. (1994) conducted a similar study in South Africa with a diallel-cross design of twelve heterotically divergent maize inbred lines, and analysis of the disease-rating data showed that both GCA and SCA variance components were highly significant. In another African study, van Rij et al. (2008) selected 27 inbreds across the different heterotic patterns used in southern Africa and crossed them in a Design II mating scheme. Although both GCA and SCA were both highly significant, more of the among-crosses variance (86%) was explained by GCA.

To better understand the field applicability of GLS resistance, and the correlation of this resistance with important agronomic factors such as flowering time and yield, more research is required. The first objective of this study was to better understand how resistance to GLS is expressed, using a larger sample size than in previous studies. To augment our knowledge of the genetics underlying the GLS resistance response, the correlation between the GLS resistance level of a set of inbred lines and their resulting testcross hybrids, in the presence of the pathogen, have been assessed. The second objective is to better understand the relationship between disease resistance and yield with this set of lines, both with and without disease pressure. This second part of the study will test the yield of this same set of hybrids, under GLS-inoculated and fungicide-sprayed treatments, and compare yield under these treatments to disease resistance values.

Materials and Methods

Development of the Research Population

Data from GLS testing locations spanning the years 2005 to 2007 (previously collected by the Goodman maize breeding program) was compiled in order to choose entries for further study of GLS resistance. GLS trials over this period were grown in three locations: the Upper Mountain Research Station in Laurel Springs, NC; the Piedmont Research Station in Salisbury, NC; and at Wood Farms in Andrews, NC. Experimental units consisted of 4.86 m long single plot rows planted with twenty-five seeds each at Laurel Springs and Salisbury, with a 1 m alley located at the end of a plot while at Andrews 3.5 m long plots containing eighteen plants were grown with a 0.8 m alley located at the end of a plot. Inter-row spacing at Laurel Springs, Salisbury, and Andrews were 0.91 m, 0.76 m, and 0.76 m respectively. Plots were subjected to standard North Carolina cultural practices, and were planted in the previous year's corn debris at Andrews and Salisbury, and with some debris present at Laurel Springs. The experiments in Salisbury and Laurel Springs were inoculated with infested oat grains provided by Syngenta AG (Basel, Switzerland) which were applied at approximately the V6 stage of growth.

Ratings were assigned to plots according to Bubeck et al., (1993), and were based upon the amount of total lesions in the overall plot, the upward spread of lesions from lower leaves, the coalescence of leaves' lesions, and the subsequent necrosis of leaf tissue. Visual GLS ratings were taken on a plot basis i.e., the visual average of all plants in a plot, on a 1-9 scale, with 1 designated as susceptible, and 9 designated as resistant. The initial ratings were taken approximately three weeks after the peak flowering time. The number of days from planting until anthesis (DTA) was recorded at the Andrews and Laurel Springs locations. Weighted Mean Disease (WMD) was estimated from repeated disease assessments that are taken during the growing season, this is functionally equivalent to standardized area under the disease progress curve (Balint-Kurti et al., 2008). In this case a higher

WMD value is desired, as plants were scored with high values corresponding to increased resistance. WMD was calculated through use of the following formula (Campbell et al., 1990):

$$\mathrm{WMD} = \sum_{i=1}^{n-1} \left(\frac{\mathrm{Rating}_{i+1} + \mathrm{Rating}_{i}}{2} \right) \left(\frac{\mathrm{t}_{i+1} - \mathrm{t}_{i}}{T} \right)$$

WMD was modeled using PROC MIXED (SAS 9.2, SAS Institute, 2008) as a completely randomized design with fixed effects for line and days to anthesis (DTA) and random effects for environments, and replications within environments. The strong correlation of maturity with the onset of significant GLS symptoms suggests inclusion of a flowering time covariate to avoid confounding of resistance and maturity. Those lines with WMD scores (from the 2005 – 2007 data) greater than two standard deviations from the LS mean of the average WMD score, 32 in all, were chosen for inclusion in the study with several lines considered to be historically significant also added to bring the total to 47 (Table 3.1). The 47 lines were placed in a paired row crossing block in the summer of 2009, at the Central Crops Research Station, in Clayton, NC. Pollinations were made to each of two hybrid testers: Pioneer 3394 (a GLS susceptible hybrid patented in 1991; US patent 5491295 A), and Pioneer 33M54 (a moderately GLS resistant hybrid patented in 2003; US patent 7005566 B1). Hybrid testers are favored by the NC State corn breeding program for their adaptability to harsh summer conditions, allowing for production of ample pollen and good seed set.

Experimental Design

The inbred lines and their respective topcrosses with the two testers were grown for GLS screening in an complete randomized block design with two replications, using the two testers as checks in both inbred and topcross screenings for 49 entries total. Inbred trials and non-yield-trial hybrids were planted in a different part of the field from the hybrids grown in yield trials due to management concerns. Each group of material was grown for GLS evaluation in the three North

Carolina locations listed above, using the same experimental units during the 2010 and 2011 growing seasons. Andrews, Salisbury, and Laurel Springs were planted on May 12, April 29, and May 12 in 2010 and May 11, April 27, and May 26 in 2011, respectively. Once ratings commenced at an environment, subsequent ratings were taken at approximately fourteen-day intervals. Andrews was rated twice in 2010 (August 17 and 28), and three times in 2011 (July 30, August 7 and 20). Salisbury was rated twice in 2010 (August 7 and 21), and three times in 2011 (August 3, 15 and 27). Laurel Springs was rated three times in 2010 (September 9, 22 and October 7), and three times in 2011 (September 3, 17 and 30). Notes on days from planting until anthesis (DTA) were also recorded for each entry at the Andrews and Laurel Springs locations. The weighted mean disease (WMD) was calculated from these DLA data points using the same method used above.

The hybrids discussed above were also placed in yield trials using a split–split block design, the first split being GLS inoculated vs. fungicide-sprayed main block treatments, and the second being the Pioneer 3394 vs. Pioneer 33M34 tester sub-block treatments. These tests were grown in a 7 x 7 lattice design with three replications, using Pioneer 3394 and Pioneer 33M54 as checks (replicated three times each) within each of the four sets of topcrosses. Randomization was restricted so that all of the forty-seven hybrids with Pioneer 33M54 were placed together, and all of the hybrids with Pioneer 3394 were placed together, field lay out in a single environment is illustrated in Figure 3.1.

For logistical reasons, the only location where yield trials could be conducted in the presence of GLS is the Piedmont Research Station in Salisbury, NC. Yield trials were planted on April 29 in 2010 and April 27 in 2011, in the same field. Half of the yield trial was inoculated with GLS inoculum, to form the diseased portion of the experiment. The fungicide Headline® (produced by BASF Ludwigshafen, Germany) was applied to the non-inoculated blocks three times at the suggested rate of 12 fluid oz. per acre, to prevent the spread of the disease to those blocks, but

complete control of the disease was not obtained. Ear height and plant height measurements were also taken in both inoculated and sprayed portions of the yield trials.

Analysis of Inbred and Testcross GLS Resistance

Disease ratings were generated from two sets of material: Inbred liens and F_1 hybrids genetic thus genetic effects of topcross hybrids were partitioned into three fixed effects: inbred parent, tester parent, and the inbred parent by tester interaction (checks were removed from data set as their parentage was unknown, leaving 47 entries). PROC MIXED in SAS version 9.2 (SAS Institute, 2008) was used to analyze WMD across the six inbred environments and eight hybrid environments (inoculated portions of the yield trials were added to the hybrid WMD analysis, and treated as field nested within environment). Data were analyzed according to a traditional randomized complete block model in which genotype, either the inbred or the inbred/tester combination, was considered a fixed treatment effect. Random effects included environment, replication, and the interaction of these with the fixed genotype effects. DTA was included in the model as a fixed covariate. As DTA was not collected at every location, DTA least squares mean (LSM) estimates calculated from the above model were substituted for the missing flowering data at Salisbury. Environment-specific row and column designations were tested in the model as random effects. First through fourth-order orthogonal polynomial trend effects in both row and column directions within each location were tested as fixed effects. Only effects with significance ($p \leq 0.01$) were retained in the spatial models, using ASReml v. 3.0 software (Tamura et al., 1988; Gilmour et al., 2009). The same model was broken down to find genotypic correlation between tropical vs. temperate inbred lines. NC474, NC480, NC488, NC494 and NC496 were removed from genotypic correlation as they were not strictly temperate or tropical lines.

