GENETIC CHARACTERIZATION OF PARTIAL RESISTANCE AND COMPARATIVE STRATEGIES FOR IMPROVEMENT OF HOST-RESISTANCE TO MULTIPLE FOLIAR PATHOGENS OF MAIZE

DISSERTATION

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ABSTRACT

Foliar diseases are important biotic constraints limiting maize production globally. Northern corn leaf blight (NCLB) incited by *Exserohilum turcicum*, gray leaf spot (GLS) incited by *Cercospora zeae-maydis* and maize streak incited by maize streak virus (MSV), are among the most destructive. Most of the maize foliar diseases are managed by means of quantitative partial resistance. Quantitative trait loci (QTL) conditioning partial-resistance to these pathogens have been identified. Validation of candidate QTL conferring partial resistance would present marker-assisted selection as a potentially viable strategy to improve host resistance. We were interested in determining the usefulness of molecular markers linked to consensus QTL controlling partial-resistance systems for improving the overall resistance level. We examined QTL for NCLB in chromosomal bins 3.06, 5.04 and 8.06; GLS QTL in bins 2.09 and 4.08; and a consensus MSV QTL in bin 1.04 as potential targets for selection in improving host resistance. We also examined the effectiveness of different selection strategies for the purpose of pyramiding resistance loci to these diseases. Field evaluations and subsequent selections were conducted independently for each disease in a population of 410 $F_{2:3}$ lines derived from hybridization between inbred line CML202 with known resistance to NCLB and MSV, and VP31 a breeding line with known resistance to GLS. Maize streak evaluations were conducted in Zimbabwe, GLS tests were

performed in Ohio, and NCLB evaluations were conducted in Uganda and Ohio. Genetic gains were calculated for simultaneous improvement of partial resistance following phenotype-based, marker-based, combined phenotype- and marker-based selection (MAS index), and random selection.

Narrow-sense heritability estimates were 0.22, 0.25 and 0.39 for MSV, NCLB and GLS, respectively. Analysis of gene action using orthogonal contrasts showed mostly dominant gene action for NCLB, GLS and MSV. For NCLB, resistance due to presence of alleles from QTL in bins 3.06 and 5.04 was detected across two seasons. The chromosomal region in bin 4.08 for GLS resistance was significant ($0.0001 \leq P \leq 0.0395$) across seasons using late-season disease assessments. The major locus conferring resistance to MSV on chromosome 1 was significant ($P<0.05$) for resistance across seasons and explained 23% of phenotypic variations in the $F_{2:4}$ generation. Phenotypic values associated with flanking markers at each locus based on interval analysis indicated that QTL in bin 4.08 for GLS, bin 1.04 for MSV, and bins 3.06 and 5.04 for NCLB significantly reduced disease severity. Our results validated the position and effect of four out of six QTL controlling partial resistance to these pathogens. This was consistent with the presence of homozygous alleles from the resistant parent. These results point out the need for validation of QTL in new populations and the potential of using marker-assisted selection for pyramiding resistance to several pathogens using target QTL.

Actual genetic gains obtained varied with the particular disease and selection treatment employed. Combining phenotypic and marker information expressed as MAS index produced the highest gains for all diseases. The MAS index reduced the mean disease ratings by 9.0% for GLS, 5.7% for NCLB and 0.6 (1-5 scale) for MSV at late season epiphytotic from the overall mean of each disease in the $F_{2:4}$ generation. In comparison to phenotypic selection, the genetic gains from genotypic selection were highest for MSV followed by GLS and then NCLB. Cumulative genetic gains for improved resistance were practically the same for both phenotypic and genotypic selection. In all cases, gains from marker-based selection represented a significant improvement over random selection that ignored QTL information. The values of predicted genetic gains were higher than actual realized gain, but the relative values for the different selection procedures were consistent with the trend for actual realized genetic gains. Estimates of costs based on lower boundary values indicated the cost of marker-based selection was lower than that of phenotypic selection. Our results indicate that markers linked to major resistance loci can facilitate pyramiding resistance to multiple diseases during early generation selections.

DEDICATION

To my Parents, Brothers and Sisters

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FIELDS OF STUDY

Major Field: Horticulture and Crop Science (Plant Breeding and Genetics)

TABLE OF CONTENTS

Comparison of Genetic Gain following Phenotype-, Marker-Based, and Combined Selection for Improved Resistance to Multiple Foliar Pathogens of Maize

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

CHAPTER 1

GENETIC AND MOLECULAR MARKER STRATEGIES TO ENHANCE BREEDING FOR MULTIPLE DISEASE RESISTANCE IN MAIZE

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop for food and feed in many parts of the world. In Africa, in addition to a strong demand as a food crop, maize is increasingly becoming an important non-traditional agricultural export crop. The demand for maize is projected to rise with increasing population growth and an expanding need for livestock feed. Production of maize grain is generally insufficient relative to the needs of food consumption in many areas. Accordingly, increasing maize production is considered essential for food security in developing countries (CIMMYT, 2002). To a large extent, fulfilling the growing need for increased and sustainable maize production will depend on preventing yield losses and maximizing yield potential of the crop (DeVries and Toenniessen, 2001). World productivity of field maize averages approximately 4.8 Mt/ha, and it has been demonstrated that yield potential is over 20 Mt/ha (FAO, 2004). Among the factors responsible for reducing yield, diseases feature prominently.

Several foliar diseases cause economic damage to maize, fortunately, the prevalence of these diseases varies depending on the region or season (Smith, 1999). The risk of crop loss due to diseases occurs because the vast majority of maize in developing countries is produced by small-scale farmers who have limited access to means of crop protection. In developed countries, the cost of crop protection reduces profit margins for production of commercial grains. As a result, maize production is continually threatened by potential outbreaks of multiple foliar diseases. Crop losses due to diseases range from trace to total devastation depending on susceptibility of the host genotype, favorable environment and time of infection. Depending on these circumstances, even those diseases that are considered minor have the potential to surge to epidemic levels.

Pratt and Gordon (2006) have recently reviewed a number of the most important diseases affecting maize production in both tropical and temperate environments. Among the foliar diseases, gray leaf spot (GLS) incited by *Cercospora zeae-maydis* Tehon & E.Y. Daniels, northern corn leaf blight (NCLB) incited by *Exserohilum turcicum* (Pass) Leonard and Suggs (Teliomorph = *Setosphaeria turcica* (Luttrell)), maize streak incited by maize streak virus (MSV) were regarded as the most persistent and destructive diseases of field maize. Other maize foliar diseases that cause significant grain loss include southern corn leaf blight (*Bipolaris maydis*), common rust (*Puccinia sorghi*), Stewart's bacterial wilt (*Erwinia stewartii*) and a number of viral diseases. The distribution of these

diseases varies, but impact is most important when infection occurs at early crop growth stages or vulnerable plant growth stages, such as grain filling. This is the case for gray leaf spot and maize streak (Bosque Perez et al., 1998; Ward et al., 1999). Compared to many other maize foliar diseases, GLS and NCLB are ubiquitous fungal diseases while MSV is the most important viral disease occurring throughout maize producing regions of sub-Saharan Africa. These diseases are ranked highly in national and international maize research agendas (Pingali and Pandey, 2001).

Developing strategies to successfully manage several diseases simultaneously presents a formidable challenge and requires caution when extrapolating arguments from one pathosystem to another. Several disease management options have been recommended to reduce the impact of maize foliar diseases. Some of the recommended practices for control of fungal diseases include conventional tillage that buries crop residues, crop rotation, fungicide application, and planting of resistant hybrids (Ward et al., 1997). Effective management of fungal diseases such as NCLB and GLS requires management tactics that focus on reducing the rate of disease development by limiting sources of primary inoculum through crop rotation and residue management. Planting of resistant cultivars can effectively reduce the rate of disease development, and the practice is widely recommended (Ward et al., 1997). Management of insect-vectored viral diseases, such as maize streak, relies on interplanting, crop rotation, pesticide application(s), and planting of resistant cultivars. Recommendations center on

cultural practices that impact the population dynamics of the insect vector, whereas breeding has focused on resistance to infection by the pathogen.

Most foliar diseases of maize are managed by means of genetic resistance (Carson et al., 2004). Development of germplasm for resistance to several diseases simultaneously is an important goal in many breeding programs, but it is difficult to achieve because of other important traits for improvement (Castor, 1992). As a result, most breeders often do not invest equal effort in improving resistance to all diseases but focus on an individual disease causing the greatest loss (Lambert and White, 1997). A number of sources of resistance have been developed for each of the diseases (Pratt and Gordon, 2006). Resistance is expressed as reduced rate of disease development and in some cases by immunity. Differences in disease reaction are generally recognized qualitatively by distinctly different lesion types associated with certain genotypes or quantitatively by development of fewer lesions or a slower rate of lesion expansion on more resistant genotypes. These components are used for assessing the level of resistance under field conditions.

Despite the fact that various sources of resistance exist for foliar diseases, hybrids and inbreds with resistance to single pathogens are not entirely useful because they are likely to be challenged by different pathogens across environments (Pratt et al., 1997). Real situations in the field require varieties having combined resistance to more than one disease, but also to other

important abiotic stress factors, and acceptable agronomic characteristics. For example, Gordon et al. (2004) attempted to evaluate a maize population for resistance to *C. zeae-maydis* in Uganda, but a natural infestation of *E. turcicum* blighted the plants to a degree that evaluation for GLS reaction was not possible (Ayiga et al., 2001). A similar case was noted in legume crops (Nene, 1988) where pigeon pea (*Cajanus cajan*) lines identified as resistant to pod borer caterpillar (*Heliothis armigera*) were found to be highly susceptible to Fusarium wilt (*F. Oxysporum* f.sp *ciceri*). These studies indicate that for germplasm to be useful, it must have relatively broad levels of resistance to multiple diseases and be adapted across environments.

Host-pathogen resistance to diseases is classified as qualitative or quantitative. Qualitative resistance is controlled by single and usually dominant genes that interact with the pathogen on a gene-for-gene basis. Unlike qualitative resistance, plants exhibiting quantitative resistance display levels of resistance that show continuous variation and, usually, incomplete expression. Quantitative resistance is controlled by multiple genes with smaller but continuous phenotypic effects or by relatively few genes with large environmental influence (Michelmore, 1995). Quantitative resistance is often assessed in the field and is considered to be durable (Lindhout, 2002). However, environmental and gene x gene interactions play important roles in the phenotypic expression of quantitative resistance loci and thus, this type of resistance requires extensive field-testing under multiple environments and growth stages.

It has been shown that multiple genes associated with partial resistance may be subject to high genotype by environment interaction; however, Schechert et al. (1999) have shown that variance attributable to high genotypic resistance tends to be larger than that of genotype by environment variance. Experiments conducted by Carson et al. (2002) to determine the effects of genotype and environment on expression of partial resistance to *C. zeae-maydis* indicated that the interaction effects arise mainly from genotype by pathogen isolate interactions. This work suggested that a more effective approach to genotype evaluation would require an increase in the number of environments. This would create a situation where the trait would be expressed at different levels allowing maximum discrimination among genotypes. Usually, genes controlling quantitative disease resistance do not prevent infection but restrict the growth of the pathogen in tissues, thus effectively slowing down disease development. Therefore, quantitative resistance is often expressed in maize as fewer and smaller lesions and a prolonged incubation period (Carson and Dyke, 1994). Partial resistance is considered more durable since it is relatively effective against all races of a potential pathogen and does not foster selection of new races (Lipps et al., 1997; Parlevliet, 2002). The durability of disease resistance is an important asset in adoption and sustained use of new cultivars.

Quantitative trait locus discovery and marker-assisted selection

The advent of molecular markers based on DNA polymorphism has enabled identification of quantitative trait loci (QTL) involved in conditioning partial

resistance. Several studies have investigated the genetic basis of quantitative resistance in maize using marker technology with a view of providing more efficient methods for improvement of partial resistance (Young, 1996). However, several authors have cautioned that success in using QTL information for breeding applications depends on the reliability and accuracy of QTL detection (Beavis, 1994; Asins, 2002; Bernardo 2002; Castro et al., 2003). Bernardo (2002) suggested that it important to validate QTL effects across different experiments to determine their consistency in order to exploit QTL for breeding purposes.

Changes in QTL effect and position as a result of differing population sizes were illustrated by Melchinger et al. (1998). That study experimentally showed that small population size was responsible for false positive QTL, an overestimation of QTL effect and biases in localization of QTL position. Typically, most reports in literature about QTL discovery have used between 100 to 200 lines or individuals, a size that may cause variations in QTL positions across populations for the same trait. The use of such small or modest population sizes is reported to reduce not only the power of detecting QTL but also to inflate estimates of QTL effects (Bernardo, 2002). To reduce the bias in estimates of QTL effects, the author recommended the validation of previously identified QTL. Approaches to verifying the effects of previously identified QTL include development of nearly isogenic lines varying for a single QTL and MAS (Flint-Garcia et al., 2003). MAS can be used to validate QTL effects by testing whether selection based on target QTL increases response to selection compared with non-selection. When a QTL

is real, significant phenotypic differences should be detected between individuals carrying alternative alleles at the putative QTL (Romagosa et al., 1999).

Markers have been used in practical breeding to assist backcrossing of resistance loci into elite cultivars previously developed through conventional means. They have also been used to select alleles with major effects for reliable marker information across multiple populations. The usefulness of genetic markers arises from the following factors: (1) markers can aid selection of target alleles whose effects are difficult to observe phenotypically, such as recessive genes, (2) combining multiple disease resistance genes in a common genotype when effects are known, (3) alleles that are not expressed in the selection environments, such as genes conferring resistance to a disease not regularly present in a particular environment and (4) genes whose phenotypic assays are more expensive than marker assays.

Frisch et al. (1989) have demonstrated that markers unlinked to target genes can be used during marker-assisted backcrossing for increasing recurrent parent genetic background, thereby potentially reducing the number of generations needed to obtain a genotype with genetic similarity to the recurrent parent. Chen et al. (2000) used a similar strategy for backcrossing the *Xa21* gene, that confers resistance to a broad-spectrum of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) races, into an elite rice cultivar. They selected for donor alleles tightly

linked to *Xa21* and for recurrent parent alleles at flanking markers outside of the gene region to reduce linkage drag.

The utility of marker-assisted selection (MAS) for most economic traits still remains unclear because of conflicting reports in the literature regarding costs and gains associated with MAS. Limitations to applications of MAS for crop improvement depend mainly on accuracy in localizing and estimating QTL effects (Beavis, 1998). Dekker and Hospital (2002) have argued that “scale up” in MAS application across breeding programs will require methods that integrate QTL information across numerous populations. However, success in MAS seems to be conditional to certain traits. For example, there are a number of studies reporting use of MAS in disease resistance improvement in various crops (Chen et al., 2000; Robert et al., 2001; Castro et al., 2003), but evidence of use of MAS directly for yield improvement is rare. Considerable evidence from disease resistance studies suggests that resistance is most often conditioned by few genes (oligogenic) with major effects rather than by involvement of many genes (polygenic). This is consistent with the more common use of MAS for improvement of disease resistance compared to other complex traits such as yield. Lande and Thompson (1990) have shown that MAS is most effective when the breeding value of an individual or a line is predicted by an index determined by both the marker score and the phenotypic value of a trait.

Although MAS may provide higher relative selection efficiency, implementation of this method also requires higher economic efficiency than conventional methods to justify its use. Evidence for higher selection efficiencies for MAS has been reported (Knapp et al., 1998; Frisch and Melchinger, 2001); however, economic efficiencies seem to vary with different programs and number of traits involved. Dreher et al. (2003) demonstrated that MAS offers a cost-effective alternative to phenotypic selection to detect a particular allele at a target locus (*opaque2* gene associated with Quality Protein Maize) when conventional selection and MAS were considered as direct alternatives. In the second stage of the same study described in Morris et al. (2003), more theoretical breeding schemes were involved and upper boundary costs were considered for comparison of field and marker-based selections. In contrast, it was determined that MAS was faster, but more costly than traditional selection. In that study, phenotypic screening of the trait was assumed to be costless, and it was concluded that the cost-effectiveness would depend on the relative cost of phenotypic to genotypic screening, time saving achieved using MAS, and the availability of operating funds. These reasons probably explain variation in the rate of adoption and application of MAS between industry and public breeding programs and between developed and developing countries.

Simulation studies by Edwards and Page (1994) compared marker-assisted selection to phenotypic recurrent selection. The authors investigated the effects of selection on single markers versus flanking markers and the effects of

recombination distance between markers and QTL. Their model for MAS did not use actual phenotypic data and assumed that all the QTL affecting the traits were accurately identified. Results from the study indicated clearly the need for tightly linked markers for selection. They showed that the utility of MAS decreased relative to phenotypic selection when recombination between markers and QTL was greater than 20%. They also found that selection on single markers was almost as good as selection on flanking markers if the markers were within 5% recombination frequency of the QTL.