The complete hybrid testcross WMD LSMs from both testers were then regressed on the LSMs of the inbreds in SAS PROC REG. The best-fitting regression line estimates the relationship between inbred and hybrid disease severity. From this regression line, the coefficient of determination, R^2, estimates how much the disease score of a hybrid depends on the disease score of its inbred parent. Genotypic correlations between the inbred and hybrid WMD were calculated with the following formula:

$$\hat{\mathbf{r}}_{gij} = \frac{\hat{\boldsymbol{\sigma}}_{Gij}}{\hat{\boldsymbol{\sigma}}_{Gi}\,\hat{\boldsymbol{\sigma}}_{Gj}}$$

in which $\hat{\sigma}_{Gij}$ is the estimated genotypic covariance between inbred and hybrid disease ratings, $\hat{\sigma}_{Gi}$ is the estimated genotypic standard deviation for inbred disease severity, and $\hat{\sigma}_{Gj}$ is the estimated genotypic standard deviation for hybrid disease severity (Holland, 2006). Correlations between hybrid resistance and inbred resistance were found through use of a multivariate mixed model in ASReml, fitting each entry as random and location as fixed. The ASReml model statement was:

$$Y_{INB}Y_{HYB} = Trait + Trait.line + Trait.env$$

Individual location LSMs for the inbreds and their corresponding testcross hybrids were input into the left side of this formula. The LSMs used to calculate the correlation are from the locations in which inbreds and hybrids were evaluated (Andrews 2010-2011, Laurel Springs 2010-2011, and Salisbury 2010-2011). In this model, Y_{INB} is the inbred GLS score, and Y_{HYB} is the hybrid GLS variate. *Trait* fits the mean for both disease variates, *Trait.line* fits the random genotype effect for each disease variate, and *Trait.env* fits the random location effect for each disease variate, producing a variance covariance matrix for both the location effects and genotype effect. It is this variance of the genotype effect that is genotypic variance.

Analysis of Yield Data

Yield, WMD, ear height, and plant height data were analyzed according to a split-split block design. The two sets of topcross hybrids and the two checks were grouped by treatment into a single experiment with 96 entries total. The inoculation vs. fungicide spray treatment factor, tester, inbred parent, and their interactions were considered as fixed effects, and year, replication, and blocking were considered as random effects. Interactions of the random effects with the fixed effects were also considered random effects. In order to separate effects of the two checks from the experimental lines dummy variables were added the data set as outlined in Piepho et al., (2006) which allowed for the separate comparison of check vs. topcross experimental lines, and the comparison of the two checks Pioneer 33M54 and Pioneer 3394 to one another. To augment this model with spatial effects, ASREML v. 3.0 was used to reduce computation time. Trend effects were modeled as first through fourth degree orthogonal polynomial fixed covariates in the row and column directions. The row and column designations of each plot within each environment were included as random factors. Only significant ($p \leq 0.01$) global effects were retained in the spatial models. If ASREML bound a variance component by zero, its corresponding random effect was dropped from the model.

To analyze the relationship between yield and resistance, yield estimates for the inoculated and sprayed portions of the experiment were regressed on the WMD estimates for both the inbred entries and the hybrids. This calculation was done using SAS PROC REG, in the same manner as hybrid disease LSMs were regressed on inbred LSMs above. Finally, the genotypic correlations between hybrid grain yield, WMD, plant height and ear height were calculated. The model used for the pairs of hybrid traits was analogous to the aforementioned bivariate treatment of inbred and hybrid GLS resistance across the number of environments. The model was also broken down to find genotypic correlation between hybrids with Pioneer 3394 vs. Pioneer 33M54, and tropical vs.

temperate inbred lines (NC474, NC480, NC488, NC494 and NC496 were removed from genotypic correlation as they were not strictly tropical or temperate lines).

Results and Discussion

In disease trials, genotype was significant (p <0.001) for both inbred lines and hybrids over all location/year combinations and in the across-environment analysis. The most resistant inbred in the combined analysis was CML5 with a WMD estimate of 8.06, and the most susceptible was PHG72 with a WMD estimate of 1.57. The average WMD over the 47 inbred lines tested was 5.87. The more susceptible hybrid tester, Pioneer 3394, had a WMD score of 3.02 while the more resistant hybrid tester, Pioneer 33M54, had a WMD estimate of 5.56. Among the Pioneer 3394 test crosses, the hybrid with the highest WMD score was derived from CML258 (WMD 6.81) and the lowest scoring cross was from PHG72 (WMD 2.44). The most resistant and susceptible crosses with Pioneer 33M54 were in combination with NC304 (WMD 7.17) and LP5 (WMD 2.54); a summary of WMD for all lines is provided in Table 3.11.

Interestingly, DTA was insignificant as a covariate in the analysis of WMD in both the inbred line (p = 0.88) and topcross (p= 0.28) analyses (Tables 3.2 and 3.5). Within inbreds, the same was true for temperate p = 0.78 and tropical entries p = 0.97 (Tables 3.3 and 3.4). Hybrids with tropical and temperate parents, again DTA was not significant in hybrids with a temperate (p = 0.54) and tropical (p = 0.22) parent (Table 3.6 and 3.7). This is contrary to much of the literature that indicates that the onset of disease is greater after flowering. The interaction of inbred parent and tester was significant (p <0.001), potentially indicating a measureable amount of heterosis in these topcrosses with respect to GLS resistance. The interaction was significant in crosses with both temperate (p <0.001) and tropical parents (p = 0.008).

Inbred and hybrid WMD were significantly correlated for both tester sets as indicated by the regression analysis (p <0 .001). The coefficient of determination was very high in crosses with

Pioneer 3394 (r^2=0.87) and in crosses with Pioneer 33M54 (r^2=0.86, Figures 3.2 and 3.3). There was a high genotypic correlation between inbred *per se* resistance and topcross resistance (94% in crosses with Pioneer 3394 and 93% in crosses with Pioneer 33M54). These high correlations suggest that indirect selection for inbred GLS resistance may be more effective at improving hybrid resistance, as opposed to direct selection for resistance in a resulting hybrid. The efficiency of response to indirect selection versus direct selection for GLS resistance can be expressed as

$$\frac{CR_{HYB}}{R_{HYB}} = \hat{r}_{GLS}\sqrt{\frac{\hat{H}_{INB}}{\hat{H}_{HYB}}}$$

where CR_{HYB} is the correlated response of hybrid resistance by selection on inbred *per se* resistance, R_{HYB} is the direct response of selection on hybrid resistance, $\hat{r}_{GLS}$ is the genetic correlation for GLS resistance between the inbred lines and their derived hybrids, $\hat{H}_{INB}$ is the entry mean heritability of inbred GLS resistance, and $\hat{H}_{HYB}$ is the entry mean heritability of hybrid GLS resistance (Falconer and Mackay, 1996). Efficiencies close to or greater than one indicate indirect selection for inbred *per se* resistance may be more effective than direct selection for hybrid resistance. The efficiency of indirect selection for GLS resistance is 0.93 in crosses with Pioneer 3394 and 0.92 in crosses with Pioneer 33M54. These values indicate that the end goal of improving hybrid resistance could be more readily achieved by selecting for resistance in the hybrids. However, the proximity to one of these efficiency values, and the very high inbred-hybrid WMD correlation, indicate breeding for GLS resistance in the inbred state would be effective.

Yield Trial Results

In the 2010 yield analysis, the treatment and inbred parent effects were significant ($p < 0.001$) as was tester effect ($p = 0.02$, Table 3.8). In 2010, the average yield was 8.6 metric tons per hectare (MT/ha) in inoculated blocks, and 10.3 MT/ha in sprayed blocks with the least significant difference

(LSD) for yield being 1.6 MT/ha thus yield was significantly reduced. The top yielding hybrid in the sprayed block was NC304 x Pioneer 33M54, with a yield of 12.5 MT/ha; the lowest yielding sprayed hybrid was PHG72 x Pioneer 3394 with a yield of 7.8 MT/ha. Under inoculated conditions, the top yielding hybrid was CML277 x Pioneer 33M54, with a yield of 11.7 MT/ha; the lowest yielding hybrid was LH93 x Pioneer 3394 with a yield of 5.1 MT/ha. The Pioneer 3394 and 33M54 testers *per se* respectively yielded 11.6 and 9.8 MT/ha in the sprayed block, and 6.9and 8.9 MT/ha in the inoculated block.