In maize, a large amount of valuable information exists in the literature concerning QTL conditioning partial resistance to diseases that may be exploited to improve resistance through gene pyramiding. A number of these QTL have been reviewed (Pratt and Gordon, 2006). In several studies, some of the QTL regions were found to be significant in more than one population, increasing the likelihood that these QTL regions could be useful for manipulation of resistance. The associated linked markers also offer the opportunity for marker-assisted selection of superior genetic combinations of resistance to multiple pathogens. The following study will consider three important foliar pathogens of maize for which QTL mapping has been extensively performed. It will also examine the effectiveness of different selection strategies for simultaneous improvement of partial resistance to multiple pathogens.

Maize streak

Maize streak is widely distributed in Africa and the adjacent islands of Mauritius, Reunion, Madagascar, Sao Tome and Principe. The incidence of the disease is estimated at greater than 60% in all maize production agroecosystems in these areas (Thottappilly et al., 1993). Outside these areas, there are reported cases of maize streak in India on *Pennisetum spp* and wheat (Seth et al. 1972). Maize streak is generally considered to be an endemic viral disease in Africa and it is not known to occur in the western hemisphere (Bosque-Perez, 2000). The disease is incited by a geminivirus that is transmitted by viruliferous leafhoppers of the genus *Cicadulina*. In addition to maize, MSV infects a wide range of hosts within the Gramineae family including finger millet (*Eleusine coracana*), oats (*Avena sativum*), sorghum (*Sorghum vulgare*) and sugarcane (*Saccharum officinarum*) (Mesfin et al., 1992).

The symptoms of maize streak include small, spherical, chlorotic spots that can develop from broken to continuous streaks along the veins. Because the virus is systemic, the symptoms appear on inoculated leaves and subsequent younger leaves (Thottappilly et al., 1993). Symptom severity depends on the genotype susceptibility and plant age at the time of infection (Bosque-Perez et al., 1998). In highly sensitive varieties, chlorotic stripping often develops into chlorosis of the entire lamina. Severe chlorosis on susceptible genotypes results in stunted growth, poor ear formation and reduced seed setting. Sometimes, chlorosis is followed by progressive necrosis and premature plant death, particularly if

infection occurs at an early stage of plant growth. The effects of maize streak on grain yield are most pronounced when young plants are infected and decrease with increasing plant age. Compared to other maize diseases, maize streak has the most devastating effects because it can result in complete crop failure (Bosque-Perez, 2000).

Distinct strains of MSV are known that vary in their degree of sequence homology (Martin et al., 2001), serological diversity and ability to induce different degrees of disease severity (Mesfin et al., 1992; Martin et al., 2002). Virulent forms of the virus incite severe stunting and chlorosis on infected susceptible hosts, and subsequently drastically reduce yield (Bosque-Perez, 2000). Varieties known to be resistant at one location may succumb to other strains of the virus at other locations (Rodier et al., 1995). Different subtypes within the strain of the virus appeared to predominate in different parts of Africa and certain of these subtypes were determined to be more pathogenic in maize (MSV-A_1, MSV-A_2 and MSV-A_5) than others (MSV-A_3 and MSV-A_4) (Martin et al., 2001). These differences are of potential importance in screening and deployment of resistant genotypes in specific geographic regions. Experiments conducted by Mawere et al. (manuscript in preparation) to investigate the stability of MSV resistance in Zimbabwe indicated significant effects of isolates and genotype by isolate interaction on MSV severity. These significant effects were regarded practically unimportant because they did not affect trends in ranking of genotypes of known reaction suggesting MSV resistance was stable in that environment.

Various cultural practices and insecticides are effective in managing maize streak (Rose, 1978). The most important among these practices are timely planting, planting barrier crops between early and late-planted maize fields to disrupt vector movement, crop rotation, avoidance of maize planting downwind from earlier planted, susceptible cereal crops and removal of infected crops. Insecticides such as dimethoate, demeton-methyl and carbofuran have been used to control insect vectors (Rose, 1978). Some of the recommended cultural practices conflict with efforts of resource-limited farmers to cope with the risks of uncertain rainfall regimes. For example, in some farming systems in southern Africa, multiple planting within a season to ensure part of the crop will be successful, facilitates the spread of MSV (Rose, 1978). It is also understandable that farmers are often reluctant to commit expensive inputs to small fields of crops that could, depending on the circumstances outside their control, fail completely. It then follows that host resistance is the most appropriate, economically viable and practical method to minimize damage caused by maize streak (Thottappilly et al., 1983).

Epidemics of maize streak are promoted by high temperatures and low rainfall which increase insect vector populations and movement (Rose, 1978). The occurrence of MSV is erratic and its effect varies from minimal damage in some seasons to total devastation of crops in other seasons. Because of erratic incidence of maize streak, progress in breeding for resistance has been slow using natural conditions. Efforts to breed for resistance have been undertaken by

national programs and international programs in Africa. Maize breeding programs at the International Institute for Tropical Agriculture (IITA), based in Nigeria, and CIMMYT, Centro Internacional de Majoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center) based in Zimbabwe, have developed many improved populations and inbred lines over years (Thottappilly et al., 1993; Bosque-Perez, 2000). This was facilitated by development of insect mass rearing for screening of germplasm (Efron et al., 1989; Pixley, 1994).

In collaboration with national programs, a number of maize populations have been developed with combined resistance to MSV and other desirable characteristics. The IRAT 297 composite created in La Reunion was one of the earlier populations bred for MSV resistance in three cycles of recurrent selection, two of which were by artificial inoculation. This composite served as a resistance donor whereby resistance was transferred to susceptible genotypes by successive backcrossing and screening under artificial infestations. Two backcrosses and two or three selfing generations were found adequate for restoring characters of the susceptible variety while concentrating resistance to MSV (Charrier et al., 2001). High yielding introduced varieties and local races grown in various African countries have been improved for MSV resistance using this conversion scheme (Efron et al., 1989). In addition, many breeding programs also use resistant sources developed from international programs to incorporate resistance into their own locally developed varieties (Soto et al., 1982).

Research by private companies, such as the Pannar seed company, based in South Africa, has also led to development of more MSV resistant sources. The commercial yellow hybrid, PAN6428 was one of the earlier hybrids bred for resistance to MSV. There was less of an effect on yield due to MSV on this hybrid when infection occurred after the 4-5-leaf stage (Barrow, 1993). This hybrid was followed by release of other high yielding hybrids, such as PAN6552; however, this genotype was still susceptible if infection occurred before the 4-5-leaf stage. The popularity of these hybrids declined because the local preference for consumption was white-grained maize. This has recently led to development of MSV resistant hybrids PAN6099 and PAN6195 that display high levels of resistance and yield competitively with other varieties grown in MSV prevalent areas. In general, research on MSV and breeding efforts to develop resistant commercially acceptable lines are continually challenged by problems associated with insect rearing for use in reliable screening trials and concerns about the potential influence of variation among MSV strains (Pixley, 1994).

There are varying reports in the literature about the inheritance, and mode of gene action, of maize streak resistance. Most of these studies have shown resistance to MSV is quantitatively inherited with a varying number of genes involved. Storey and Howland, (1967) ascribed maize streak resistance to a single gene lacking dominance while Fourie and Piennar (1983) indicated that resistance was simply inherited with strong dominance effects. On the other hand, Engelbrecht (1975) found that five dominant genes were involved, but Kim

et al. (1989) reported that resistance in inbred IB32 was quantitatively inherited through additive gene action of several genes. More recent studies on genetic control of MSV resistance in population CVR3-C3 have shown that multiple genetic systems for resistance may exist (Rodier et al., 1995). Both unimodal and bimodal frequency distributions of symptom ratings were observed when progeny developed by self-pollination within resistant, partially-inbred lines following inoculation with MSV. This result suggested the possible existence of two different genetic systems controlling resistance; one with major genes for complete resistance, the other with minor genes controlling partial resistance.

Genomic regions associated with quantitative resistance to GLS, NCLB and MSV have been identified in several studies using different populations and environments with a view of eventually improving host resistance. For MSV, Kyetere et al. (1995; 1999) identified a major QTL on the short arm of chromosome 1 (1S - bin1.04) and designated it *msv1*. The same locus was identified by Welz et al. (1998) in a population derived by crossing CML202, an MSV resistant inbred, and Lo951, a susceptible inbred, using a different viral isolate. Additional studies by Pernet (1999a and 1999b) using two other resistance sources, identified a major QTL in the same genomic location on chromosome 1S, and proposed that MSV resistance was under the control of two genetic systems, one arising from a major gene on the short arm of chromosome 1 and the other conditioned by minor genes on chromosomes 2, 3 and 10, that confer quantitative resistance. Based on the consistent results of these studies,

we concluded that different sources of resistance contain what is very likely to be the same resistance factor, *msv1*, that accounts for a large phenotypic variance associated with resistance. Thus, *msv1* was considered to be a suitable candidate QTL for MAS for improving maize streak resistance.

Northern corn leaf blight

Northern corn leaf blight occurs throughout maize producing regions wherever moderate temperatures and high humidity prevail (Carson, 1999; Smith, 1999). NCLB is a destructive disease, particularly in the humid mid-altitude and highland regions of Africa (Ngwira et al., 1999; Smith, 1999; Bigirwa et al., 2001; Okori et al., 1999). The disease is caused by *Exserohilum turcicum* (Pass) Leonard and Suggs [Teliomorph = *Setosphaeria turcica* (Luttrell)]. The pathogen overseasons in corn residue left on the soil surface as either conidia or mycelium and conidia are dispersed by wind or rain splash. When warm and moist conditions occur, new conidia are produced on crop residues and dispersed to leaves of maize plants. Infection occurs when there is free water on the leaf surface for 6-18 hours and moderate to warm temperatures of 65 to 80°F (18-27°C). Lesion development and subsequent sporulation occurs within one to two weeks (Levy and Cohen, 1983; White, 1999).

Exserohilum turcicum occurs as several physiological races. These races are designated as 0, 1, 2, 12, 23 and 23N, and were assigned according to the resistance genes that their virulence matches (Leornard, 1989). The prevalence

of the races varies with geographical region. Races 1 and 0 are reported to be predominant in the Mid-western Corn Belt (Pratt et al., 1993; Lipps et al., 1997) but race 0 has been reported to be most prevalent in Uganda (Adipala et al., 1993a; Bigirwa et al., 1993). Borchardt et al. (1997) reported the presence of at least two races in the neighboring country of Kenya. Using isolates collected widely in both tropical and temperate environments, Borchardt et al. (1997) detected high genotypic diversity among populations sampled. In addition, high sexual recombination capacity of this fungus was observed, implying a potential for development of new races of the pathogen.

Resistance to *E. turcicum* is governed in both a race-specific (qualitative) and a race-nonspecific (quantitative) manner (Raymundo and Hooker, 1982; Welz and Geiger, 2000). Presently, five dominant genes are reported to individually condition resistance in maize to *E. turcicum* (Leornard, 1993). These genes are designated as *Ht1, Ht2, Ht3, Htm1* and *Htn1*, and are determined from one another by the differential reaction they sustain to the known physiological races of the pathogen. Race 0 is avirulent against lines with any of the *Ht* genes. Race 1 is virulent against lines with *Ht1* gene. Various other combinations of virulence also occur (Leornard, 1993). Maize lines carrying these genes are known to express a qualitative form of resistance characterized by lesions that are chlorotic, rather than necrotic when challenged by an avirulent race (Raymundo and Hooker, 1982). In contrast to other forms of resistance genes, *Htn1* is

reported to act quantitatively by delaying the onset of lesion development until adult plant stages, usually after flowering (Gevers, 1975).

Studies have shown that the race-specific *Ht* genes tend to be sensitive to environmental conditions, particularly in evaluations conducted in tropical environments, since they failed to protect genotypes lacking a partial resistance background (Adipala et al., 1993b; Lipps et al., 1997; Welz and Geiger, 2000). Adipala et al. (1993b) showed that when different sources of resistance were challenged by isolates of *E. turcicum*, the level of resistance conferred by the *Ht* gene in inbreds carrying them was low compared to progenies with partial resistance. In a comparison of S_1 progeny with variable levels of race-nonspecific resistance the relative ranks of resistance to race 0 and 1 of *E. turcicum* were similar in evaluations conducted both in temperate and tropical environments (Pratt et al., 1993).

Major genes (race-specific), particularly the *Ht*1 gene, have been used in managing NCLB but emphasis in many breeding programs has been placed on exploiting quantitative resistance. Quantitative resistance has been widely used because of its durability and effectiveness (Meyer et al., 1991; Pratt et al., 1993; Lipps et al., 1997). Partial resistance to NCLB is expressed as a reduction in disease severity (Brewster et al., 1992). Known components of partial resistance that lead to reduced disease development include increased incubation and latent period, and reduced lesion size, lesion expansion rate, and infection

efficiency (Carson and Dyke, 1994). Carson and Dyke (1994) determined the effects of temperature and light intensity on components of partial resistance and found that most components were stable, but the amount of sporulation within lesions was variable. The prolonged incubation period expressed by younger plants was strongly correlated with low blight severity on adult plants in the field (Schechert et al., 1997). In contrast to qualitative resistance conferred by *Ht* genes, quantitative resistance is largely insensitive to varying light and temperature conditions (Carson and van Dyke, 1994). The stable expression of quantitative resistance allows resistance evaluations to be conducted without precise control of environmental conditions. Other desirable attributes of this type of resistance include effectiveness against all physiological races of *E. turcicum*. Partial resistance may also be used in combination with race-specific resistance (Lipps et al., 1997). A high level of resistance was obtained in inbred Mo17*Ht*1 that had a combination of both the *Ht* gene and polygenic resistance.

Components of quantitative resistance to NCLB are generally highly heritable. Adipala et al. (1993) found consistent and significant positive correlations between components of partial resistance on seedling plants in growth chamber studies and measures of disease severity on adult plants in the field. Hakiza et al. (2004) estimated heritability for some of the components of partial resistance derived from inbred H99, a corn-belt adapted inbred, and found that despite its shorter maturity and poor adaptation to tropical environments, it expressed a high level of partial resistance. Estimates of heritability for various components of

partial resistance were moderate to high and suggested that substantial genetic gains could be obtained for quantitative resistance. Studies on the nature of gene action have indicated that additive gene action was of major importance in the inheritance of quantitative resistance (Schechert et al., 1997). The significance of dominance depended on the genetic material and the stage of plant development when disease responses were assessed. Experiments conducted by Carson (1995) on inheritance of latent period showed that most of the variation among generation means was explained by additive gene action in juvenile plants, but Schechert et al. (1997) observed dominant gene action gradually became more important over the course of the epidemic in adult plants.

Several genetic and mapping studies have been conducted to identify QTL conditioning resistance to infection by *E. turcicum*. These studies used diverse sources of resistance representing those from the US Corn Belt, Africa and Europe. In a population derived from inbred Mo17, Dingerdissen et al. (1996) mapped QTL conferring NCLB resistance on chromosome arms 3L, 5S, 7L and 8L. The authors' findings regarding major QTL influencing resistance to NCLB largely agreed with those of Freymark et al. (1993; 1994) who also reported significant QTL affecting disease severity on 1S, 3L, 7L and 8L. The exact map positions were similar despite large differences in disease intensity, population sizes and genetic materials used in the experiments. Similar studies by Schechert et al. (1999) using CML202 as the resistance source found 12 QTL significantly influenced NCLB resistance across three environments. Only three

QTL on chromosomes 5, 8 and 9 were effective over the entire growing season. The other QTL were detected only during an early or late stage of host plant development. Welz et al. (1998) detected 13 QTL for NCLB resistance in a population derived from a resistant European line, D32. Only five of these QTL on chromosomal bins 1.06, 3.07, 4.03, 5.04 and 8.06 were found to be significantly associated over multiple disease ratings. In general, three QTL regions on chromosomes 3 (bin 3.06), 5 (bin 5.04), and 8 (8.06) were consistently significant in the different populations (Table 1.1), increasing the likelihood these QTL regions are shared in different populations. The consistency of several QTL for resistance to NCLB across different populations has been thoroughly investigated by Welz and Geiger (2002). Thus, the consistency of resistance loci (consensus) makes them suitable candidates for MAS for improvement of resistance.