In 2011, the inbred parent effect for yield was significant ($p<0.001$), but the tester and treatment effects were not significant ($p=0.72$ and $p=0.17$ respectively; Table 3.9). The average yield was 9.9 MT/ha in the inoculated block, and 10.5 MT/ha in the sprayed block. In this environment, the LSD was 1.8 MT/ha, and an increase in disease pressure throughout the location in 2011 may have not allowed the sprayed block to reach its full yield potential, thereby resulting in the lack of means separation. The overall top yielding hybrid in the sprayed block was CML277 x Pioneer 33M54 at 13.1 MT/ha; the lowest yielding sprayed hybrid was NC250 x Pioneer 3394 at 8.3MT/ha. Under inoculated conditions, the top yielding hybrid was NC320 x Pioneer 33M54 with a yield of 13.7 MT/ha, and the lowest yielding hybrid was A632 x Pioneer 3394 with a yield of 6.4 MT/ha. The Pioneer 3394 and Pioneer 33M54 testers respectively yielded 11.2 and 10.8 MT/ha in the sprayed block, and 8.8 MT/ha and 10.2 MT/ha in the inoculated block.

As there are two years of data, the error term for yield in the combined analysis for the main block factor (the location by year interaction) has one degree of freedom; thus providing little power for testing the effects of the spray and inoculation treatments. Similarly the tester effect also has one degree of freedom in the combined analysis. The sprayed vs. inoculated treatment factor for yield was not significant ($p=0.31$). Also the effect of tester was not significant ($p = 0.57$), though the inbred parent factor was significant ($p <0.001$). The interaction of inbred parent with tester was significant

(p= 0.007), indicating different levels of heterosis in crosses with the two testers (Table 3.10). Average yield across all hybrids was 9.29 MT/ha in inoculated blocks and 10.34 MT/ha in sprayed blocks with an overall LSD of 1.6 MT/ha.

The overall top yielding hybrid under sprayed conditions was NC320 x Pioneer 3394 (12.9MT/ha), while the hybrid with the lowest yield in the sprayed blocks was PHG72 x Pioneer 3394 (8.1 MT/ha). Under disease pressure, the highest yielding hybrid was NC320 x Pioneer 33M54 (12.5 MT/ha), while the lowest yielding hybrid under disease pressure was LH93 x Pioneer 3394 (5.8 MT/ha). Pioneer 3394 and Pioneer 33M54 yielded 11.4 MT/ha and 10.2 MT/ha under sprayed conditions, and 8.0 MT/ha and 9.6 MT/ha under inoculated conditions, respectively. In general, the highest yielding hybrids in the inoculated blocks were those with the most disease resistance. Though average yield between the two treatment groups was not significantly lowered some individual hybrids showed a significant reduction in yield. These include Pioneer 3394 crossed with A632, B73, CML281, F42, FR1064, Ki11, LH132, LH93, LP5, Mo17, NC292, NC304, NC320, NC462, NC488, NC496, PHG72, and Tzi1, and inbreds LP5, 90156(GP-273), NC374, VO613Y, and Mo17 crossed with Pioneer 33M54 (Table 3.11).

With a linear regression, the relationship between both inbred parent and hybrid GLS resistance can be better defined. In Figure 3.4, the yields of Pioneer 3394 hybrids under inoculation have been regressed on the WMD of these same hybrids. There is a correlation between increased disease resistance of a hybrid and its resulting level of yield ($R^2 = 0.77$, $p<0.001$). The same phenomenon is illustrated in Figure 3.5 where yields of Pioneer 33M54 hybrids under inoculation have been regressed on the WMD scores of the Pioneer 33M54 hybrids ($R^2 = 0.64$, $p<0.001$). In crosses with both testers, there is a positive correlation between the GLS resistance level of the inbred and the yield of the corresponding hybrids under disease pressure. Yield of crosses with Pioneer 3394 and inbred WMD was significantly correlated ($p<0.001$) with a $R^2 = 0.72$ (Figure 3.6). In

crosses with Pioneer 33M54, yield was also correlated ($p<0.001$) with the inbred WMD with an $R^2 = 0.50$ (Figure 3.7).

GLS resistance and yield also have a positive genotypic correlation in this sample of lines. The overall genotypic correlation of GLS resistance across the sprayed and unsprayed treatment blocks with yield was 0.85. Within the inoculated block, the genotypic correlation between yield and WMD of all lines was 0.90. The genotypic correlation between GLS resistance and yield in inoculated blocks reveals a genotypic correlation of 0.86 in the Pioneer 33M54 topcross set and 0.92 ($p<0.001$) in the Pioneer 3394 topcross set. Genotypic correlations between disease resistance and other agronomic traits were also significant (Table 3.12).

Breaking down the genotypic correlations of agronomic traits between hybrids with a temperate or tropical parent shows disease resistance in temperate lines is more highly correlated with yield (0.78) than in tropical lines (0.61). This is likely due to a wider range of disease scores among temperate lines and a narrower range of yields among tropical topcrosses. This is also possibly due to different levels of heterosis (temperate x tropical crosses as compared to temperate x temperate). The correlation between ear height, plant height and yield is also of less magnitude in the hybrids with a temperate parent than in hybrids with a tropical parent (Table 3.13 and 3.14), this may be due to a smaller range of plant and ear heights in hybrids with a temperate parent, and a large range among tropical hybrids, or differential levels of heterosis.

Overall this study has revealed a close relationship between inbred per se and topcross GLS resistance. The high correlation between the disease resistance level of a given inbred line and its resulting hybrid progeny demonstrates that indirect selection among inbred lines may be useful in the development of disease-resistant hybrids. Under sprayed conditions topcrosses with resistant parentage generally had higher or equivalent yield than topcrosses with susceptible parentage. Under inoculated conditions topcrosses with a resistant parent had lower yield losses than hybrids with a

susceptible parent, indicating that the use of resistant parents can maintain yield in the presence of disease. On average, limited yield loss was observed when hybrids had WMD scores of about seven; this may represent a general target number for hybrid development programs.

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Table 3.1. List of lines used in this study with PI Number and region of origin.

Inbred Line	PI Number	Material Origin
(RMP85 x OH43E)F3S15	N/A	Temperate
(NC258xNC296)F4S8	N/A	Temperate
90156(GP-273)	561603	Tropical
A632	648423	Temperate
B73	648426	Temperate
CML5	Ames 27071	Tropical
CML10	Ames 27072	Tropical
CML108	Ames 27082	Tropical
CML258	Ames 27090	Tropical
CML277	595550	Tropical
CML281	Ames 27092	Tropical
CML288	N/A	Tropical
CML295	N/A	Tropical
F42	601026	Temperate
FR1064	IL Found seeds	Temperate
Ki11	Ames 27124	Tropical
Ki14	Ames 27259	Tropical
LH93	601171	Temperate
LH132	601004	Temperate
LP5	601378	Temperate
Mo17	648430	Temperate
Mo18W	550441	Temperate
NC250	550555	Temperate
NC258	531084	Temperate
NC292	Ames 27142	Temperate
NC300	Ames 27146	Tropical
NC302	Ames 27147	Tropical
NC304	Ames 27148	Tropical
NC320	Ames 27156	Temperate
NC354	Ames 27173	Tropical
NC368	Ames 27180	Temperate
NC374	N/A	Temperate
NC460	N/A	Tropical
NC462	N/A	Tropical
NC474	N/A	Mixed
NC480	N/A	Mixed
NC488	N/A	Mixed
NC494	N/A	Mixed
NC496	N/A	Mixed
NC508	N/A	Tropical
NC522	N/A	Tropical
Nei9004b	Ames 26253	Tropical
PHG72	601319	Temperate
Tzi5	540742	Tropical
Tzi1	506243	Tropical
Tzi17	506252	Tropical
VO613Y	N/A	Tropical

Note: (RMP85 x OH43E)F_3S_{15} (known internally as 3199-1/06) and (NC258xNC296) F_4S_8 (known internally as 9328-blk/02) are experimental inbred lines from the NC State inbred line development program. VO613Y is referenced by Gordon (2006).