Gray leaf spot

Gray leaf spot, caused by the fungal pathogen *Cercospora zeae-maydis* Tehon & E.Y. Daniels, is perhaps the most important disease of maize in the US (Carson, 1998) and sub-Saharan Africa (Ward et al., 1999). For many years after it was identified in 1924 by Tehon and Daniels in Southern Illinois near the Mississippi River, it was considered a minor disease. It became a major concern only in the 1970s (Nowell and Ward, 1997). Since the mid l980's, GLS has posed an increasing challenge to maize production globally (Ward et al., 1999). The recent increase in prevalence of gray leaf spot has been attributed mainly to increased

use of conservation tillage practices and or the more frequent monoculture of corn. These factors provide a potential reservoir for inoculum and favor the survival of the pathogen from one season to the next. It has been demonstrated that *C. zeae-maydis* can survive for a long time on corn debris and initiate epidemics in subsequent seasons (de Nazareno et al., 1993; Asea et al., 2002). In tropical environments, where little conservation tillage is practiced, multiple overlapping plantings of maize, combined with widespread use of maize residue as mulching material for other crops, such as banana and coffee, create opportunities for pathogen spread and increased severity (Asea and Adipala, 2001; Okori et al., 2004).

The development of GLS is highly influenced by microclimatic conditions (Payne and Waldron, 1983; Bigirwa et al., 2001). The fungus is commonly known as a leaf-blighting pathogen that spreads most rapidly after tasseling, but it can appear earlier in the season if weather conditions are favorable. The presence of intense disease pressure is an essential prerequisite to evaluate the level of GLS resistance in genotypes. Donahue et al. (1991) found that GLS prevails in high relative humidity environments and with extended leaf wetness (6-18 hrs). Recent studies have shown that, in addition to these environmental factors, cultural practices such as time of planting also influence disease development. Bhatia and Munkvold (2002) observed that later-planted maize tends to develop higher disease severity because plants experience initial infection at an earlier growth stage and, thus, there is a greater opportunity for multiple infections

before the plants reach physiological maturity. Weather conditions later in the year also may favor additional disease spread. However, risk assessment models developed by Paul and Munkvold (2004) indicated that plant maturity was not an important predictor of disease severity in the field.

The genetic basis of resistance to *C. zeae-maydis* has been examined in numerous studies. Thompson et al. (1987) indicated that resistance to *C. zeae-maydis* in hybrids was conditioned mainly by additive effects, was not complex, could be evaluated effectively using inbreds per se, and could be transferred by backcrossing and appropriate selection techniques. Their study also provided evidence for relatively few genetic factors involved in resistance because a high level of resistance was retained in the progeny during backcrossing into a susceptible cultivar. Based on partial diallel analysis among inbred lines, Huff et al. (1988) reported that general combining ability was greater than specific combining ability. A three-locus model derived from diallel analysis explained most of the observed variation in inbreds and their single crosses, F_2 and backcross generations (Elwinger et al., 1990). Freppon et al. (1996) examined the chlorotic lesion type phenotype conferred by inbred NC250A in segregating progeny lines at mid-epiphytotic and concluded that a single gene governed the chlorotic lesion response; however, transition of the lesion phenotype from chlorotic to necrotic in some progenies later in the season was not consistent with a single gene model.

The detection and location of resistance loci for GLS have been extensively conducted across diverse environments using populations derived from different resistance sources. In contrast to consistent identification of QTL controlling resistance to maize streak in different resistance sources, the QTL for GLS are dispersed on all the chromosomes in these populations. Regardless, some QTL were consistent on the same chromosomal positions across populations and generations. Earlier studies by Bubeck et al. (1993) using inbreds NC250A and ADENT as resistance sources mapped QTL for GLS on five chromosomes in three $F_{2:3}$ mapping populations, but only one QTL on chromosome 2 was found to be consistent across populations and environments. Similarly, Saghai-Maroof et al. (1996) found 3 QTL on chromosomes 1, 4 and 8 which together explained about 54% of the variation in F_2 and $F_{2:3}$ generations. The QTL on chromosome 8 was found to display recessive gene action. Using inbred 061 as a resistant parent, Clements et al. (2000) evaluated BC_1S_1 populations for two seasons at two sites. From this population he identified five QTL significantly associated with resistance to *C. zeae-maydis* across all environments and rating periods. The resistant parent donated all alleles contributing to increased resistance at the five regions on chromosomes 1, 2, 5 and 7. Later ratings indicated additional significant effects associated with resistance on chromosomes 3, 4 and 8. Gordon et al. (2004) identified two QTL on the long arms of chromosomes 2 and 4 that confer resistance to GLS in the South African inbred VO613Y. The two loci together explained more than 50% of the phenotypic variation. Our own personal observations in the field show that this inbred is highly resistant to GLS under

high epiphytotic conditions compared to other common resistant sources in tests conducted in Ohio and tropical environments in Uganda. Despite the disparity of maturity effect and genetic background, the close proximity of probes linked to resistant loci in these different resistance sources suggests that they share similar genomic regions associated with resistance on chromosomes 2 and 4. A summary of these QTL positions in different sources of resistance is shown in table 1.1.

Multiple pathogen resistance

There are few published studies on application of markers linked to QTL for pyramiding quantitative resistance to multiple foliar pathogens. Pyramiding qualitative resistance genes with different race specificities has been proposed as one way to increase the likelihood of achieving resistance to multiple pathogens and enhancing durability (Mundt, 1990). The potential problem associated with using qualitative resistance for crop protection even against the same pathogen is that it may become ineffective in the long-term following shifts in pathogen populations to biotypes that do not carry the corresponding genes. The defeat of *Ht* genes for resistance to *E. turcicum* in maize is a classical example of the loss in effectiveness of major qualitative resistance over time. The *Ht*1 resistance was widely utilized, but virulent races of *E. turcicum* developed within a period of approximately two decades to cause more epidemics (Smith, 1999). Nevertheless, deployment of major genes is likely to continue since protection did last for nearly 20 years. Crop protection by qualitative resistance

probably lasted for such a long time because quantitative resistance backed it up in the 1980's and 90's (e.g. prevalent use of inbred Mo17).

Durable resistance is important because it is much more likely to remain effective for a considerable time, despite wide exposure to different biotypes of a pathogen. This type of resistance is often characterized by reduced incidence for viral diseases and is associated with a lower rate of pathogen build-up (Parlevliet, 2002). Insurance against severe damage conferred by "durable resistance" is needed because there is an increased trend of maize monoculture and hybrid production that results in reduced genetic diversity, thus predisposing crops to increased risks. In rice, the *Xa-4* gene for resistance to bacterial blight caused by *Xanthomonas oryzae* played an important role in protection against severe damage; however, it was later defeated by virulent new races (Mew et al., 1992). Huang et al. (1997) pyramided three genes conferring resistance to bacterial blight and the resultant lines showed more, and a wider spectrum, of resistance than lines carrying single genes. This was attributed to interaction and complementation between the three resistance genes. Races of some pathogens are not known but it is assumed partial resistance will provide adequate, durable protection.

Host resistance to many pathogens causing economic damage is under polygenic control governed in a quantitative manner. Pyramiding multiple alleles for quantitative resistance may also be an approach to increasing the level of

host resistance. Moreover, quantitative resistance is usually race nonspecific and not subject to erosion (Leonard, 1993). Pyramiding quantitative resistance genes is likely to increase the probability that a cultivar will show durable resistance. This approach has been demonstrated in barley using QTL previously mapped for stripe rust (Castro et al. 2003), but data to support this for maize foliar diseases is lacking.

Parvlevleit (1983) has argued for finding selection methods that combine both major gene and partial resistance to enhance durability of single resistance genes. Conventional selection for quantitative resistance in the field is usually difficult because trait expression is strongly influenced by the environment, genotype-by-environment interaction, and inadequate disease development. As a result, quantitative resistance is more difficult to select, typically requiring large-scale, replicated and multi-environment testing in order to make progress. Marker-based selection may be useful for improving durable resistance but the specific strategies to achieve that goal may also require combining both phenotypic and genotypic information to account for the quantitative nature of inheritance of resistance. Combining both phenotypic and genotypic data is likely to improve selection for all resistance factors, including the minor QTL with small effects often associated with a large experimental error.

Marker-assisted selection has been used successfully in a number of studies involving improvement of maize and other crops. For example, using a back-

cross strategy, Ribaut et al. (2000) improved the performance of maize inbred CML247 to drought. They used PCR-based markers to screen a large population of about 2,000 plants at each selection cycle. After 2 backcrosses and 2 self-pollinations, the best genotype was fixed for the five target regions (12% of the genome) as well as 7% from the donor genome outside the QTL regions. Similarly, Walker et al. (2002) also used the back-cross method for pyramiding a QTL conditioning corn earworm resistance in soybean PI 229358 and a synthetic *Bacillus thuringiensis cry1Ac* transgene from the recurrent parent into BC_2F_3 plants by MAS. They utilized SSR markers to select for regions which mapped to the QTL of interest to determine which line to advance. After 2 backcrosses and 3 cycles of self-pollination, they were able to successfully select plants with the desired combinations of genes.

Edwards and Johnson (1994) reported successful gain in yield-related traits in sweet corn based on 3 cycles of marker-only recurrent selection. They wanted to improve an index of traits, including agronomic characteristics and quality traits. Marker scores were estimated in the testcross generation, and lines were selected on the basis of combined marker scores and directly observed trait values. The selected lines then formed the basis of a recurrent selection program based on indirect selection for marker score only. Three rounds of selection were performed per year. Results indicated that gains were made in most of the important traits under selection.

The predicted gains from rapid recurrent selection for yield in the previously described study were confirmed in subsequent experiments. In these experiments, each F_2 population underwent several cycles of recurrent selection based on marker-only selection (Johnson, 2004). In the original population, selection was based on an index in which both observed trait values and marker scores were included. The results suggested that three cycles of selection on marker alone would add a four-fold gain in yield over that obtainable by one cycle of selection based on an index of observed yield and marker score (Johnson, 2004).

Although sources of resistance have been identified for each of the diseases in maize, development of multiple resistant cultivars has been slow and reliable control against many diseases simultaneously is lacking (Castor, 1992). The lack of well-defined selection criteria for many diseases, emerging races of pathogens, and difficulty in screening germplasm for reaction to several pathogens, have hampered breeding progress. For many years, selection for resistance based on conventional methods has been employed to improve resistance, yet there is a wealth of information regarding chromosomal regions conditioning resistance to diseases that could provide new tools for selection that could expedite combining multiple resistance factors.

This study elected to examine the feasibility of improving a population derived from the CIMMYT maize inbred line CML202 for multiple disease resistance and

agronomic traits in both tropical and US corn-belt environments. This line has a high level of partial resistance to *E. turcicum* and MSV as confirmed in previous genetic studies, but it is late maturing (Welz et al., 1998; Schechert et al., 1999; Vivek et al., 2000). The inbred is widely used in many tropical breeding programs for production of hybrids and new inbred lines due to its excellent combining ability for disease resistance and yield (Schechert et al., 1999). In Uganda, the Cereal Research Program in cooperation with CIMMYT has released two hybrids (UH-1 and UH-2), each with CML202 as one of the inbred parents. However, there are conflicting reports about the reaction of inbred CML202 to infection by *C. zeae-maydis*. For example, it was reported to be moderately resistant (K. Pixley, personal communication) while studies in Ethiopia (Wegary et al., 2003) have shown that it is susceptible in that highland environment. Generally, we considered the inbred to possibly display intermediate resistance to infection by *C. zeae-maydis*. Because of severity of GLS in sub Saharan Africa today, it was considered essential to add one or more resistance factors to assure it would possess durable resistance.

Previously, resistance to GLS was identified and characterized in inbred VO613Y, developed in South Africa. Two loci conferring resistance to GLS were identified on chromosome 2 (bin 2.09) and chromosome 4 (bin 4.08) (Gordon et al., 2004). The inbred has potential to be a donor for improved resistance to GLS. Our observations in the field have shown that the inbred is highly resistant to GLS compared to other corn-belt resistant sources previously used in all other

studies and is broadly adapted to conditions in both Africa and US Corn-Belt. Because CML202 is a confirmed source of resistance to MSV and *E. turcicum* and QTL for resistance have been mapped, there is opportunity to develop multiple disease resistance in CML202 derived lines with acceptable agronomic traits for adaptation in both temperate and tropical environments. There will also be an opportunity to backcross elite lines derived from the population to develop an earlier maturing CML202 inbred line with multiple resistance. In addition, we also sought to compare different selection procedures to determine which procedure would achieve better genetic gains.

The specific objectives of this study were to:

(i) Estimate heritability of partial resistance to *E. turcicum*, *C. zeae-maydis* and MSV in a population derived from inbred CML202

(ii) Identify PCR-based flanking markers in consensus QTL regions that are polymorphic and linked to QTL controlling partial resistance to infection by *E. turcicum*, *C. zeae-maydis* and MSV

(iii) Characterize modes of action for quantitative resistance to *E. turcicum*, *C. zeae-maydis* and MSV

(iv) Characterize partially inbred lines with molecular markers associated with resistance loci for developing multiple disease resistant lines

(v) Compare genetic gains and associated costs between phenotype- and marker-based selection procedures in the above population.

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Reference	Resistance source	Marker Interval	Bin	Chromosome arm
GLS (One discovered and consensus)				
Gordon et al., 2004	VO613Y	*bnlg1520-umc36*	2.09	(2L)
Gordon et al., 2004	VO613Y	*umc127*	4.08	(4L)
Bubeck et al., 1993	ADENT	*umc19-bnl7.65*	4.08	(4L)
Bubeck et al., 1993	B73	*umc47*	4.05	(4L)
Saghai-Maroof et al., 1996	B73	*npi444-umc15*	4.08	(4L)
NCLB (Consensus)				
Welz et al., 1999a	CML202	*umc361- bnl15.20*	3.06	(3L)
Welz et al., 1999b	D145	*umc3b-umc17a*	3.06	(3L)
Freymark et al., 1993	Mo17	*umc60-bnl15.20*	3.06	(3L)
Schechert et al., 1999	CML202	*bnl8.01-umc389b*	3.06	(3L)
Freymark et al., 1993	Mo17	*bnl10.06-bnl7.71*	5.03	(5S)
Freymark et al., 1993	Mo17	*umc1-bnl5.40*	5.03	(5S)
Welz et al., 1999a	CML202	*umc001-bnl5.40*	5.04	(5S)
Welz et al., 1999b	D145	*csu36a-bnl7.71*	5.04	(5S)
Welz et al., 1999a	CML202	*bnl12.30-umc030*	8.05	(8L)
Freymark et al., 1993	Mo17	*bnl7.08-bnl8.26*	8.04	(8L)
Freymark et al., 1993	Mo17	*umc323-umc30*	8.06	(8L)
Welz et al., 1999b	D145	*umc17b-npi268a*	8.06	(8L)

Continued

Table 1.1. Discovered and consensus QTL for resistance loci associated with gray leaf spot, maize streak and northern leaf blight.

Table 1.1 continued

MSV (Consensus)				
Kyetere et al., 1999	Tzi4	*bnl12.06a-npi262*	1.04	(1S)
Welz et al., 1998	CML202	*umc302-umc167*	1.04	(1S)
Pernet et al., 1999	D211	*asg30-csu92*	1.05	(1S)
		npi232a-umc44a	1.05	(1S)
Pernet et al., 1999	CIRAD390	*asg30-umc177*	1.05	(1S)

CHAPTER 2

VALIDATION OF CONSENSUS QTL, GENETIC EFFECTS AND HERITABILITY OF RESISTANCE TO MULTIPLE FOLIAR PATHOGENS OF MAIZE

Abstract

Maize (*Zea mays* L.) production in sub-Saharan Africa is threatened by the potential outbreak of multiple foliar diseases such as northern corn leaf blight (NCLB) incited by *Exserohilum turcicum*, gray leaf spot (GLS) incited by *Cercospora zeae-maydis* and maize streak incited by maize streak virus (MSV). Quantitative trait loci (QTL) conditioning partial-resistance to these pathogens have been identified. Validation of candidate QTL conferring partial resistance would present marker-assisted selection as a potentially viable strategy to improve host resistance. We were interested in determining the usefulness of molecular markers linked to consensus QTL controlling partial-resistance systems for improving the overall resistance level. We examined QTL for NCLB in chromosomal bins 3.06, 5.04 and 8.06; GLS QTL in bins 2.09 and 4.08; and a consensus MSV QTL in bin 1.04 as potential targets for selection. We developed and evaluated 410 $F_{2:3}$ and selected 228 $F_{2:4}$ families derived from parents with

complementary resistance. Field evaluations were conducted independently for each disease in the 2003 and 2004 growing seasons. Maize streak evaluations were conducted in Zimbabwe, GLS tests were performed in Ohio, and NCLB evaluations were conducted in Uganda and Ohio. Narrow-sense heritability estimates were 0.22, 0.25 and 0.39 for MSV, NCLB and GLS, respectively. Analysis of gene action using orthogonal contrasts showed mostly dominant gene action for NCLB, GLS and MSV. For NCLB, resistance due to presence of alleles from QTL in bins 3.06 and 5.04 were detected across two seasons. The chromosomal region in bin 4.08 for GLS resistance was significant ($0.0001 \leq P \leq 0.0395$) across seasons using late-season disease assessments. The major locus conferring resistance to MSV on chromosome 1 was significant ($P<0.05$) for resistance across seasons and explained 23% of phenotypic variations in the $F_{2:4}$ generation. Phenotypic values associated with flanking markers at each locus based on interval analysis indicated that QTL in bin 4.08 for GLS, bin 1.04 for MSV, and bins 3.06 and 5.04 for NCLB significantly reduced disease severity. Our results validated the position and effect of four out of six QTL controlling partial resistance to these pathogens. This was consistent with the presence of homozygous alleles from the resistant parent. These results point out the need for validation of QTL in new populations and the potential of using marker-assisted selection for pyramiding resistance to several pathogens using target QTL.