Table 3.2. ANOVA of 2010-2011 combined analysis of WMD of inbreds

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	46	214.1	22.02	<.001
DTA	1	364.6	0.02	0.875
env 5 C1	1	128.9	8.11	0.005

Random Effects		
Source	Component	Component÷Comp.SdErr
Env	0.80	1.52
Rep(Env)	0.02	1.16
Env*Inbred Parent	0.42	6.29
Col(Env)	0.07	1.46
Error Variance	0.38	6.6

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. Env 5 C1 represents the first-order orthogonal polynomials for column in environment five.

Table 3.3. ANOVA of 2010-2011 combined analysis of WMD of temperate inbreds

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	17	79.8	21.37	<.001
DTA	1	156.1	0.07	0.779
Env 5 C1	1	141.1	343	0.068
Random Effects				
Source	Component	Component÷Comp.SdErr		
Env	1.45	1.52		
Rep(Env)	~0	0		
Env*Inbred Parent	0.32	2.99		
Col(Env)	~0	0		
Error Variance	0.54	6.84		

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. Env 5 C1 represents the first-order orthogonal polynomials for column in environment five.

Table 3.4. ANOVA of 2010-2011 combined analysis of WMD of tropical entries

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	23	108	3.81	<.001
DTA	1	180.5	0.00	0.966
env 5 C1	1	42.6	2.79	0.102
Random Effects				
Source	Component	Component÷Comp.SdErr		
Env	0.50	1.46		
Rep(Env)	0.01	.59		
Env*Inbred Parent	0.44	4.82		
Col(Env)	~0	0		
Error Variance	0.38	7.75		

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. (NC474, NC480, NC488, NC494 and NC496 were not included as they were not strictly tropical lines). Env 5 C1 represents the first-order orthogonal polynomials for column in environment five.

Table 3.5. ANOVA of 2010-2011 combined analysis of WMD of experimental hybrids

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	46	202.8	71.26	<.001
Tester	1	142.4	273.41	<.001
Tester* Inbred Parent	46	185.6	3.85	<.001
DTA	1	1051.5	1.19	0.277
Env 3 C1	1	1277.8	10.31	.001

Random Effects		
Source	Component	Component÷Comp.SdErr
Env	0.99	1.03
Field(Env)	0.49	1.01
Env* Inbred Parent	0.05	4.34
Env*Tester *Inbred Parent	0.02	1.77
Row(Field*Env)	0.02	3.7
Error Variance	0.27	23.58

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. The analysis does not include the two checks. Env 3 C1 represents the first-order orthogonal polynomials for column in environment five.

Table 3.6. ANOVA of 2010-2011 combined analysis of WMD of hybrids with a temperate parent

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	17	68.8	87.84	<.001
Tester	1	62.4	324.88	<.001
Tester* Inbred Parent	17	67.4	2.81	<.001
DTA	1	569.1	0.37	0.540
env 3 C1[#]	1	334.1	31.42	<.001

Random Effects		
Source	Component	Component÷Comp.SdErr
Env	1.24	1.04
Field(Env)	0.57	0.99
Env*Inbred Parent	0.03	1.88
Env*Tester*Inbred Parent	0.01	0.82
Row(Field*Env)	0.02	1.79
Error Variance	0.27	13.91

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. Env 3 C1 represents the First order orthogonal polynomials for column in environment three.

Table 3.7. ANOVA of 2010-2011 combined analysis of WMD of hybrids with a tropical parent

F Statistics Fixed Effects				
Source of Variation	NumDF	DenDF	F value	P value
Inbred Parent	23	101.6	9.33	<.001
Tester	1	92.0	57.54	<.001
Tester* Inbred Parent	23	98.9	2.06	0.008
DTA	1	693.1	1.53	0.219
env 3 C1[#]	1	722.0	0.37	0.538

Random Effects		
Source	Component	Component÷Comp.SdErr
Env	0.88	1.03
Field(Env)	0.45	1.02
Env*Inbred Parent	0.02	1.81
Env*Tester *Inbred Parent	0.02	1.54
Row(Field*Env)	0.03	2.80
Error Variance	0.25	15.99

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom. (Hybrids with NC474, NC480, NC488, NC494 and NC496 were removed from tropical analysis as they were not strictly tropical lines). Env 3 C1 represents the First order orthogonal polynomials for column in environment three.

Table 3.8. ANOVA analysis of yield in 2010

F Statistics Fixed Effects				
Source	NumDF	DenDF	F value	P value
Treatment	1	8.8	61.73	<.001
Inbred Parent	46	356.3	14.18	<.001
Checks vs. Top Crosses	1	355.5	0.1	0.74
Check1 vs Check2	1	133.7	0.04	0.84
Tester	1	12.7	7.42	0.02
Inbred Parent*Tester	46	357.6	1.30	0.1
Treatment*Inbred Parent	46	359.2	1.23	0.15
Treatment * Checks vs. Top Crosses	1	358.0	5.03	0.03
Treatment*Check1 vs. Check2	1	136.2	18.54	<.001
Treatment*Tester	1	12.7	4.27	0.06
Treatment*Inbred Parent*Tester	46	360.8	0.92	0.6
Random Effects				
Source	Component	Component÷Comp.SdErr	DF	
Reps(Treatment*Tester)	.08	1.21	8	
Row(Treatment)	0.14	2.32	50	
Error Variance	0.95	12.97	327	

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 3.9. ANOVA for yield in 2011

F Statistics Fixed Effects				
Source	NumDF	DenDF	F value	P value
Treatment	1	11.2	2.16	0.17
Inbred Parent	46	351.8	19.03	<.001
Checks vs. Top Crosses	1	354.9	0.01	0.92
Check1 vs. Check2	1	67.3	0.96	0.331
Tester	1	16.4	0.13	0.72
Inbred Parent*Tester	46	350.2	1.21	0.205
Treatment*Inbred Parent	46	353.1	1.9	<.001
Treatment * Checks vs. Top Crosses	1	360.4	4.9	0.029
Treatment*Check1 vs. Check2	1	68.6	2.36	0.132
Treatment*Tester	1	16.4	1.9	0.19
Treatment*Inbred Parent*Tester	46	355.8	1.19	0.194

Random Effects			
Source	Component	Component÷Comp.SdErr	DF
Reps(Treatment*Tester)	0.17	1.48	8
Row(Treatment)	0.29	2.82	50
Column(Treatment)	0.07	1.71	22
Error Variance	1.11	12.7	316

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 3.10. ANOVA of 2010-2011 combined analysis of yield

F Statistics Fixed Effects				
Source	NumDF	DenDF	F value	P value
Treatment	1	1	3.49	0.31
Inbred Parent	46	45.6	14.32	<.001
Checks vs. Top Crosses	1	8.4	0	0.978
Check1 vs. Check2	1	859.6	0.56	0.46
Tester	1	1.4	0.65	0.57
Inbred Parent*Tester	46	852.8	1.61	0.007
Treatment*Inbred Parent	46	855.0	2.2	<.001
Treatment * Checks vs. Top Crosses	1	62.3	6.24	0.016
Treatment*Check1 vs. Check2	1	864.5	18.79	<.001
Treatment*Tester	1	20.7	6.89	0.013
Treatment*Inbred Parent*Tester	46	858.3	1.1	0.299
Random Effects				
Source	Component	Component÷Comp.SdErr	DF	
Year	0.17	0.30	1	
Year*Treatment	0.33	0.66	1	
Year*Tester	0.05	0.58	1	
Year*Inbred Parent	0.11	2.58	46	
Reps(Treatment*Tester *Year)	0.07	1.41	26	
Row(Treatment*Year)	0.25	4.06	100	
Column(Treatment *Year)	0.03	1.5	44	
Error Variance	1.03	19.70	753	

NumDF are the numerator degrees of freedom, DenDF are the denominator degrees of freedom.