INTRODUCTION

Diseases represent a major threat to production of maize (*Zea mays* L.) worldwide. In many agroecosystems, low yields are attributable to devastating effects of foliar diseases (Leonard et al., 1993; Ward et al., 1999; Okori et al., 1999; Pratt and Gordon, 2006). The causal agents are widespread, with some tending to be more or less prevalent in particular regions, or during certain seasons (Smith, 1999). NCLB, incited by *Exserohilum turcicum*; GLS, incited by *Cercospora zeae-maydis*; and maize streak, incited by maize streak virus, are three of the most destructive diseases (Adipala et al., 1993; Leonard et al., 1993; Bosque-Perez et al., 1998; Ward et al., 1999). Epidemics of the fungal diseases continue to occur due to favorable climatic conditions, planting of susceptible varieties and widespread adoption of conservation tillage practices. Maize streak occurs throughout sub-Saharan Africa. Losses due to each of these diseases may exceed 30% in endemic areas (Raymundo and Hooker, 1981; Thottappilly et al., 1993; Saghai Maroof et al., 1993; Bosque-Perez et al., 1998; Bosque-Perez, 2000) but combined losses could be higher. Unfortunately, these diseases may occur simultaneously in sub-Saharan Africa, resulting in dramatically increased risk of crop losses.

Successful management of multiple diseases presents a unique challenge. Integrated management using various control methods has been suggested (Ward et al., 1999). The methods emphasized include use of cultural practices

such as timely planting, burning of crop residues, conventional tillage, application of fungicides and host resistance. The availability, feasibility and cost-effectiveness of these methods are likely to vary in different production settings (commercial vs. subsistence) and regions (tropical vs temperate) (Pratt et al., 2003). Host resistance is generally considered the most practical, cost-effective and environmentally acceptable means for managing maize diseases (Fehr, 1987; Pratt et al., 2003; Carson et al., 2004). Most hybrids and inbreds grown in temperate and tropical environments rely mainly on quantitative (partial) resistance to manage diseases (Carson et al., 2004). Partial resistance is considered more durable because it is relatively effective against all races of a potential pathogen and does not foster selection of new races (Lipps et al., 1997; Parlevliet, 2002).

In maize, there already exists information concerning the genetic basis of host resistance and pathogen virulence for several pathosystems. A number of loci conferring partial resistance have been identified and some have been observed in multiple studies (Table 2.1). For NCLB, Welz and Geiger (2000) investigated the consistency of QTL controlling partial resistance and found several were significant in more than one population. Such robust marker-QTL associations provide consensus that some QTL positions and effects previously identified are reproducible. This knowledge presents marker-assisted selection for these candidate QTL as a potentially viable strategy to pyramid and improve

quantitative (partial) resistance. Selection based on such QTL information can also broaden the spectrum of resistance and enhance durability.

Most maize breeders employ quantitative resistance to protect crops against diseases and cultivar development would benefit from increased knowledge of QTL-marker associations and genetic effects of resistance. The objectives of this study were to: (i) identify polymorphic markers linked to previously reported consensus QTL controlling partial resistance to *E. turcicum*, and MSV, (ii) validate QTL and identify polymorphic markers for *C. zeae-maydis* resistance, (iii) characterize partially inbred lines with molecular markers associated with resistance loci, (iv) characterize modes of action for quantitative resistance to *E. turcicum*, *C. zeae-maydis* and MSV, (v) estimate heritability of partial resistance to *E. turcicum*, *C. zeae-maydis* and MSV.

Materials and Methods

Plant materials. Resistance to maize streak and *E. turcicum* has been identified in CIMMYT inbred CML202. VP31 is a partially inbred line ($F_{2:4}$) derived from a South African inbred (VO613Y) and is considered to be a reliable source of resistance for GLS (Gordon et al., 2004) with much earlier maturity than its parent VO613Y. The initial hybrid between CML202 and VP31 was self-pollinated to develop a segregating F_2 population. An F_3 population consisting of 410 families was developed by pollinating F_2 plants to form $F_{2:3}$ lines. Self-pollination of single plants within each of the $F_{2:3}$ families was performed to

create $F_{2:4}$ lines. Genetic structure was maintained in each generation by descent from individual F_2 plants.

Field trials. The $F_{2:3}$ and $F_{2:4}$ lines were evaluated for partial resistance to three diseases in field trials at the Fry and Schaffter Farms of the Ohio Agricultural Research and Development Center (OARDC) near Wooster, Ohio (GLS and NCLB, respectively) in 2003 and 2004, Namulonge Agricultural and Animal Production Research Institute, Uganda (NCLB) in 2004 and the Center for Maize and Wheat Improvement (CIMMYT) Experimental Station at Mt. Pleasant in Zimbabwe (maize streak) in 2003 and 2004. Because of limitations in available land, $F_{2:3}$ progenies were initially evaluated for GLS using an augmented experimental design constructed using a randomized complete block design as described by Federer et al. (2001). Inbred checks Pa405 and B73 were included. The 410 $F_{2:3}$ progenies were distributed randomly in 10 blocks, each containing 49 entries (resistant and susceptible local checks, the parents and 41 $F_{2:3}$ progenies). Inbreds Pa405 and H100 were planted as susceptible checks while inbreds VO613Y and CML202 served as resistant checks for GLS and NCLB, respectively. The check treatments and the parental lines were replicated 20 times (2 replicates per block) to allow for calculation of an error term and to account for any spatial variation in the field.

At other sites, and in later evaluations of $F_{2:4}$ progenies, plots were planted in a simple randomized complete block design with two replicates. The planting

material consisted of the lines, parents, and local checks. At Wooster, each plot was a single-row into which 20 seeds were sown. To increase the spread of *C. zeae-maydis* and *E. turcicum*, kernels of B73 (known to be susceptible to both fungal pathogens) were planted as guard rows surrounding the experimental plots and between every four ranges. The GLS nursery at Fry Farm consisted of plots planted into soil that had a large amount of maize debris (>50% soil surface coverage) on the surface from the previous no-till plots while the site at Shaffter Farm for NCLB was plowed following alfalfa (*Medicago sativa*). Chlorpyrofos insecticide (*O,O*-diethyl*O*-3,5,6-trichloro-2-pyridyl phosphorothioate) was applied in the furrow at planting in both Wooster plots. Weeds were controlled by 1,3,5-Triazine-2,4-diamine (Bullet, Monsanto Company) and hand weeding was performed as necessary in all plots. Plots in Namulonge were established in a field maintained in maize-soybean rotation. The soil type in this location is deep tropical red clay loam characteristic of the lower slopes of Buganda catena. The region is located in the mid-altitude Lake Victoria Crescent agroecological zone, with a mean temperature of 20^{o}C (Wortmann and Eledu, 1999). The area is characterized by a bimodal rainfall regime with an annual precipitation of 1,200 mm. The field was ploughed and later disc-harrowed before planting to achieve a fine seedbed. Two seeds were planted per hill at a distance of 30 cm between plants and 75 cm between rows, and 2 wk after planting, plots were thinned to one plant to maintain a plant stand of 54,000 plants per hectare. Plots in Zimbabwe were established similar to those in Uganda but they were thinned only after maize streak symptoms appeared. Standard management practices for

maize production in the sub-humid natural region II of Zimbabwe were employed. Plots were fertilized with 32 kg N, 56 kg P_2O_5 and 28 kg K_2O ha^{-1} at planting and supplemented with 69 kg N ha^{-1} 6 wk after planting.

Inoculum preparation. Conidia of *C. zeae-maydis* were isolated from senesced maize leaves collected the previous season from diseased fields near Wooster and Apple Creek, Ohio. Leaves were air-dried and stored in polythene bags at room temperature until used. Portions of infested leaf tissue were cut into segments and surface sterilized in 9:1 (v:v) water/sodium hypochlorite solution for about 30 s. Washed leaf segments were placed on a metal screen in 8.5-cm diameter Petri dishes with three layers of water-saturated filter paper in the bottom and placed in high humidity under fluorescent light for 3 d to initiate sporulation. After incubation, single conidia were aseptically transferred from the lesion surface with a sterilized glass needle to V8 agar (350 ml of V8 vegetable juice, 3 g of $CaCO_3$, 20 g of agar, 650 ml of de-ionized water per liter) in Petri dishes. Cultures were maintained at 28°C with 12 hr of cool-white fluorescent light per 24 h period. Plugs from 10 to 25 d old sporulating colonies were used to colonize sorghum (*Sorghum bicolor*) kernels in 1000 ml autoclavable plastic containers filled half-full. The containers were shaken once wk^{-1} to loosen the inoculated kernels and facilitate uniform colonization. Infested kernels were air-dried on greenhouse benches for 3 to 4 d and thereafter kept dry at 10°C until used. The colonized kernels were used for inoculation. Treatment materials and inbred checks were inoculated twice at one-week intervals, starting at the V6

growth stage, by placing approximately 5 to 20 infested sorghum kernels into the leaf whorls of all plants. In 2004, plants were inoculated at V8 growth stage. After each inoculation, low volume overhead irrigation sprinklers applied water once daily at dusk for approximately 30 min to extend the period of leaf wetness. This was done to enhance disease establishment until environmental conditions were conducive.

E. turcicum inoculum was produced from isolates obtained from infected maize leaves from Namulonge, Uganda and Licking County, Ohio for inoculation of plots in the respective locations. Portions of infected leaf tissues were surface sterilized in 1% sodium hypochlorite for 30 s, rinsed in distilled water, and placed in high humidity under fluorescent light for 3 d to initiate sporulation. Single conidia were then picked from conidiophores with a sterile glass needle and placed on lactose casein hydrolysate agar (37.5 g lactose, 3 g casein hydrolysate, 1 g KH_2PO_4, 5 g $MgSO_4$, 2 ml microelements, 15 g agar dissolved in 1 l of de-ionized water) in Petri plates. Cultures were maintained at room temperature for 15 d until the plates were fully colonized. Colonized media sections from the culture were placed onto sorghum kernels as described above. Inoculations were performed using the same method as described for *C. zeae-maydis* starting at the V6 growth stage. Before inoculations were performed, race-specific virulence of the isolates was established by inoculating known differentials of maize inbred lines H4460, H4460*Ht1*, H4460*Ht3*, A619, A619*Ht* and A619*Ht2* grown in the greenhouse at the OARDC. Based on the specific

reactions of these differentials, isolates were confirmed to be a mixture of races 0 and 1 in approximately equal proportions for trials conducted in Ohio. Trials conducted in Uganda were inoculated using a method similar to that described above. Earlier pathogen race monitoring in Uganda indicated the presence of only race 0 (Adipala et al., 1993; Bigirwa et al., 1993) although the racial profile of the pathogen today is unknown.

For MSV inoculation, plants were artificially infested with viruliferous leafhoppers (*Cicadulina mbila*, Naude) at the V3 stage 2 wk after planting in the field. Leafhoppers were reared on millet (*Pennisetium americanum*) seedlings maintained at 26^{o}C and 65% relative humidity. Millet plants in rearing cages were removed and replaced with maize plants showing symptoms of MSV for the *C. mbila* populations to feed on. After the insects acquired virus (48 h), the cages containing leafhoppers were covered using dark sheets with small openings for light penetration. All adult insects that moved towards the light source were removed with a modified hand-operated vacuum cleaner. Recovered insects were anaesthetized with carbon dioxide and then transported to the field. Three viruliferous, anaesthetized leafhoppers were placed into the leaf whorl of each plant and allowed to feed on maize seedlings for one wk. The seedlings were then sprayed with a systemic dimethoate insecticide (2-dimethoxyphosphino-thioylthio-*N*-methylacetamide) to prevent damage from insect pests.

Disease severity assessment

Disease assessments for GLS and NCLB were made on a whole-plot basis commencing 53 d after the first inoculation. The first disease score was made at the R1 growth stage (Ritchie et al., 1989), the time when approximately 50% of plants had visible silk exertion. Standard assessment diagrams developed by Ward et al. (1997) were used as a guide in estimating disease severity. The scale assigns a percentage leaf area affected (PLAA) score based on visual estimates of the percent leaf surface area covered by lesions on single plants. Instead of individual plant assessments, visual estimates were made on whole-plots because each plot constituted a family and reaction of the plants within a family to infection was similar. A total of three assessments were made at one-wk intervals. The three scores were used to calculate area under disease progress curves as AUDPC = $\Sigma[(x_i + x_{i+1})/2](t_{i+1} - t_i)$ (Campbell and Madden, 1990) and they were standardized by dividing by the total number of d used for disease assessment, where x_i is disease rating on date i, and t_i is the time in days on which x_i was recorded. MSV symptoms were allowed to develop and severity scores were rated 52 d after infestation when a range of host responses were observable. Disease symptom severity for MSV was rated on a commonly used standard scale of 1-5 with 0.5 increments, where 1 was the most resistant, showing no symptoms of infection, and 5 was the most severely affected (Welz et al., 1998). Disease ratings of the $F_{2:4}$ population were performed using the same procedure as for the $F_{2:3}$ population.

Genotypic analysis. Simple sequence repeat (SSR) oligonucleotide primer pairs were used to detect polymorphisms between the parents in chromosomal regions where the major NCLB and MSV consensus QTL, and GLS QTL, had been mapped (Table 2.1). For NCLB resistance, the consistency of QTL across populations has been previously evaluated and the consensus regions were determined to be in bins 3.06, 5.04 and 8.06 (Welz and Geiger, 2000). These regions were chosen as candidate QTL in this study. Primer sequences were obtained from the maize genetics and genomics database (http://www.maizegdb.org/). The probes tested for parent genotyping were selected because of their proximity to markers linked to resistance QTL in previous studies. The rate of polymorphism between the two parents was found to be 19%. The markers flanking each locus were in the same bin position that was considered to be the consensus region for that QTL. Two informative markers flanking each locus where used for screening the population to detect if there was a relationship between these markers and resistance. Finally, the genetic distance between each flanking marker was calculated and the effect of the previously identified target QTL was reevaluated based on data from both the $F_{2:3}$ and $F_{2:4}$ populations.

Statistical analysis. For genotypic analysis, ten field-grown $F_{2:3}$ individuals per family, and five $F_{2:4}$ individuals per family, were sampled. In both generations, genotypic data from markers were scored as: homozygous for one parental allele; homozygous for the other parental allele; and heterozygous. Chi-square

analyses were carried out to test the distribution of observed frequencies against those expected for the segregation model (1:2:1). Independence of residuals and normality tests for phenotypic data were evaluated by the Ryan-Joiner test (similar to Shapiro-Wilks test) and equality of variance as well as summary statistics were calculated at 95% Bonferroni confidence interval using the MINITAB statistical package. Residuals of disease ratings for NCLB were not normally distributed and consequently data were transformed by a log10 (datum +1) transformation to normalize variance as described by Gordon et al. (2004).

Associations between individual marker loci and disease severity were tested with single-factor analysis of variance using the PROC GLM procedure (Version 9.1, SAS Institute), with a threshold significance level of P = 0.05. For tests of marker-trait association within the population, the statistical model used was $X_{ij} = \mu + B_i + M_j + G_k(M_j) + \varepsilon_{ijk}$, where X_{ijk} is the trait value for the k^{th} genotype of the j^{th} marker class in the i^{th} block, μ is the population mean, B_i is the effect of the i^{th} block, M_j is the effect of the jth marker class, and ε_{ijk} is the experimental error. Block was considered a random effect in the model. In all cases, the appropriate F test for declaring marker significance was equal to the mean square for marker classes/mean square for genotype within maker. For tests of epistasis, the statistical model was $X_{ij} = \mu + M1_i + M2_j + (M1_k * M2_j) + \varepsilon_{ijk}$, where M1 and M2 were markers on different loci. The F test for epistasis was equal to the mean square for marker interaction/ mean square for the residual error.

Total phenotypic variation explained by the marker loci was calculated by estimating variance components by restricted maximum likelihood (REML) using the VARCOMP procedure of SAS (2003 Version 9.1, SAS Institute, Cary, NC). Because the experiments for $F_{2:3}$ MSV and NCLB and all $F_{2:4}$ disease evaluations were replicated, the phenotypic variation explained by the effect of QTL were expressed as the variation due to the marker divided by the total variation (V_m/V_p). Orthogonal contrasts were used to test for significance and estimates of additive and dominance gene action associated with each locus using the PROC GLM procedure.

Broad-sense heritability estimates for disease resistance were calculated from a standard analysis of variance with total variance portioned into genotypic and phenotypic components. Broad-sense heritabilities were estimated by variance component analysis of the $F_{2:3}$ and $F_{2:4}$ generations as described by Holland et al. (2003): $h^2 = \sigma^2_g /(\sigma^2_e/re + \sigma^2_{ge}/e + \sigma^2_g)$ where σ^2_g is the genotypic variance component, σ^2_{ge} is genotype by environment, σ^2_e is the experimental error variance component, e = number of environments and r = number of replications per environment. Narrow-sense heritability estimates were computed by calculating the correlation coefficients between $F_{2:3}$, and $F_{2:4}$, family means during mid and late epiphytotic and AUDPC. Correlation rather than regression coefficients were used as estimates of heritability because the parents ($F_{2:3}$) and progenies ($F_{2:4}$) were evaluated in different years and/ or macroenvironments

(NCLB). Parent-offspring correlation has been recommended as a more reliable method than regression for estimating narrow-sense heritability (Foolad et al., 2002). The authors advocate this method because it reduces the scale of phenotypic variation in progeny relative to the parent due to the environmental differences. The standard error (SE) for each h^2 estimate based on correlation was calculated as SE $[h^2 (F_{2:4})] = [(1-r^2_{F2:4})/(n-2)]^{1/2}$ (Foolad et al., 2002), where n is the number of $F_{2:4}$ families and r is the correlation coefficient.

Results

Gray leaf spot. Weather conditions and high levels of inoculum, including that from infested debris from the previous crop, were favorable for early gray leaf spot development during both seasons. High disease severity resulted and clear genotypic responses were observed. At two wk postanthesis, the susceptible inbred Pa405 was already severely affected (>70% PLAA). By the last rating (63 DAI), disease severity had reached 91% PLAA on Pa405 and 76% on the susceptible inbred B73. Several susceptible progeny lines were more than 50% affected. The most resistant checks and progenies had less than 10% PLAA (Table 2.2).

There were highly significant differences ($P<0.0001$) in reaction to GLS among the progenies in both generations. The reactions of parental inbreds were similar and intermediate compared to the susceptible and resistant check inbreds,

respectively. The difference between the parents and the grand mean of all families were not significantly different (P>0.05). In general, disease severity was continuously distributed with significant (P<0.001) transgressive segregation in both seasons. This may have been observed because the parents carried different resistance genes that were either unlinked, or linked in coupling. Final disease severity ratings for both parents ranged from 29 to 33% in both growing seasons. Differences in resistance reaction of CML202 compared to VP31 were noted. The resistance reaction of VP31 was characterized by fewer and smaller lesions occurring on the entire plant while on CML202 lesions appeared later in the season, were larger, and mainly blighted leaves below the ear. It was evident that the parental inbred CML202 contributed some level of GLS resistance to the population. The check inbred VO613Y, from which resistance of VP31 was derived, had a relatively low disease score of 13% PLAA by the end of the growing season. The most resistant $F_{2:3}$ family had 8% PLAA and the most susceptible family had 80% PLAA. Overall, 156 lines had final disease severity less than 30% (less than either parent) and 87 lines had final severity scores of greater than 45% PLAA.

Northern corn leaf blight. Weather conditions in Uganda favored development of severe NCLB epidemics. As a result, a large portion of the $F_{2:3}$ progenies were extensively blighted (> 50%). The resistant and susceptible parents differed significantly (P<0.0001) for severity of NCLB in both seasons. Parental inbred CML202 expressed a relatively high level of resistance with only 8% final PLAA

at leaf senescence in the Uganda trial and 0% final PLAA by the end of the season in Ohio (Table 2). The susceptible parental line, VP31, was severely damaged (>60% PLAA) in both seasons and locations with disease scores similar to the most susceptible checks (inbreds B73 and H100). In Uganda, only three progenies had 0% PLAA by the end of the season while 156 progenies had final PLAA >50%. The distribution of severity ratings did not fit a normal curve and no transgressive segregation was observed for NCLB resistance in either the $F_{2:3}$ or $F_{2:4}$ generations.

Maize streak. Disease evaluation for maize streak following artificial inoculation resulted in infection of all families and check inbreds. Both trials for $F_{2:3}$ and $F_{2:4}$ progenies were conducted at CIMMYT, Zimbabwe. Results indicated that there was a differential reaction of the family lines ranging from resistant to susceptible. The CML202 parent previously reported as 2.2 on a 1 to 5 severity assessment scale (Welz et al., 1988) had a mean score of 3.7, and VP31 had a mean score of 4.3, in the first season. The average rating for $F_{2:3}$ families was 4.2 with a range of 2.0 to 5.0 (Table 2.2). The majority of the population was skewed towards susceptibility with 105 families having a score of 5.0. None of the plants escaped infection since all had disease scores greater than 2.0 during the first season. In the second season, CML202 had a mean score of 1.3 and the susceptible inbred checks (≥4.2) were not significantly different from 5.0. The $F_{2:4}$ progeny mean was 2.2 and the final ratings of 35 progenies were not significantly

different from that of CML202. Across two seasons, the parental means were 2.5 for CML202 and 3.1 for VP31.

Correlation among traits. Correlations between disease severity rating time points were highly significant and positive for each disease ($0.70 \leq r \leq 0.97$). The highest correlations among disease variables were obtained between the mid-epiphytotic assessment and disease progress curves (AUDPC). Among ratings across different diseases, positive but non-significant ($P>0.05$) linear correlations were obtained for all the ratings from early to late epiphytotic, indicating that some progenies were generally resistant to each disease, conversely, some inbreds remained consistently susceptible to all diseases. Phenotypic correlations between resistance traits and days to silking, also used as a measure of maturity, were significantly ($P<0.05$) negatively correlated ($-0.41 \leq r \leq -0.50$). Thus, earlier maturing genotypes tended to have higher disease severity than later maturing genotypes in the initial disease assessments.

Broad-sense heritability estimates. Variance components were used to estimate broad-sense heritability for each disease in the population. The heritability estimates based on disease severity for GLS and maize streak were higher in $F_{2:4}$ than $F_{2:3,}$ but estimates for NCLB were generally similar across generations. Heritability estimates for all diseases ranged from 0.42 to 0.90 and were more variable for maize streak than for NCLB or GLS. The lowest and highest estimates of heritability across the two generations were obtained for

maize streak (Table 2.2). In general, during both seasons, estimates of heritability were similar for disease ratings from mid-epiphytotic to late epiphytotic, indicating similarity in genotypic responses as the season progressed (Table 2.2 and 2.3).

Narrow-sense heritability estimates of disease resistance. There were variations in the magnitude of narrow-sense heritability estimates for the three diseases. Narrow-sense heritability estimates based on the correlation coefficients from parent-offspring regression at final disease ratings were 0.22, 0.25 and 0.39 for maize streak, NCLB and GLS, respectively. Across all disease assessment times, heritability estimates were greater for GLS ($0.34 \leq r_p \leq 0.39$) compared to NCLB ($0.06 \leq r_p \leq 0.25$) and maize streak ($0.22 \leq r_p \leq 0.29$) (Table 2.3). Heritability generally increased at later ratings, corresponding perhaps to increased disease severity as the season progressed. The highest heritability estimates were attained from the AUDPC that accounted for the season long disease progress. Overall, there were modest, but significant, similarities between the $F_{2:3}$ families and the corresponding $F_{2:4}$ families in reaction to GLS, NCLB and maize streak, as evidenced by low to moderate correlation coefficients ($0.06 \leq r_p \leq 0.39$; $P<0.0001$).

Genotypic analysis. The flanking markers for each locus used for genotyping were grouped together based on reference genetic maps, indicating cosegregation of these markers in the population for the disease traits. Most of

the markers segregated 1:2:1 as expected. The QTL positions for NCLB resistance on chromosomes 3, 5 and 8 were significantly ($P<0.05$) associated with resistance at mid- and late-maturity disease ratings. Interestingly, both flanking markers at the QTL position on chromosome 5 (bin 5.04) were significantly associated with resistance at all severity ratings and they were stable across environments and generations (Table 2.4 and 2.5). Single-factor analysis of variance for GLS resistance showed that markers flanking the QTL position on chromosome 4 (bin 4.08) were significantly ($P<0.05$) associated with resistance at most rating times across generations, but the significance of a GLS locus in bin 2.09 was marginal and more variable. The same markers in bin 4.08 were significant for overall disease rating expressed as AUDPC. Single factor analysis of variance showed significant association ($P<0.05$) between markers used and resistance at most consensus loci previously reported (Table 2.4 and 2.5) but in some cases only one marker in an interval was significant. This justified the use of interval analysis to determine whether some of these regions were still associated with resistance.

Analysis of gene action using orthogonal contrasts showed that mostly dominant gene action was present for NCLB and maize streak. The magnitude of gene action varied with the QTL position. The QTL in bin 5.04 for resistance to *E. turcicum* showed consistently higher significant effects for dominance compared to the QTL in bin 3.06 (Table 2.6). Dominance was expressed starting from early disease ratings, about 2 wk postanthesis to maximum epiphytotic near leaf

senescence. In general, the values of dominant effects were greater for the overall disease rating expressed as AUDPC than for individual ratings. Consistently, stronger additive gene action was detected for the QTL position on chromosome 8 (bin 8.06). Resistance due to the presence of alleles from QTL positions 3.06 and 5.04 was maintained from mid- and late-season ratings and no evidence for epistatic interactions were detected among these loci.

Comparison of genotypic classes for NCLB resistance showed that families homozygous for the CML202 alleles in bins 3.06 and 5.04 where significantly more resistant than families homozygous for the VP31 allele. However, resistance from the QTL position in bin 8.06 was contributed by VP31 (susceptible) (Table 2.7). There were no significant differences for disease ratings on lines that were heterozygous or homozygous for chromosome 3 and 5 alleles. Similarly, mean severity scores for maize streak indicated that lines homozygous for CML202 allele as well as those heterozygous, were significantly ($P<0.05$) more resistant compared to lines homozygous for the susceptible parent. The mean severity of genotypic classes for GLS also showed that lines with homozygous alleles at bin 4.08 from the resistant parent were more resistant and bin 2.09 had a contrasting effect (Table 2.7). The effects of VP31 alleles on bin 2.09 were additive and the mean severity ratings were not significantly different among genotypic classes.

Discussion

Our study used a large population and promoted disease development by artificial inoculations. As a result of high levels of disease, highly resistant and susceptible families were identified. No plants escaped infection among the susceptible check inbreds. In all cases, the coefficients of variation were less than 28%. Results were largely consistent with previous studies on inheritance of partial resistance to foliar diseases in maize although the susceptible parent contributed a resistance allele in two cases. Dominant gene action was evident, with no epistasis effects detected, broad-sense heritabilities were medium to high, partial resistance was stable across environments and a small number of quantitative trait loci appeared to have large effects on resistance levels.

The effectiveness of some NCLB QTL for partial resistance in conferring high levels of resistance was confirmed by the reaction of progeny lines in tests conducted in both temperate and tropical environments. Partial resistance to *E. turcicum* in CML202 is controlled by multiple genes (Schechert, 1999, Welz and Geiger, 2000). This inbred has been used in many breeding programs as a donor of NCLB resistance due to its high combining ability for both resistance and yield (Schechert et al., 1999). Our results also show that based on resistant reactions of some families, quantitative resistance is highly effective, and sufficiently heritable to be recovered in subsequent generations.

Although CML202 seems to be a source of resistance to multiple diseases, there have been conflicting reports on the reaction of the inbred to GLS. For example, it has been reported to be highly resistant (Schechert et al., 1999), or moderately resistant (K. Pixley, pers. comm.) whereas in some studies, it has been described as susceptible to GLS (Wegary et al., 2003). Our results during high epiphytotic seasons indicated that the inbred was moderately resistant. Based on transgressive segregation, and the observation of distinct resistant host responses to infection, we hypothesize that CML202 contributed resistance to the population in this study from unknown factor(s) not linked to, and perhaps different from, those derived from VO613Y.

Differences in allelic effects were detected for both loci conferring resistance to GLS. Interval analysis clearly indicated the QTL on chromosome 2 had opposite allelic effects for resistance. The favorable alleles that contributed to resistance were not derived from the intended donor parent. Gordon et al. (2004) have previously shown that markers linked to the locus on chromosomal bin 2.09 were significant in one (Wooster, Ohio) of two locations. This locus also displayed recessive inheritance. Our results agree with these earlier findings and suggest that this locus may be less reliable in selecting for resistance in breeding applications.

The reaction of progenies derived from CML202 to MSV were similar to the reaction of progenies derived from other resistance sources such as Tzi4

(Kyetere at al., 1999). In our study, the resistant parent CML202 and none of the 410 $F_{2:3}$, nor any selected $F_{2:4}$, families were completely resistant to maize streak. This suggests that resistance conferred by CML202 provides partial protection against maize streak. Previously, Kyetere et al. (1999) noted that the resistant parent, Tzi4 varied in reaction across years. In trials conducted in Nigeria, Tzi4 had a severity score of 1.0 based on 1-5 scale but had a mean score of 3.3 in trials conducted in Zimbabwe (Kyetere et al., 1999). He attributed the variation to heterogeneity among different accessions. Results from our study showed that CML202 had a mean score of 2.5 across seasons which was comparable to a mean of 2.2 previously reported by Welz et al. (1998). In the two seasons, the mean of VP31 was 3.1 indicating that the line was somewhat resistant to MSV. It was derived from a South African line, VO613Y whose reaction to MSV was unknown. This suggests that VP31 also contributed resistance to the population in agreement with transgressive segregation observed for reaction to MSV resistance.

Strains of MSV that may vary in their ability to induce symptoms are a potential concern in breeding for host resistance (Martin et al., 2001). Resistant varieties that have succumbed to strains of the virus associated with particular locations have been reported (Rodier et al., 2002). In our field trials conducted in Zimbabwe, considerable variation was noted in the reactions of many progenies across the two growing seasons. It was hypothesized that environmental conditions were largely responsible; however, isolate variability may have been

partly responsible for these differences. Occurrence of prolonged drought in the first season may have affected the level of disease expression. High temperatures and low rainfall are known to influence the development of MSV (Rose, 1978). Experiments conducted by Mawere et al. (manuscript in preparation) to investigate the stability of MSV resistance in response to infection by isolates collected across Zimbabwe showed significant effects of isolates, and genotype by isolate interaction, on MSV severity. These significant effects were found to be practically unimportant because they did not affect trends in ranking of genotypes with known reactions, suggesting MSV resistance was stable. In our trials, we also observed the highly resistant and susceptible lines maintained their resistance rankings fairly consistently.

An influence of individual NCLB QTL has been reported to affect different components of resistance (Schechert et al., 1999; Welz and Geiger, 2000), and our findings were in agreement with these earlier studies. Schechert et al. (1999) indicated that NCLB resistance on chromosome 3 was conferred by two QTL, 22 cM apart and linked in repulsion. These loci affected primarily lesion number. QTL on chromosome 5 affected both lesion number and incubation period (IP). The QTL on chromosome 8 was found to only affect IP. Our ratings were made based on PLAA, a trait that is closely related to number of lesions. This relationship possibly explains the significant effects of markers on chromosome 3 and 5 and a stronger and more consistent effect of chromosomal bin 5.04 across generations and environments. The locus in bin 5.04 was also stable for all

assessment times and appeared non-specific to the expression of resistance early or late in the season, making it more desirable for breeding purposes. Although one marker (*umc2169*) on the QTL position in bin 3.06 was non-significant in both generations, interval analysis between flanking markers indicated this region was associated with resistance.