Table 3.11. LSM estimates of WMD of inbred and hybrid lines, and the yield of each of cross by tester and GLS treatment

	WMD			GLS Yield MT/ha		Sprayed Yield MT/ha	
Inbred Line	Inbred	Pioneer 3394	Pioneer 33M54	Pioneer 3394	Pioneer 33M54	Pioneer 3394	Pioneer 33M54
(RMP85 x OH43E)F_3S_{15}	6.34	5.49	6.23	9.95	9.84	9.95	10.26
(NC258x NC296)F_4S_8	6.63	5.49	6.11	10.11	10.27	11.58	11.57
90156(GP-273)	7.14	6.10	6.51	9.04	8.52	9.91	10.27
A632	2.78	2.83	4.04	6.52	8.34	8.68	8.91
B73	2.47	3.02	4.46	7.08	7.30	9.43	8.00
CML 5	8.06	6.47	6.99	10.10	9.76	10.38	9.53
CML 10	7.45	6.38	6.81	11.24	10.34	12.26	11.55
CML 108	7.29	5.98	6.54	9.87	10.85	10.53	9.70
CML 258	7.85	6.81	6.93	10.57	10.92	11.20	11.21
CML 277	7.41	5.63	6.46	10.97	11.66	11.87	12.21
CML 281	7.48	5.95	6.88	8.76	10.27	10.80	10.56
CML 288	7.06	6.28	6.08	9.95	10.56	11.06	10.25
CML 295	6.77	6.02	6.34	9.81	10.54	10.50	10.84
F42	2.48	3.35	4.17	6.39	7.35	8.50	8.27
FR1064	3.64	3.16	4.29	6.34	7.39	9.28	8.01
Ki11	5.46	4.60	5.70	6.88	9.07	9.91	8.91
Ki14	5.18	6.06	6.33	9.05	10.57	10.00	10.31
LH93	2.05	2.62	3.76	5.82	8.22	9.13	9.71
LH132	3.47	3.29	4.59	6.58	7.59	8.81	8.67
LP5	1.76	2.54	3.41	6.25	7.55	8.94	9.29
Mo17	4.09	3.60	4.71	6.82	8.58	9.73	10.66
Mo18W	6.91	6.02	6.48	8.83	9.09	10.06	9.37
NC250	6.55	5.32	6.11	7.67	8.70	8.75	9.08
NC258	6.57	5.51	5.85	10.44	10.33	10.90	11.83
NC292	2.32	3.11	4.64	6.67	7.79	8.64	8.98
NC300	6.68	6.05	6.07	10.45	9.12	10.78	9.88
NC302	6.86	6.52	6.72	9.12	9.74	9.79	9.78

Table 3.11 Continued

	WMD			GLS Yield MT/ha		Sprayed Yield MT/ha	
Inbred	Inbred	Pioneer 3394	Pioneer 33M54	Pioneer 3394	Pioneer 33M54	Pioneer 3394	Pioneer 33M54
NC304	6.38	6.70	7.17	9.86	10.90	11.64	11.54
NC320	6.20	5.68	6.50	10.73	12.45	12.88	11.98
NC354	6.00	5.98	6.26	10.54	11.46	11.54	10.85
NC368	5.52	4.31	5.52	8.92	8.79	10.20	9.60
NC374	6.45	4.70	5.35	8.52	8.18	10.07	9.96
NC460	7.01	6.32	6.47	9.92	10.68	11.40	11.16
NC462	6.18	6.20	6.87	8.21	9.62	10.66	10.64
NC474	6.19	5.23	6.04	9.08	8.86	9.67	9.31
NC480	7.40	6.51	6.79	10.00	11.20	11.00	11.24
NC488	6.40	5.23	6.05	8.50	9.21	11.32	10.00
NC494	6.16	6.32	7.00	11.14	12.34	11.80	12.31
NC496	6.65	5.84	6.28	9.15	10.05	11.04	10.20
NC508	6.83	6.50	6.79	10.16	10.78	11.28	10.81
NC522	6.90	6.09	6.43	10.87	10.72	12.05	11.84
Nei9004b	7.23	5.56	6.01	9.25	9.65	10.13	10.43
PHG72	1.57	2.44	3.52	6.41	7.12	8.13	8.65
Tzi1	6.86	5.87	6.63	9.29	9.95	11.09	10.29
Tzi5	7.53	6.23	6.47	9.83	9.38	10.48	9.74
Tzi17	6.91	6.40	6.78	10.10	10.91	11.44	11.75
VO613Y	6.81	6.12	6.52	9.68	9.27	10.89	11.19
Hybrid Testers							
Pioneer 3394	NA	3.02	NA	7.96	NA	11.37	NA
Pioneer 33M54	NA	NA	5.56	NA	9.64	NA	10.23
Mean	5.87	5.29	5.93	8.95	9.61	10.45	10.1
St Err of Diff	0.33	0.21	0.42	0.83	0.83	0.83	0.83
LSD .05	0.47	0.42	0.84	1.63	1.63	1.63	1.63

Note: (RMP85 x OH43E)F_3S_{15} and (NC258xNC296) F_4S_8 are experimental inbred lines from the NC State inbred line development program.

Table 3.12. Genotypic correlations of agronomic traits for 2010-2011 across hybrids

	Ear Height	Plant Height	Yield	WMD
Ear Height	1			
Plant Height	0.74***	1		
Yield	0.70***	0.76***	1	
WMD	0.54***	0.78***	0.85***	1

*** Significant at p=0.001

Table 3.13. Genotypic correlations of agronomic traits for 2010-2011 across hybrids with a temperate parent

	Ear Height	Plant Height	Yield	WMD
Ear Height	1			
Plant Height	0.87***	1		
Yield	0.25NS	0.004NS	1	
WMD	0.46*	0.15NS	0.78***	1

NS not Significant at p=.05, * Significant at p= .05, *** Significant at p=0.001

Table 3.14. Genotypic correlations of agronomic traits for 2010-2011 across hybrids with a tropical parent

	Ear Height	Plant Height	Yield	WMD
Ear Height	1			
Plant Height	0. 52***	1		
Yield	0.37***	0.49***	1	
WMD	-0.0072NS	0.57***	0.61***	1

NS not Significant at p=0.05, *** Significant at p=0.001

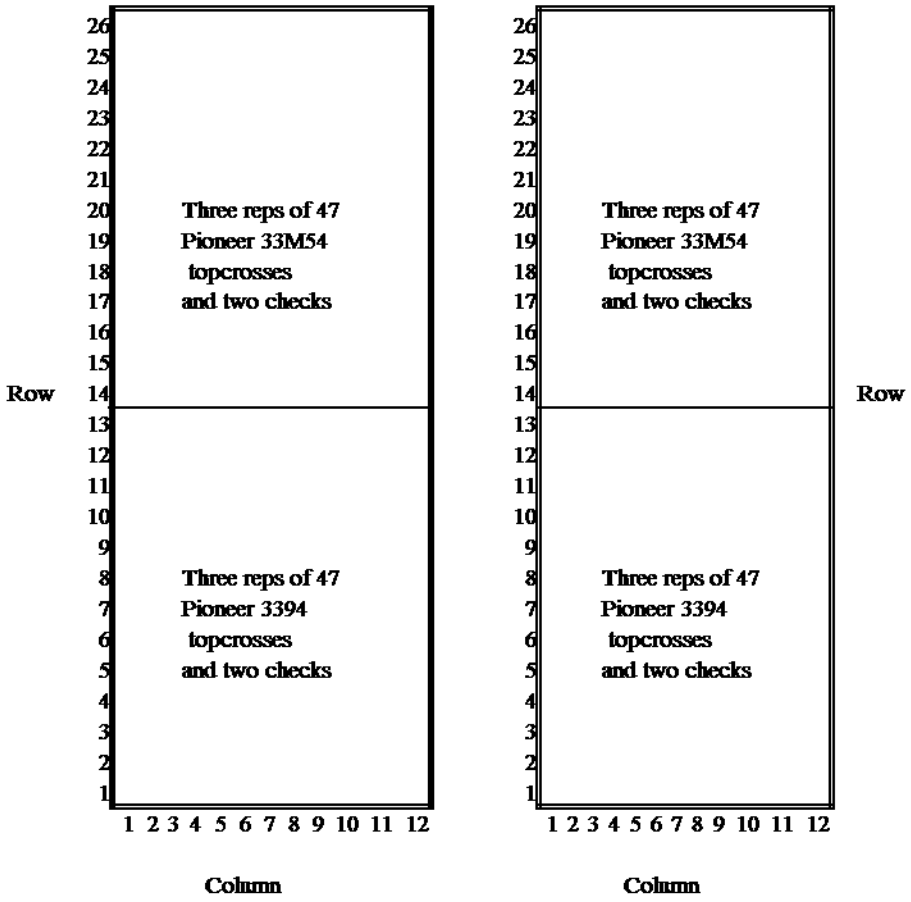

Figure 3.1. Map of layout of split-split block yield trials in one environment

Fields were laid out in two strips 12 columns (plots) wide and 26 row (ranges) long, using a split – split block design. The first split in the experimental design is GLS inoculated vs. fungicide-sprayed main block treatments. The second split is Pioneer 3394 vs. Pioneer 33M34 tester sub-block treatments. For each treatment 96 genotypes were tested including two check hybrids and 47 experimental lines each crossed to two testers.