We observed consistently negative correlations between maturity and resistance to the three different pathogens suggesting high levels of resistance were associated with late maturity. In spite of the significant correlation between resistance and maturity, resistant early, and susceptible late, lines were observed. Single factor analysis of variance indicated non-significant relationships between resistance and plant maturity. For GLS, there is always concern of confounding severity ratings with differences in plant maturity because of late-season disease development, mainly from anthesis through grain filling (Ward et al., 1999). Our results suggest that maturity period may not be important in selecting for resistance to *C. zeae maydis*. Gray leaf spot is a polycyclic disease if a sufficient level of inoculum is initially present, suggesting epidemics increase as the season progresses primarily due to sporulation within lesions when conditions are favorable. Later in the season, the abundance of conidia within the crop canopy and in air from neighboring fields increases the number of infections. Some of the resistant, late maturing plants do not merely escape early infection during the physiologically susceptible stage. Paul and Munkvold (2004) found similar relationships in pre-planting risk models

developed for GLS. They demonstrated that the relationship between maturity and GLS intensity had non-significant, negative logistic regression indicating that maturity was not an important predictor of disease severity.

The estimates of broad-sense heritability for all diseases were moderate to high (0.58 to 0.90) suggesting reasonable progress in selection is possible for disease resistance traits in this population. The estimates of heritability were in agreement with those reported previously for disease traits: 0.59 to 0.82 for NCLB (Hakiza et al., 2004; Welz and Geiger, 2000) using PLAA; 0.28 to 0.80 for GLS (Gordon, 2003) using PLAA; and 0.62 to 0.93 for MSV (Welz et al., 1998) using standard 1-5 scale estimated independently in different genetic backgrounds. Moderate estimates of heritability based on parent-offspring coefficients indicate that resistance to pathogens was heritable and early-generation selection could result in improved germplasm under high disease pressure evaluations. A low initial narrow-sense heritability was observed for NCLB probably because of the fact that during evaluations conducted in the second year in Ohio, leaf blights were observed late in the season, but blight occurred earlier in evaluations conducted in Uganda. A highly variable estimate of heritability was observed for maize streak. The high variability could be attributed to variable reaction of progenies due to extreme drought during the first season that may have affected virus multiplication, plant growth, and resultant symptom expression.

The current study indicated the potential of using several target QTL for MAS to pyramid quantitative resistance to multiple pathogens. Marker-based selection can be implemented on seedling plants without disease challenge, thereby potentially shortening the generation interval by allowing for selection in winter nurseries or greenhouses in the off-season. The parents in our study were confirmed sources of resistance to different diseases similar to founding parents as described by Servin et al. (2004) for pyramiding quantitative alleles from complementary genotypes. They demonstrated pairwise mating of lines with known genotypes at target loci in a step-wise fashion optimized the accumulation of favorable alleles in coupling, and fixing them into the homozygous state by self-pollination. Such a strategy for MAS would increase population mean genetic values for one or more traits. In our study, because the resistant family lines were selected independently for each disease, we are now implementing pairwise crosses of a similar nature for combining resistance QTL.

Clustering of disease resistance genes is common in maize and other crops (McMullen and Simcox, 1995; Pflieger et al., 2001; Yuan et al., 2003; Randall et al., 2005) and suggests several potential applications to enhance the efficiency of MAS for pyramiding resistance as emphasized in integrated disease management. It is interesting to note that some QTL characterized as having minor effects are located on the same bin positions for major QTL for other diseases. For example, Pernet et al. (1999) detected QTL with minor effects for MSV in bins 3.06, 5.03 and 8.07. These regions also harbor major QTL for

resistance to NCLB. Similarly, the QTL region for NCLB in bin 3.05 occurs in the same region as QTL or genes conferring resistance to maize mosaic virus (*mv1*), sugarcane mosaic virus (*Scmv1*), wheat streak mosaic virus (*Wsm2*) and maize chlorotic dwarf virus (*Mcd1*) (Yuan et al., 2003; Jones et al., 2004). Associations between qualitative resistance genes or resistance gene analogs (RGA) and QTL are also common (Pflieger et al., 2001; Quint et al., 2003; Randall et al., 2005). Gordon et al. (2004) found two RGA were linked to *umc127* marker in bin 4.08, QTL region for resistance to GLS. Similarly, the QTL for NCLB on chromosome 8L occurs in the same region as race-specific genes *Ht2* and *Htn1* (McMullen and Simcox, 1995). Because of these relationships, it is not surprising that some of the candidate QTL with major effects considered for MAS in this study had significant effects on resistance and probably explain low but positive correlations of resistance to different diseases. These relationships also provide opportunity to aid in selecting desirable alleles at QTL and to make the most desirable allelic combinations.

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Table 2.1. Discovered and consensus QTL for resistance loci associated with gray leaf spot, maize streak and northern corn leaf blight.

Reference	Resistance source	Marker interval	Bin	Chromosome arm
GLS (One discovered and consensus)				
Gordon et al., 2004	VO613Y	*bnlg1520-umc36*	2.09	(2L)
Gordon et al., 2004	VO613Y	*PIC21-umc127*	4.08	(4L)
Bubeck et al., 1993	ADENT	*umc19-bnl7.65*	4.08	(4L)
Saghai-Maroof et al., 1996	B73	*npi444-umc15*	4.08	(4L)
NCLB (Consensus)				
Welz et al., 1999a	CML202	*umc361- bnl15.20*	3.06	(3L)
Welz et al., 1999b	D145	*umc3b-umc17a*	3.06	(3L)
Freymark et al., 1993	Mo17	*umc60-bnl15.20*	3.06	(3L)
Schechert et al., 1999	CML202	*bnl8.01-umc389b*	3.06	(3L)
Freymark et al., 1993	Mo17	*bnl10.06-bnl7.71*	5.03	(5S)
Freymark et al., 1993	Mo17	*umc1-bnl5.40*	5.03	(5S)
Welz et al., 1999a	CML202	*umc001-bnl5.40*	5.04	(5S)
Welz et al., 1999b	D145	*csu36a-bnl7.71*	5.04	(5S)
Freymark et al., 1993	Mo17	*bnl7.08-bnl8.26*	8.04	(8L)
Welz et al., 1999a	CML202	*bnl12.30-umc030*	8.05	(8L)
Freymark et al., 1993	Mo17	*umc323-umc30*	8.06	(8L)
Welz et al., 1999b	D145	*umc17b-npi268a*	8.06	(8L)

Continued

Table 2.1. Discovered and consensus QTL for resistance loci associated with gray leaf spot, maize streak and northern corn leaf blight.

Table 2.1 continued

MSV (Consensus)				
Kyetere et al., 1999	Tzi4	*bnl12.06a-npi262*	1.04	(1S)
Welz et al., 1998	CML202	*umc302-umc167*	1.04	(1S)
Pernet et al., 1999	D211	*asg30-csu92*	1.05	(1S)
		npi232a-umc44a	1.05	(1S)
Pernet et al., 1999	CIRAD390	*asg30-umc177*	1.05	(1S)

	GLS			NCLB			MSV[a]	DTS[d]
	Sev_{53}[b]	Sev_{62}	SAUDPC[c]	Sev_{54}	Sev_{65}	SAUDPC	Sev_{max}	days
Parental lines								
CML202	27.5	32.8	27.2	4.9	7.7	5.1	3.7	104.0
VP31	26.8	31.8	27.5	59.7	60.7	53.6	4.3	84.4
Inbred checks								
B73	66.8	76.3	65.5					83.7
Pa405	88.5	91.0	86.6					83.2
H100				80.0	85.2	81.5		
Tzi4				9.5	12.0	8.6		
Genetic materials								
$F_{2:3}$	30.0	36.0	30.8	25.9	32.1	23.3	4.2	98.1
Mean[e]	33.7	39.6	34.2	33.7	39.6	34.2	4.2	96.6
SD	10.1	11.8	9.8	10.1	11.8	9.8		8.7
CV(%)	27.0	15.7	22.5	46.9	32.1	43.5	18.6	1.3
LSD	25.5	17.4	21.6	24.0	24.7	20.1	1.7	

continued

Table 2.2. Means of disease severity values (0-100%) of the parent lines, check inbred lines and $F_{2:3}$ families inoculated simultaneously with *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus, and estimates of variance components and heritabilities among $F_{2:3}$ families evaluated at Wooster, Ohio, Namulonge, Uganda and CIMMYT Zimbabwe, respectively.

Table 2.2 continued

Variance components ($F_{2:3}$ families)							
σ^2_F [f]	67.60	110.03	67.44	161.3	191.12	126.12	0.22
σ^2_e	53.95	32.96	29.57	29.34	172.47	113.52	0.61
H^2	0.70	0.77	0.69	0.85	0.69	0.69	0.42

[a] 1-5 standard scale was used for MSV severity ratings

[b] Sev_{53}, Sev_{62}, Sev_{55}, Sev_{65}, Sev_{max} = severity assessed 53, 62 days after inoculation for GLS, 54, 65 days after inoculation for NCLB and maximum severity for MSV

[c] SAUDPC = standardized area under the disease progress curve

[d] DTS = days to silking

[e] Experimental mean including the check inbreds

[f] σ^2_F, σ^2_e, H^2 Estimates of variances between families, residuals and broad-sense heritability

	GLS			NCLB			MSV	
	Sev_{55}[a]	Sev_{63}	SAUDPC[b]	Sev_{50}	Sev_{62}	SAUDPC	Sev_1	Sev_2
Parental lines								
CML202	25.0	29.0	25.2	0.0	0.0	0.0	1.0	1.3
VP31	25.0	30.0	24.0	47.5	47.5	38.8	1.5	1.8
Inbred checks								
B73	39.4	65.0	42.4	38.3	40.0	32.6	2.1	3.0
Pa405	87.5	95.0	81.4				2.8	4.0
VO613Y	7.5	15.0	8.0				1.8	2.5
H100				72.5	75.5	62.0	2.0	3.3
Genetic materials								
$F_{2:3}$	27.0	35.5	27.3	10.2	14.2	9.4	2.2	2.4
Mean	27.4	36.2	27.8	10.7	14.7	9.8	2.2	2.4
CV(%)	19.8	21.1	19.4	83.5	67.4	75.3	39.5	42.9
LSD	10.7	15.1	10.6	17.6	19.4	14.6	1.8	1.9

Continued

Table 2.3. Means of disease severity of the parents, check inbreds and $F_{2:4}$ families inoculated simultaneously with *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus as well as estimates of variance components and heritabilities among $F_{2:4}$ families evaluated at Wooster, Ohio (GLS, NCLB) and CIMMYT Zimbabwe (MSV).

Table 2.3 continued

Variance components ($F_{2:4}$ families)[c]								
σ^2_F	50.80	100.99	58.15	55.11	99.18	47.98	0.41	0.48
σ^2_e	29.10	55.18	29.57	80.19	98.38	54.74	0.09	0.14
H^2	0.78	0.79	0.80	0.58	0.67	0.64	0.90	0.87
$h^2_{(F3:F4)}$	0.34	0.39	0.39	0.06	0.25	0.24	0.22	0.29
SE [$h_{(F3:F4)}$]	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07

[a]Sev_{53}, Sev_{65}, Sev_{50}, Sev_{62}, Sev_1 and Sev_2 = severity assessed 53, 62 days after inoculation for GLS, 50, 60 days for NCLB and maximum severity for MSV assessed at two weeks interval

[b]SAUDPC = standardized area under the disease progress curve, DTS = days to silking

[c]σ^2_F, σ^2_e, H^2, h^2 Estimates of variances between families, residuals, broad and narrow-sense heritability at the experimental design

Marker	Bin	P (M/Gen(M))			V_m/V_P	Trait	QTL effect
		Mid epiphytotic	Late epiphytotic	SAUDPC[b]			
umc1551	2.09	0.7021	0.8591	0.6374	0.000	GLS	NS
umc2077	2.09	0.1973	0.0166	0.0948	0.025	GLS	+[a]
umc1086	4.08	<0.0001	<0.0001	<0.0001	0.088	GLS	+++
umc1559	4.08	0.0022	0.0004	0.0002	0.059	GLS	+++
umc1644	3.06	0.0537	0.0329	0.0545	0.013	NCLB	+
umc2169	3.06	0.1875	0.1271	0.2034	0.004	NCLB	NS
phi330507	5.04	0.0140	0.0708	0.0305	0.022	NCLB	++
umc1221	5.04	0.0346	0.1652	0.0475	0.015	NCLB	++
umc1724	8.06	0.1273	0.2005	0.1780	0.007	NCLB	NS
mmc0181	8.06	0.0067	0.0357	0.0146	0.025	NCLB	+++
umc1169	1.04	-	0.0160[c]	-	0.021	MSV	+++
bnlg2086	1.04	-	0.0054	-	0.047	MSV	+++

[a]+++, ++, +, NS = Marker effect is significant at all, two, one and none disease assessment times, respectively, [b]SAUDPC = standardized area under disease progress curves, GLS = gray leaf spot, MSV = maize streak virus and NCLB = northern corn leaf blight.[c] ratings were made only once at the end of the season

Table 2.4. ANOVA for the effects of markers on disease severity and standardized area under disease progress curves in $F_{2:3}$ population from CML202 x VP31 evaluated in 2003 for GLS in Ohio, NCLB in Uganda and MSV in Zimbabwe.

Marker	Bin	P (M/Gen(M))			V_m/V_P	Trait	QTL effect
		Mid epiphytotic	Late epiphytotic	SAUDPC[b]			
umc1551	2.09	0.0821	0.5005	0.1677	0.001	GLS	NS
umc2077	2.09	0.0028	0.1254	0.0040	0.045	GLS	++[a]
umc1086	4.08	0.2011	0.0395	0.1349	0.026	GLS	+
umc1559	4.08	0.0272	0.0124	0.0219	0.038	GLS	+++
umc1644	3.06	0.0365	0.0223	0.0191	0.029	NCLB	+++
umc2169	3.06	0.8327	0.5097	0.6238	0.000	NCLB	NS
phi330507	5.04	<0.0001	0.0004	0.0002	0.067	NCLB	+++
umc1221	5.04	<0.0001	<0.0001	<0.0001	0.079	NCLB	+++
umc1724	8.06	0.8900	0.8195	0.8814	0.000	NCLB	NS
mmc0181	8.06	0.9264	0.6923	0.8243	0.000	NCLB	NS
umc1169	1.04	<0.0001	<0.0001	-	0.169	MSV	+++
bnlg2086	1.04	<0.0001	<0.0001	-	0.228	MSV	+++

[a]+++, ++, +, NS = Marker effect is significant at all, two, one and none disease assessment times, respectively, [b]SAUDPC = standardized area under disease progress curves, GLS = gray leaf spot, MSV = maize streak virus and NCLB = northern corn leaf blight.

Table 2.5. ANOVA for the effects of markers on disease severity and standardized area under disease progress curves in $F_{2:4}$ population from CML202 x VP31 evaluated in 2004 for GLS in Ohio, NCLB in Uganda and MSV in Zimbabwe.

Chr. Bin			$F_{2:3}$			$F_{3:4}$		
Position	Marker	Parameter	Sev_{53}[a]	Sev_{62}	SAUDPC[b]	Sev_{55}	Sev_{63}	SAUDPC
MSV (1-5 scale)								
1.04	umc1169	a	0.067	-	-	0.187**	0.154*	-
		d	0.302**	-	-	0.822***	0.552***	-
		Action	D[c]			D	D	
1.04	bnlg2086	a	0.069	-	-	0.043	-0.002	-
		d	0.393**	-	-	1.027***	0.812***	-
		Action	D			D	D	
GLS (0-100%)								
2.09	umc2077	a	1.093	2.068		1.651***	1.416*	1.541***
		d	-0.360	-0.675		2.299***	1.925*	0.772
		Action						A
2.09	umc1551	a	-0.475	-0.200		1.405***	1.274*	1.235**
		d	-0.393	0.548		0.810	-0.788	0.772
		Action				A	A	A
4.08	umc1086	a	-1.583	-1.201		0.222	0.704	0.148
		d	-4.348	-5.886		-2.035**	-4.066***	-2.378***
		Action				D	D	D

Continued

Table 2.6. Genetic effects associated with putative quantitative trait loci affecting resistance to maize streak, gray leaf spot and northern corn leaf blight on severity in 410 $F_{2:3}$ families and 202 $F_{2:4}$ families evaluated for each disease separately at CIMMYT Zimbabwe, OARDC Ohio and Namulonge Uganda, respectively.

Table 2.6 continued

4.08	umc1559	a	-0.370	0.328		0.244	0.573	0.148
		d	-4.037	-5.187		-3.001***	-4.667***	-3.245***
		Action				D	D	D
NCLB (0-100%)								
3.06	umc1644	a	0.029*	0.031**	0.428*	0.099**	-0.098**	0.095***
		d	0.077**	0.066**	1.229**	0.054	0.073	0.062
		Action	D	D	D	A	A	A
3.06	umc2169	a	-0.012	-0.008	-0.209	0.001	-0.027	-0.014
		d	-0.067*	-0.064**	-1.008**	-0.041	-0.057	-0.052
		Action	D	D	D			
5.04	umc1221	a	0.018	0.009	0.428*	0.027**	0.085**	0.085**
		d	0.095***	0.059**	1.229**	0.235***	0.222***	0.213***
		Action	D	D	D	D	D	D
5.04	phi330507	a	0.031*	0.019*	0.464*	0.106**	0.097**	0.091**
		d	0.097***	0.067**	1.357***	0.201***	0.172***	0.177***
		Action	D	D	D	D	D	D
8.06	umc1724	a	-0.035*	0.243*	-0.505**	-0.018	-0.013	-0.016
		d	-0.046	0.262*	-0.627	0.014	-0.032	-0.008
		Action	A	A	A			
8.06	mmc0181	a	-0.064***	-0.046***	-0.948***	-0.015	-0.033	-0.023
		d	-0.008	-0.017	-0.182	0.014	-0.007	0.002
		Action	A	A	A			

[a]Sev_{53}, Sev_{63}, Sev_{55}, Sev_{62}, = severity assessed 53, 62 days after inoculation for GLS in first season and 55, 62 days during the second, [b]SAUDPC = standardized area under the disease progress curve, [c]D = dominant gene action, A = additive gene action

Disease	Interval (Chrom.)		Max. severity[a]	Overall severity[a]
NCLB	bin 3.06	HH_c[b]	25.4A	18.9A
		HO	28.9A	19.9A
		OO_v	26.1A	19.3A
	bin 5.04	HH_c	24.8A	18.6A
		HO	26.7BA	19.4BA
		OO_v	29.5B	20.4B
	bin 8.06	HH_c	31.3A	20.8A
		HO	26.0B	19.0B
		OO_v	25.9B	19.4B
MSV	bin 1.04	HH_c	4.0A	-
		HO	4.2A	-
		OO_v	4.4B	-
GLS	bin 2.09	HH_c	34.8A	29.9A
		HO	36.7A	30.1A
		OO_v	35.8A	30.9A

Continued

Table 2.7. Mean disease severity for 410 $F_{2:3}$ progenies inoculated with *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak based on marker interval analysis for major QTL bin positions derived from CML202 and VP31.

Table 2.7 continued

bin 4.08	HH_c	37.7A	33.2A
	HO	36.4A	30.4A
	OO_v	31.7B	26.7B

[a]Means followed by the same letter are not significantly different at P = 0.05

[b]HH_c = homozygous for the CML202 allele; HO = heterozygous and OO_v = homozygous for VP31 allele

CHAPTER 3

COMPARISON OF GENETIC GAIN FOLLOWING PHENOTYPE-, MARKER-BASED, AND COMBINED SELECTION FOR IMPROVED RESISTANCE TO MULTIPLE FOLIAR PATHOGENS OF MAIZE

Abstract

Foliar diseases are important biotic constraints limiting maize production globally. Northern corn leaf blight (NCLB) incited by *Exserohilum turcicum*, gray leaf spot (GLS) incited by *Cercospora zeae-maydis* and maize streak incited by maize streak virus (MSV), are among the most destructive. Most foliar diseases of maize are managed by means of quantitative partial resistance. We used flanking markers at consensus regions for QTL conditioning partial resistance to these diseases to determine their effectiveness in improving host-resistance. Our objective was to examine the effectiveness of different selection strategies for the purpose of pyramiding resistance loci to these diseases. Genetic gains were calculated for simultaneous improvement of partial resistance following phenotype-based, marker-based, combined phenotype- and marker-based selection (MAS index), and random selection. Field evaluations and subsequent selections were conducted independently for each disease in a population of 410

$F_{2:3}$ lines derived from hybridization between inbred line CML202 with known resistance to NCLB and MSV, and VP31 a breeding line with known resistance to GLS. Maize streak evaluations were conducted in Zimbabwe, GLS tests were performed in Ohio, and NCLB evaluations were conducted in Uganda and Ohio. Our results showed that actual genetic gains varied with the particular disease and selection treatment employed. Combining phenotypic and marker information expressed as MAS index produced the highest gains for all diseases. The MAS index reduced the mean disease ratings by 9.0% for GLS, 5.7% for NCLB and 0.6 (1-5 scale) for MSV at late season epiphytotic from the overall mean of each disease in the $F_{2:4}$ generation. In comparison to phenotypic selection, the genetic gains from genotypic selection were highest for MSV followed by GLS and then NCLB. Cumulative genetic gains for improved resistance were practically the same for both phenotypic and genotypic selection. In all cases, gains from marker-based selection represented a significant improvement over random selection that ignored QTL information. The values of predicted genetic gains were higher than actual realized gain, but the relative values for the different selection procedures were consistent with the trend for actual realized genetic gains. Estimates of costs based on lower boundary values indicated the cost of marker-based selection was lower than that of phenotypic selection. Our results indicate that markers linked to major resistance loci can facilitate pyramiding resistance to multiple diseases during early generation selection.

INTRODUCTION

A number of foliar diseases limit maize (*Zea mays* L.) productivity worldwide. Several of the diseases are important in broad areas and others are more specific in their occurrence (Castor, 1992; Pratt and Gordon, 2006). The prevalence of these diseases is influenced by several interactive factors including the extent to which susceptible hosts are grown, the presence of pathogens, insect vectors and environmental conditions. Gray leaf spot (*Cercospora zeae-maydis*) and northern corn leaf blight (*Exserohilum turcicum*) are cosmopolitan diseases that have become established in many maize growing regions. Conservation tillage favors the survival of these fungal pathogens because conidia of both overseason on crop debris (Payne et al., 1987; de Nazareno et al., 1993; Adipala et al., 1994; Asea et al., 2002). In addition, MSV is one of the most destructive diseases of maize throughout sub-Saharan Africa (Thottappilly et al., 1993; Bosque-Perez et al., 1998). These diseases may be considered among the most damaging, and epidemics recur due to favorable weather conditions, planting of susceptible cultivars and continuous maize cropping (Carson et al., 2002).

Genetic studies concerning resistance to pathogens causing foliar diseases of maize have shown resistance is often quantitatively inherited, in some instances involving only a few major loci. Several studies have independently identified quantitative trait loci (QTL) conditioning resistance to these diseases in different

maize inbreds (Bubeck et at., 1993; Freymark et al., 1993; Saghai Maroof et al., 1996; Kyetere et al., 1999; Pernet et al., 1999; Welz et l., 1999; Clements et al., 2000; Gordon et al., 2004). Some of the QTL regions with major to moderate influence are common across different populations (Asea et al., this volume). For instance, one major QTL explaining half (or more) of the phenotypic variation was detected for resistance to maize streak in four diverse sources of resistance. Similarly, three QTL regions conditioning resistance to NCLB during the adult plant stage, and two for GLS, are common in different sources of resistance. A summary of consistent QTL regions from different studies is shown in Figure 1.1.

Quantitative traits are reported to have the most potential for marker-assisted selection (MAS) because phenotypic selection of traits with relatively low heritability is costly and often ineffective, due in part to confounding environmental effects. For the same reason, markers associated with QTL have been difficult to find and, once identified, have exhibited limited usefulness across a range of genetic backgrounds and environments. Actual use of marker-trait associations for improving quantitative traits is seldom documented, but several simulation studies have suggested a greater potential for their applications (Lande and Thompson, 1990; Dudley, 1993; Hospital and Charcosset, 1997; Moreau et al., 1998). Consequently, MAS has been advocated as a method of selection to improve quantitative traits. Dreher et al. (2003) also demonstrated that MAS offers a cost-effective alternative to phenotypic selection to detect a particular allele at a target locus (*o2*, the opaque endosperm 2 locus

associated with Quality Protein Maize) when phenotypic and MAS were considered as direct alternatives. An economic analysis of the Dreher et al. (2003) study that involved more theoretical breeding schemes determined that MAS was faster, but more costly than phenotypic selection (Morris et al. 2003). In addition to differences in efficiencies, there may also be differences in relative costs depending on the trait.

Despite the promising results from these theoretical studies, few documented experiments have been conducted for actual improvement of traits with MAS. Castro et al. (2003) combined three resistance alleles for stripe rust resistance QTL in barley and validated their effects in reducing disease severity. They demonstrated that pyramiding resistance alleles from more than one QTL substantially increased the probability of recovering the resistant phenotype. MAS also may have advantages over phenotypic selection in achieving improved host resistance because genes that are “masked” can be identified and recovered. Marker-based selection is also likely to confer an advantage when phenotypic screening is particularly expensive or ineffective due to inadequate disease development.

Improved levels of selection efficiency have been demonstrated for race-specific resistance in *Phaseolus* beans (Miklas et al., 2000) and for bacterial blight resistance in rice using markers linked to major genes (Sanchez et al., 2000). However, most breeders rely mainly on quantitative resistance for managing

maize diseases and they focus mostly on selection of resistant breeding lines from inoculated field trials (Carson et al., 2004). Pyramiding multiple alleles for quantitative resistance may increase the likelihood of improving host resistance and enhancing durability. The challenges in pyramiding quantitative resistance include: (1) the need for multiple genes to confer resistance, (2) the influence of environmental conditions on expression of resistance, (3) possible interactions among resistance genes, (4) the need to screen large populations to identify multiple disease resistant recombinants, and (5) the logistics of screening plants for resistance to three different diseases. These factors affect the accuracy of selection and thus genetic gain for improved quantitative resistance. Recently, Servin et al. (2004) have suggested an optimal strategy for pyramiding favorable alleles by combining into a single genotype a series of target genes identified in different parents. They proposed an optimal succession of crosses over several pedigree generations until a desired genotype is obtained. This systematic approach may also help to address some of the challenges related to combining multiple resistance genes.

An investigation for utility of molecular-markers linked to QTL controlling complex partial resistance systems in maize is useful for pyramiding resistance factors for multiple diseases. In tomato, Robert et al. (2001) have used markers linked to QTL for improving quantitative resistance to blackmold (*Alternaria alternata*). In their study, they selected five QTL conditioning resistance for introgression into cultivated tomato and found that lines carrying alleles at the QTL were

associated with increased genetic response for resistance. Similar studies by Bouchez et al. (2002) have used marker-assisted introgression of favorable alleles at three QTL for earliness and yield improvement in maize and found that the QTL effects were in agreement with those from earlier mapping studies. Flint-Garcia et al. (2003) demonstrated that MAS was a potentially valuable selection tool compared with phenotypic selection for stalk strength (rind penetrometer) and European corn borer (*Ostrinia nubilalis*) resistance. They showed that QTL in several populations were effective in improving both traits related to stalk lodging. In all the populations they studied, MAS was effective in selecting for both resistance and susceptibility. These studies have demonstrated the value of MAS for increasing the genetic response for traits with low heritability and its usefulness for manipulation of resistance conditioned by several QTL.

We demonstrated that some of the candidate QTL previously identified for resistance to maize foliar pathogens were significantly associated with resistance in a breeding population (Asea et al., this volume) and could be used for MAS to improve host resistance. MAS should further validate QTL effects if selection based on target QTL effectively changes the mean trait value when compared to non-selected controls (Flint-Garcia et al., 2003; Romagosa et al., 1999). In this study, we wished to compare the effectiveness of MAS for target QTL with other selection procedures as an approach for improving resistance to multiple pathogens. Our specific objectives were to: (i) examine the effectiveness of combining target QTL to three maize pathogens using phenotype-, marker-based

and combined phenotype- plus marker-based selection procedures (ii) compare genetic gains and (iii) examine the associated costs for the different procedures.

Materials and Methods

Plant materials. The partially inbred lines developed for this study were derived from a cross between CML202 and an $F_{2:4}$ line (VP31) with known resistance to GLS (Gordon et al., 2004). CML202 has a relatively high level of partial resistance to *E. turcicum* and MSV as confirmed in previous genetic studies (Welz et al., 1988; Schechert et al., 1999). The inbred is also widely used in many tropical breeding programs for production of hybrids and new inbred lines due to its excellent combining ability for disease resistance and yield (Schechert et al., 1999). CML202 has white, semi-dent kernels and it was developed by the Centro Internacional de Majoramiento de Maiz y Trigo (CIMMYT; International Maize and Wheat Improvement Center) with tropical materials originating in West Africa (Welz et al., 1988). It is late maturing and generally well adapted to growing conditions in the humid mid-altitude zones of eastern and southern Africa. The GLS resistance source VP31 was derived from a resistant line selected from a cross between single plants of South African inbred VO613Y and Corn Belt inbred Pa405, that are resistant and susceptible to infection by *C. zeae-maydis*, respectively (Gordon et al. 2004). VP31 is yellow-grained with dent kernels and matures considerably earlier than CML202. Crosses were made between inbred CML202 and VP31 using standard pollination techniques. The F_1 plants from this cross were self-pollinated and F_2 plants were grown and self-

pollinated in winter nurseries in Puerto Rico, Hawaii and at Wooster, Ohio (greenhouse) to generate F_2 derived F_3 ($F_{2:3}$) lines. This process produced 410 $F_{2:3}$ family lines that were subsequently used in this study. All $F_{2:3}$ family lines and selected $F_{2:4}$ family lines were evaluated in field trials conducted at Wooster, Ohio (GLS and NCLB); Namulonge, Uganda (NCLB); and the CIMMYT Mt. Pleasant Experimental Station in Zimbabwe (maize streak). Field plots were established and inoculations and disease assessments were performed, using the same methods previously described (Asea et al., this volume).

Early generation selection for resistance to infection by multiple pathogens. The $F_{2:3}$ families were evaluated for resistance to infection by three foliar pathogens during the 2003 growing season in Wooster, Ohio; Namulonge, Uganda; and at Mount Pleasant, Zimbabwe. The strategy employed was to prioritize each disease trial in one location, where that disease was endemic, to facilitate better disease development, disease assessment and separation of the families based on their reactions. After evaluation, the $F_{2:3}$ progenies were selected independently for resistance to infection by each foliar pathogen based on standard deviation with the following number of families advanced to the $F_{2:4}$: 38 for GLS, 37 for maize streak and 38 for NCLB. This selection corresponded to selection intensity of approximately 10% for each disease at a truncation point of 1.0 standard deviation unit from the mean for GLS and NCLB and 2.0 standard deviation units for maize streak. Also, 38 families with desired resistant parental alleles at each locus were selected for each trait using flanking markers.

Concomitantly, an equal number of lines were randomly selected to produce an unselected control population using the random number generation procedure. Some of the randomly selected families overlapped with those previously selected for other traits resulting in only 26 random selections that did not overlap.

To help eliminate negative agronomic attributes, mild culling (10%) for late maturity, stalk lodging, and plant height was employed before selection for host resistance. This was intended to emulate simultaneous progress that would be made while developing a resistant population with acceptable host resistance and agronomic traits in a pedigree breeding program. This process resulted in a total of 228 $F_{2:4}$ selected families that were grown and inoculated together with the randomly selected progenies. Subsequently, treatments for comparisons were 1) phenotype-based selection, 2) marker-based selection, 3) combined phenotype- and marker-based selection (MAS index) and, 4) random selection. We used the term “MAS” for reference to a selection scheme using a combination of both phenotypic value and QTL information, in essence augmenting phenotypic data with genotypic data. Marker-assisted selection assumed that some of the QTL for a given trait were not known and the contribution of minor QTL would also be captured. The term “marker-based selection” was used interchangeably with genotypic selection.

Genotypic analysis. Genomic DNA was extracted from the family lines based on the method described by Saghai-Maroof et al. (1984). Three-tenths g of lyophilized ground leaf tissue was used per 8 ml CTAB (Hexadecyltri-Methylammonium Bromide) extraction buffer (100 mM Tris pH 7.5, 0.7M Nacl, 1% CTAB, 10 mM NaEDTA and 1% B-mercaptoethanol). The mixture was incubated at 65^{o}C for 60 min with occasional mixing to denature proteins. 4.5 ml of 24:1 chloroform:octanol (v/v) was added and the solution was mixed by inversion to form an emulsion. This emulsion was centrifuged for 10 min at 1800 g in a tabletop centrifuge (Beckman model TJ-6 centrifuge) at room temperature. The supernatant was removed and 6 ml of cold isopropanol added and mixed gently by tube inversions to precipitate DNA. The precipitated DNA was lifted out with a glass hook, transferred to 1.5 ml Eppendorf tubes containing 1 ml of wash solution (76% ethanol and 0.2 M NaOAC) for 20 min, and then dipped in a rinse solution (76% ethanol and 10 mM NH_4Oac) for 5 s. DNA of each sample was dissolved in 400 ml TE (10 mM Tris, pH 8, and 1 mM EDTA) overnight in 4^{o}C cooling room on a rocker. The solution was centrifuged for 10 min at 16000 g in a tabletop Eppendorf centrifuge model 5415C and the supernatant was transferred to a clean-labeled 1.5 ml Eppendorf tube. DNA was drawn from these tubes and diluted to 1:5 in water and stored in a refrigerator (4^{o}C) for further use. The primer sequences used in this study were obtained from the maize genetics and genomic database (www.maizegdb.org) and purchased from Research Genetics (Huntsville, AL). PCR methods were performed as described by Davis et al.