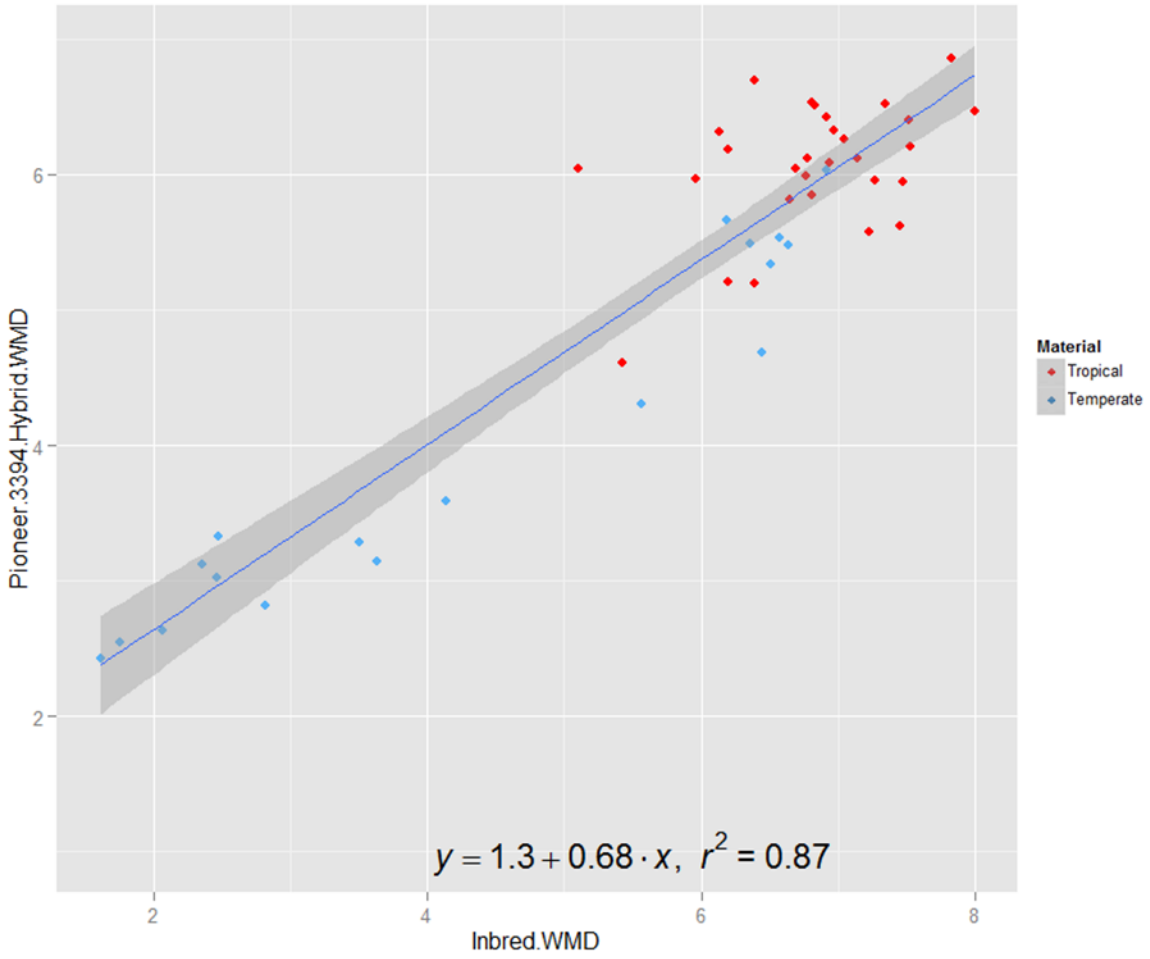

Figure 3.2. Regression of WMD of Pioneer 3394 topcrosses on WMD of inbreds.

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant.

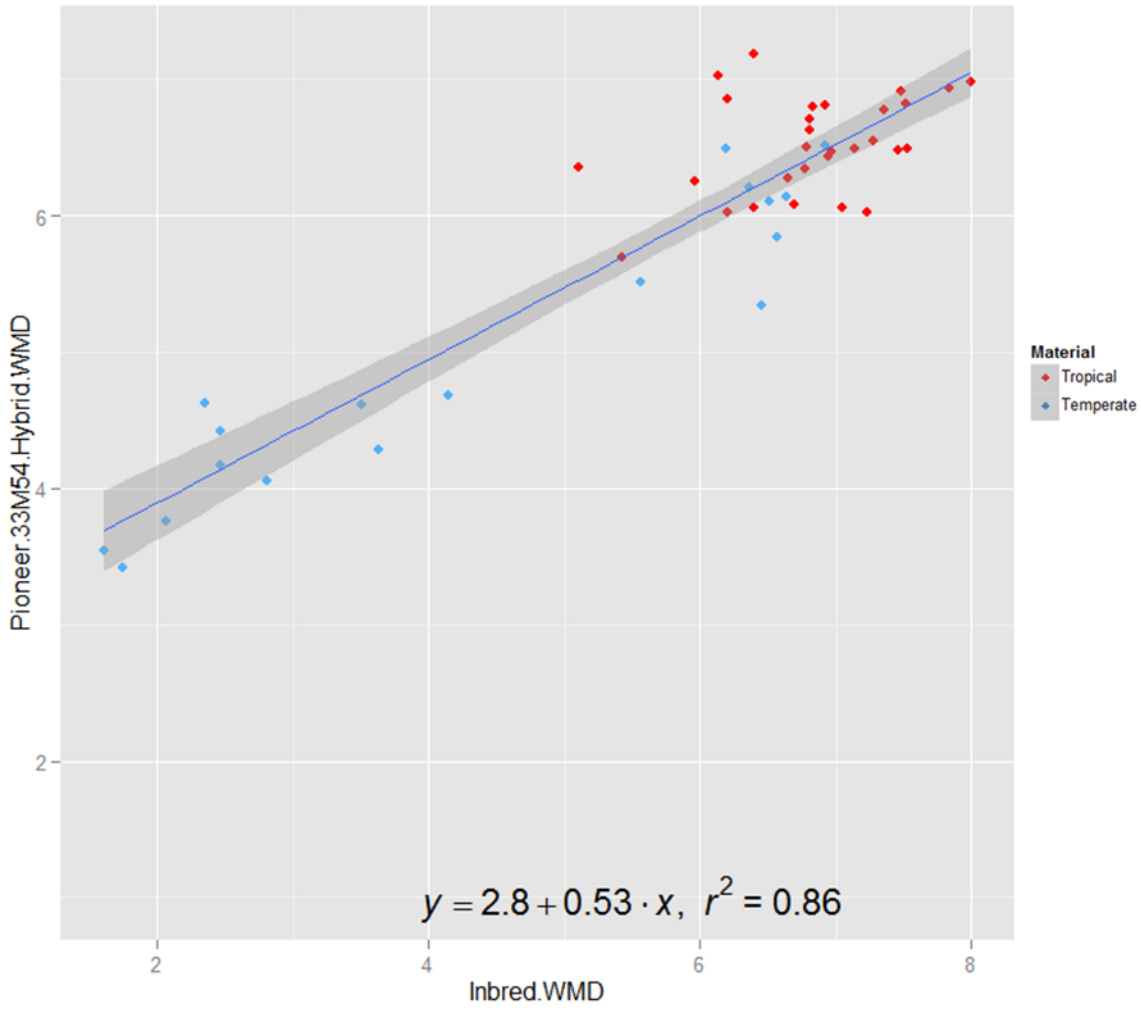

Figure 3.3. Regression of WMD of Pioneer 33M54 topcrosses on WMD of inbreds

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant.

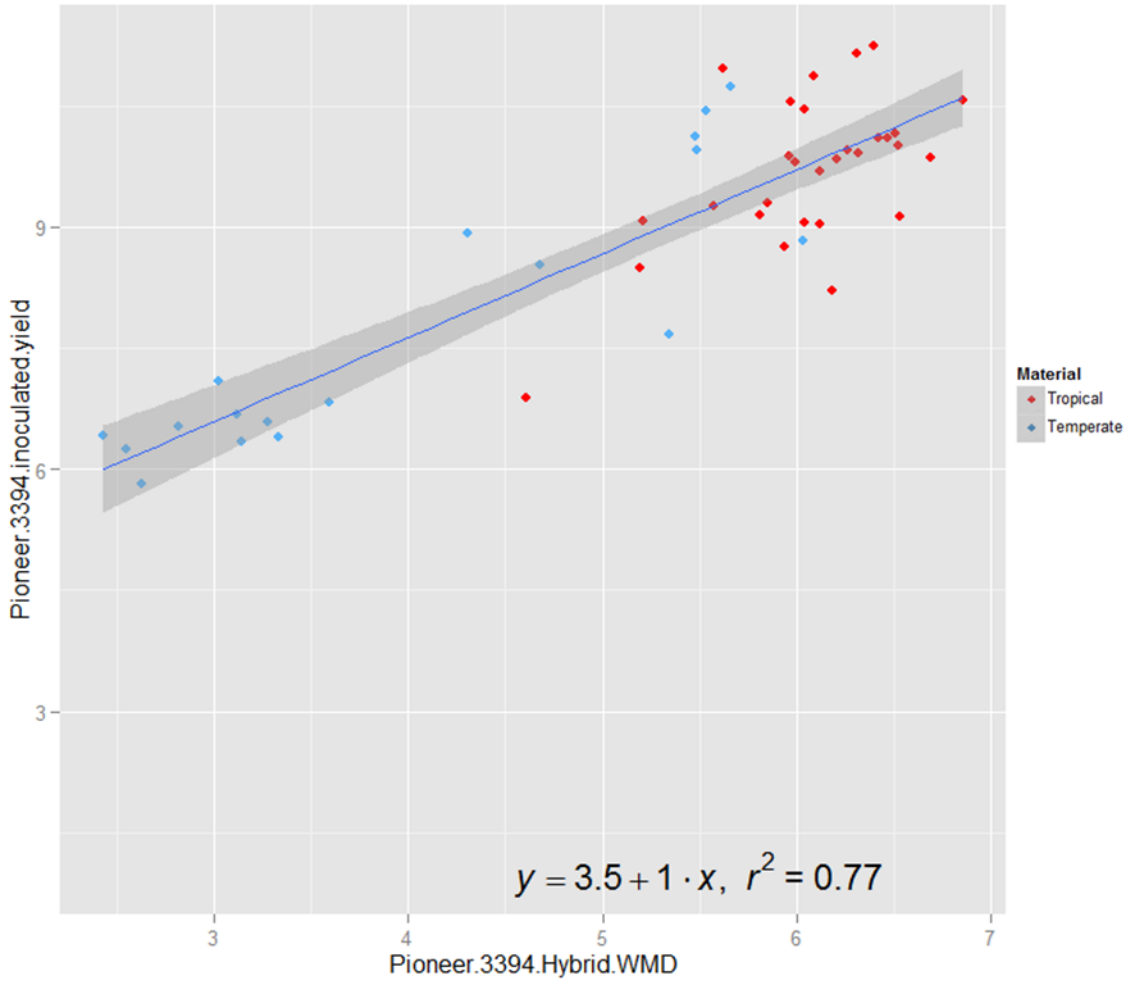

Figure 3.4. Regression of yield of inoculated Pioneer 3394 hybrids on WMD of Pioneer 3394 hybrids

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant with highest yields.

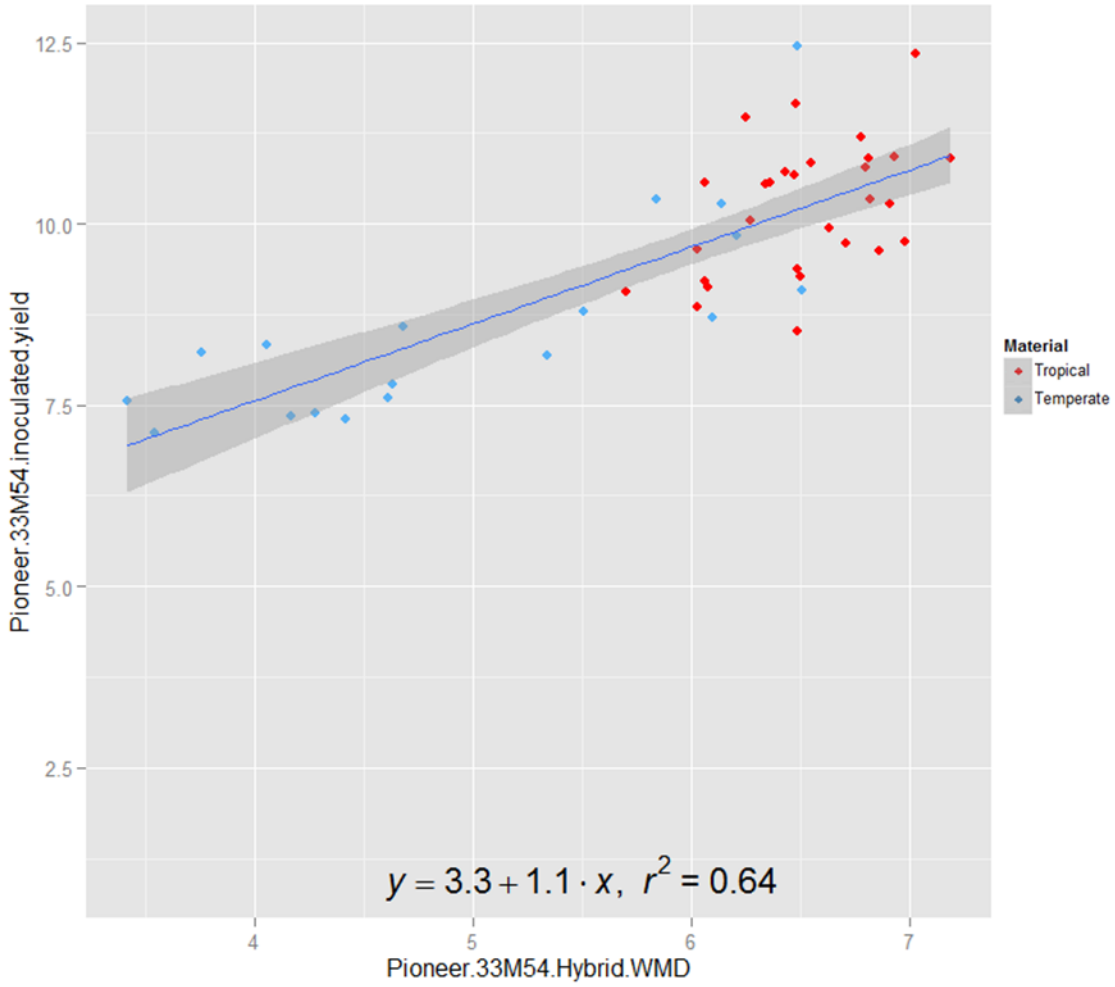

Figure 3.5. Regression of yield of inoculated Pioneer 33M54 hybrids on WMD of Pioneer 33M54 hybrids

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant with highest yields.

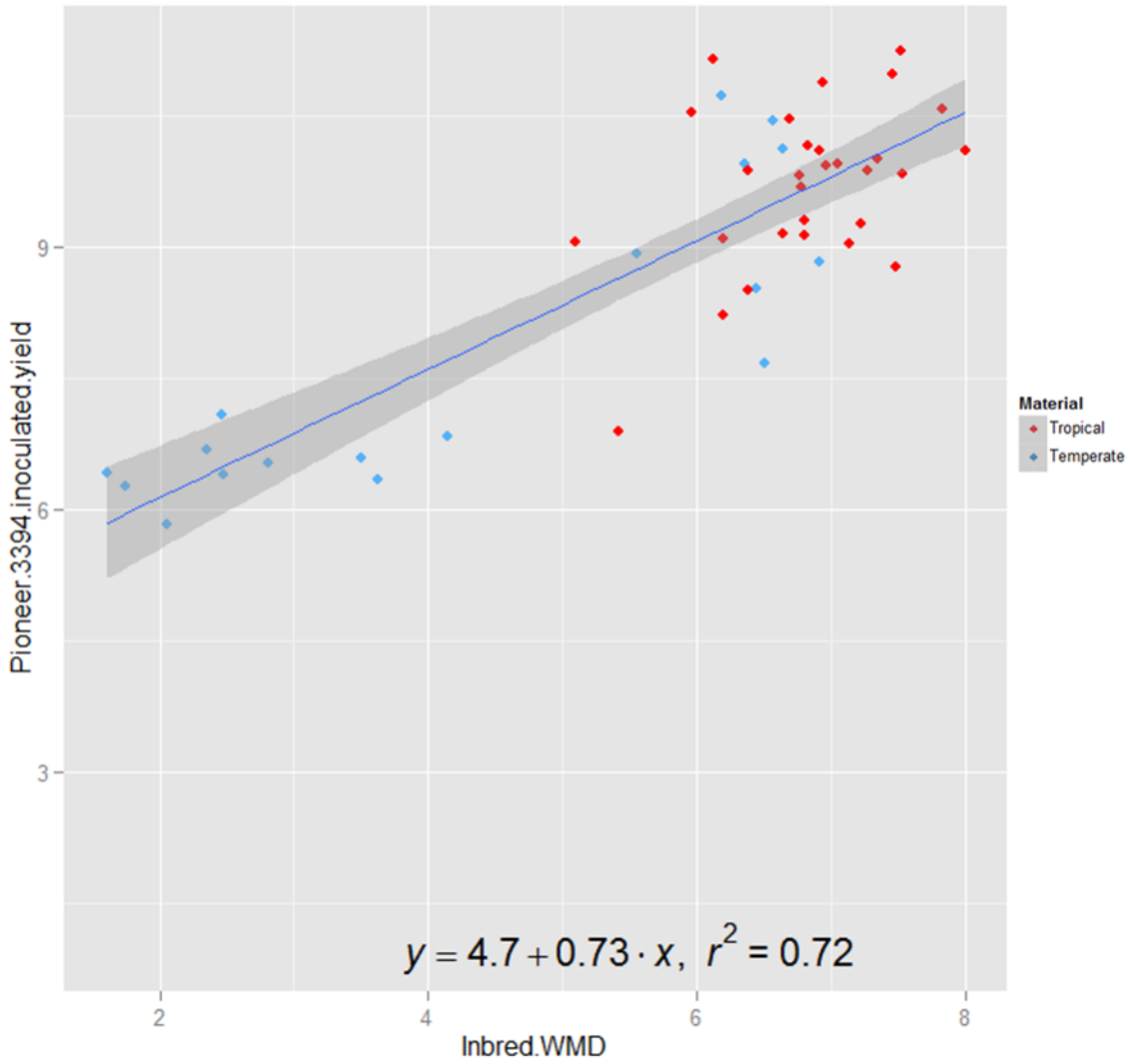

Figure 3.6. Regression of yield of inoculated Pioneer 3394 hybrids on inbred WMD

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant with highest yields.

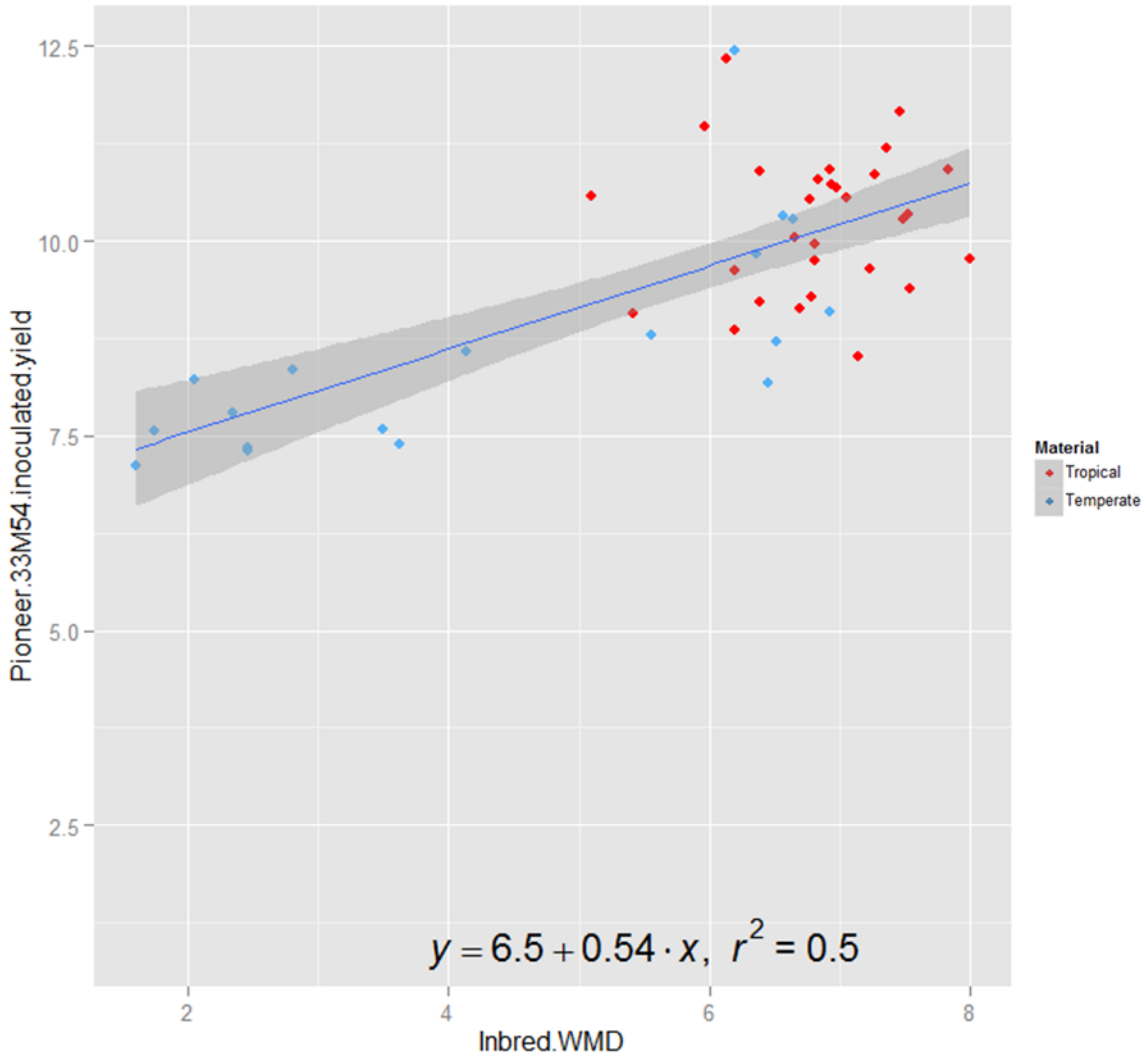

Figure 3.7. Regression of yield of inoculated Pioneer 33M54 hybrids on inbred WMD

Blue line represents the regression line, equation given above. Grey area represents the 95% confidence interval of prediction equation. Blue dots are temperate lines, the red dots tropical lines. Also of note is the location of the two types of germplasm, tropical or temperate, around the regression line. In general the tropical line topcrosses are the most disease resistant with highest yields.