(1999). Electrophoresis was performed on agarose gels (Ameresco, Solon, OH) with concentrations ranging from 3.5 to 4.5%.

Marker analysis. Selection of desired resistant parental alleles at the QTL positions were performed using flanking molecular markers to delimit approximately 5-20 cM around each QTL. The net molecular score was calculated by summing across marker loci the product of the coded marker genotype (0, 0.5 and 1) as described for marker-based selections (Lande and Thompson, 1990; Eathington et al., 1997; Moreau et al., 2000). A value of 0 was given to a locus lacking a resistance allele, 0.5 for heterozygous and 1 for homozygous resistant loci. The selection index used for ranking the families incorporated the information from both the phenotypic and genotypic data through a combined score of the form $I_i = b_z Z_i + b_m M_i$, as described by Eathington et al. (1997), where I_i is the index value for the ith family, Z_i is the phenotypic mean, b_z is the weight given to the phenotypic mean, M_i is the net molecular score. In each index, b_z was set to one, while b_m was calculated as $[(1/h^2) - 1]/(1-p)$, where p is the proportion of additive genetic variance explained by the marker (Lande and Thompson, 1990). Because additive genetic variance explained by each marker was less than 30%, broad-sense heritability estimates were moderately high, and each candidate locus was considered important, the value of b_m was approximately the same for all the loci and was consequently set to one. The sums of selection indices across each QTL for families were ranked

and used to select the highest families equivalent to that selected previously using the phenotype-based selection method.

Statistical analysis. Statistical analyses were performed for each trait independently. Genetic variance and error variance were estimated using the PROC GLM procedure of SAS (SAS, 2003). To obtain the performance of the genotypic value of each family, analyses of variance were conducted on a family-mean basis and total variation was portioned into the effects of replication, family genotypes and errors for each disease as: $Y_{ij} = \mu + B_i + F_j + \varepsilon_{ij}$, where μ = overall mean, B_i = effect of block, F_j = family effect, ε_{ij},= experimental error.

Associations between individual marker loci and disease severity were tested with single-factor analysis of variance using the SAS PROC GLM procedure, with a threshold significance level of P = 0.05. For genotypic analysis, ten $F_{2:3}$ individuals per family and five $F_{2:4}$ individuals per family were sampled. For tests of marker-trait association within the population, the statistical model used was $X_{ijk} = \mu + B_i + M_j + G_k(M_j) + \varepsilon_{ijk}$, where X_{ijk} is the trait value for the k^{th} genotype of the j^{th} marker class in the i^{th} block, μ is the population mean, B_i is the effect of the i^{th} block, M_j is the effect of the jth marker class, and ε_{ijk} is the experimental error. The appropriate F test for declaring marker significance was equal to the mean square for marker classes/mean square for genotype within marker.

The realized genetic gain (R) was calculated as the difference between the mean of selections (k) and the grand mean of all (n) genotypes: $R = (\Sigma_k Y)/k - (\Sigma Y)/n$,

where Σ_k = summation over the k selections, Σ = summation over all genotypes and n = genotypes tested in both seasons before and after selections. The standard error of R was calculated as: $[(n-k)/nk]^{1/2}$(SEM), where SEM is the standard error of the mean for an individual genotype in the test of $F_{2:4}$ and n-k was equal to the number of genotypes that were not selected. Predicted gains under selection were calculated as described by Allard (1964) as $G_s = (k)(\sigma_A)(\sigma_a^2)/(\sigma_A^2)$ where k = selection differential, σ_A = phenotypic standard deviation of the trait mean, σ_a^2= genetic component arising from genetic differences among families and σ_A^2 = total phenotypic variance. The relative efficiency of marker-based selection over phenotypic selection was calculated as $RE_{MBS:PS} = \frac{\sqrt{V_M / V_A}}{h}$, where V_M is marker variance due to marker scores, V_A is additive genetic variance and was calculated using narrow-sense heritability based on parent-offspring regression, h is the square root of heritability. The efficiency of marker-assisted selection over phenotypic selection was calculated as $RE_{MAS:PS} = \sqrt{\frac{V_M / V_A}{h^2} + \frac{(1 - V_M / V_A)^2}{1 - h^2 (V_M / V_A)}}$, where h^2 is the heritability of the trait (Bernardo, 2002).

Results

Natural conditions were generally favorable for development of GLS in Ohio and NCLB in Uganda and Ohio nurseries during both seasons of evaluation. Susceptible check inbreds were significantly damaged by fungal infections (greater than 70% PLAA) and resistant check inbreds had low disease severities (<10%). There was a wide variation in the reaction of families tested. Such wide ranges of reactions are reported to be typical of partial resistance. Quantitative resistance may result in symptoms ranging from small fleck lesions to highly expressed necrotic lesions on some progenies, as was observed for both GLS and NCLB. Controlled artificial inoculation using viruliferous leafhoppers at the CIMMYT experimental station in Zimbabwe resulted in infection of all progeny lines. Infections resulted in differential reaction of the family lines ranging from resistant to susceptible classes. Evaluations of $F_{2:3}$ progeny lines resulted in a population mean of 36% PLAA (0-100%) (11.8 standard deviation units) for GLS, 32% PLAA (0-100%) (16.3 standard deviation units) for NCLB and 4.0 (0-5 scale) (0.8 standard deviation units) for maize streak that were subsequently selected for further evaluations.

Genetic gains under selection

Due to large population size and a wide range of reactions to pathogens causing each of these diseases, there was marked differences in the family lines representing each selection method. Nevertheless, twenty families were commonly identified by more than one selection procedure and only six families

were commonly selected for resistance across three diseases at 10% selection intensity. There were differences in the amount of gain realized for disease ratings at mid, final, late season and overall epiphytotic assessments during selection. Overall, the amounts of genetic gains were higher at late season disease severity presumably due to maximum differences in the reactions of progenies associated with this assessment time (Table 3.1). Comparatively higher genetic gains were realized for GLS than for NCLB and maize streak for all disease rating times.

Table 3.1 shows a comparison of actual realized genetic gains calculated for the four types of selection procedures used in this study. Actual realized genetic gain varied with the selection treatments employed, indicating differences in effectiveness of selection treatments. For the three diseases studied, the highest genetic gains were realized from combining phenotypic and marker information expressed as MAS index. The MAS index reduced the mean disease ratings by 9.1% for GLS, 5.6% for NCLB and 0.6 (1-5 scale) for maize streak using epiphytotic assessments from the overall mean of each disease evaluated in the $F_{2:4}$ generation. The reduction in mean disease ratings were marginally higher when MAS index was adjusted by using only statistically significant markers at each locus for the candidate QTL.

For GLS, genotypic selection resulted in lower disease ratings of 3.7 and 5.0% PLAA from $F_{2:3}$ to $F_{2:4}$ generation at mid and maximum disease assessment

times, respectively, compared to phenotypic (3.4 and 5.0%) and random selection (-0.8 and -0.2%). Overall, marginally higher gains were realized for genotypic selection over phenotypic selection, although differences were not significant (P>0.05) (Table 3.1). Gains from using both methods (MAS) represented a significant improvement over random selection. Similar results were obtained for maize streak where slightly higher genetic gains were realized for genotypic selection over phenotypic selection. For NCLB resistance, results from using different selection treatments indicated that phenotypic selection was a better method of selecting for resistance and resulted in a population with lower mean disease ratings compared to either genotypic or random selection. The gains were increased in all cases when phenotypic and genotypic selections were combined (MAS index). In most cases, random selection (simulating no selection) resulted in higher disease ratings compared to the overall mean of the $F_{2:4}$ progenies and consequently was, as anticipated, not effective for improving disease resistance.

Predicted genetic gains calculated using both narrow-sense and broad-sense heritabilities for the three disease resistance traits were greater than actual realized gains for all ratings. Predicted genetic gains were similar at late season epiphytotic and overall disease ratings for both GLS and NCLB and ranged from 6.8 to 8.2% PLAA for GLS and 6.5 to 8.4% PLAA for NCLB (Table 3.2). Variance component estimates used to calculate predicted genetic gains are shown in table 3.2. The three disease resistance traits varied in broad-sense heritability:

0.77 for GLS, 0.69 for NCLB and 0.42 for maize streak. NCLB, with the highest heritability, had a predicated gain from phenotypic selection that was correspondingly higher relative to other selection methods.

Calculation of relative efficiencies of selection for the three disease traits indicated that efficiencies for marker-based selection were generally less compared with phenotypic selection (Table 3.3). The efficiencies for marker-based selection were practically equal to that for phenotypic selection for maize streak and GLS at both assessment dates. The lower values reflect correspondingly lower variance explained by the markers and moderately high heritability for the disease traits. These results indicate that marker-based selection will be particularly effective in situations where there is poor disease development or selections must be made in off-season nurseries where disease may be absent, and consequently the heritability during selection is zero or very low. In all cases, the efficiencies of MAS were greater than for phenotypic selection.

Cost comparison of selection schemes

A comparison was also made between phenotypic and genotypic selection schemes relative to their costs. The economic analysis was based on a spreadsheet budget similar to one used by Dreher et al. (2003) that provided a basis for comparing costs of different selection schemes for disease resistance. Additional costs that were added to estimate the cost of selection for disease

resistance included the cost of inoculum preparation and performing inoculations, and the cost of insect rearing and infestations to illicit maize streak. During the 2003/04 season, field costs totaled $5,300 per acre at Ohio, USA. This value was only for one disease trait and represented lower boundary costs because it was mainly based on overhead costs. Our estimates indicated that costs of genotyping were lower than phenotypic selection for resistance even when all the traits were selected simultaneously. In addition, the cost of genotyping is more likely to benefit from economies of scale when sample size increases.

In disease trials, time commitment during inoculations and disease ratings, in addition to other agronomic field requirements, added to labor costs. These factors together increased field costs for conventional selection relative to genotyping costs for the same traits. These costs are likely to vary in different programs (primarily due to differences in labor costs), but the results indicated that genotyping was relatively cheaper compared with selection under field conditions for three disease traits.

We used only markers associated with previously validated QTL regions and the cost of QTL mapping was not considered as a factor in cost comparison. It was evident that the efficiency of genotypic selection compared to that of phenotyping was higher based on cost-effectiveness and the time required to obtain data. Not only was genotyping cost effective, but it also resulted in higher genetic gains for the population during one cycle of selection for resistance to GLS and NCLB.

Maximizing genetic gain under the assumption of unlimited resources to invest in selection for resistance would be obtained using data from MAS.

Discussion

The results of this study indicated the effectiveness of MAS as an approach for improving resistance to multiple foliar pathogens of maize. Selections based on target QTL that were consistent across populations were effective in improving the level of host-resistance. The magnitude of the extra genetic gains obtained from selections in response to infections varied among the three diseases studied. In comparison to total progeny mean and random selections, genetic gains using target QTL were higher for resistance to all three pathogens. These gains are likely to be maintained in subsequent generations because the target QTL for selection were significantly associated with resistance across generations. The marginally significant QTL in $F_{2:3}$ were highly significant in the $F_{2:4}$ generation, indicating the effectiveness of the QTL in discriminating the progenies into resistance classes following selection. The largest increase in resistance to the pathogens was obtained from the MAS index. The greatest gain from QTL information in genotypic selection was achieved for GLS and MSV, consistent with the oligogenic nature of inheritance. Genetic contributions for improved host resistance were maximized using information from both phenotypic and genotypic selection procedures. These results support the speculation of Lande and Thompson (1990) that MAS is expected to be more effective when the breeding value of an individual or a line is predicted by an

index determined by both the marker score and the phenotypic value of a trait. We have also demonstrated the effectiveness of major QTL that confer resistance to maize foliar pathogens for improving host resistance as was postulated by Welz and Geiger (2000).

Improved host resistance is usually the primary means for managing damage and preventing losses caused by maize diseases (Ward et al., 1999; Carson et al., 2004). For NCLB, several major genes (*Ht, Ht2, Ht3, Htm1* and *Htn1*) have been identified that confer race-specific resistance to *E. turcicum* (Leonard, 1993; Welz and Geiger, 2000). These sources are not as widely used as quantitative sources of resistance for managing NCLB (Moon et al., 1999). Qualitative resistances are usually ephemeral because new races of *E. turcicum* may overcome them (Leonard, 1993; Lipps et al., 1997). The challenge to using quantitative resistance is that it requires extensive and accurate field-testing, making it also difficult to select. Our findings have shown MAS should enhance introgression of resistance factors into commonly grown susceptible cultivars. In addition to pyramiding several race-specific genes, combining the effects of race-specific genes with QTL that confer partial resistance, and combining marker-based strategies with traditional phenotype-based approaches, should enhance durability.

Interactions between the effect of genotypes and pathogen isolates are often responsible for causing variations in the level of host resistance in genotypes

evaluated across seasons or locations (Carson et al., 2002; Rodier et al., 1995). In this study, we observed significant variations in the performance of the progenies across the two seasons in response to infection by MSV. During NCLB evaluation, it was also noticed that certain families appeared to have high resistance equivalent to the resistant checks, in trials conducted in Ohio, but these were severely blighted when tested in Uganda. In contrast to performance of progenies in response to infection by *E. turcicum* and MSV, reaction of families was similar across seasons following infection by *C. zeae-maydis*. Unlike *E. turcicum*, no races have been determined for *C. zeae-maydis* that can cause significant variation in reaction of inbreds across locations or years. Wang et al. (1998) have identified two sibling types of the pathogen named as type I and II that vary in their aggressiveness. Type II is reported to be the most prevalent isolate in both the US (Dunkle and Levy, 2000; Carson et al., 2002) and Africa (Dunkle and Levy, 2000; Okori et al., 2003). This indicates that an approach to maximize gain from selection is to evaluate progenies in high disease pressure environments and use highly aggressive isolates of the pathogen to obtain highly resistant lines that remain consistent in progeny rankings across locations and seasons.

Predicted and actual realized genetic gains calculated for this study were for only one cycle of selection. Pooling estimates from successive cycles of selection would probably provide higher estimates of genetic gains. These gains would likely be higher when introgressing resistance to susceptible varieties. We also

observed that despite variations in reactions of some inbreds to MSV, parental inbred VP31 was moderately resistant across years. The line VP31 was derived from a South African line, VO613Y, and it is probable that it also contributed resistance to the population. It is not known if that resistance is allelic or different from *msv1* in CML202. The predicted genetic gains for the methods compared in this study may not have been realized, but the relative values for the different methods were consistent with the trend for actual realized genetic gains and thus, provide a useful estimate of the relative effectiveness of the different selection procedures. Results obtained also demonstrated statistical and practical equivalence of phenotypic and genotypic selection for resistance to the three pathogens. Both selection methods produced family lines that were significantly more resistant than both parents and equaled the resistance of the most resistant check inbred. This was in contrast to random selection (control) in which the performance of the $F_{2:4}$ family lines was not different from the base $F_{2:3}$ population.

Our results confirm the effects and location of the candidate QTL controlling resistance to each disease with the exception of the resistance QTL for NCLB on chromosome 8. The greater genetic response due the QTL effects following genotypic selection also indicates the stability of the resistance factors as a consequence of selection. In practical sense, while conventional phenotypic selection is often the most common criteria for improving host resistance, MAS may be preferable to achieve greater response. In situations where a particular

disease is not yet present but introduction is likely, marker-based selections may offer the opportunity to deploy resistance in order to safeguard local germplasm against future epiphytotics. In our case, comparison of costs indicated genotype-based selection was relatively cheaper compared with selection under field conditions for three disease traits. The cost estimates were simplifications based only on marginal costs but represented realistic estimates during evaluation and selection for disease resistance. The cost-effectiveness and time efficiency obtained using a genotype-based selection scheme compared to phenotype-based selection agrees with reports on other traits comparing the cost of the two selection schemes (Dreher et al., 2003; Moreau et al., 2000).

As part of managing damage caused by maize diseases, it is important to consider a wide complex of pathogens because several are important in broad areas and they may occur simultaneously (Pratt and Gordon, 2006). There are several inbred lines that are singly resistant to each of these pathogens but their usefulness for broad adaptation is limited because of the potential impact of these diseases. Developing multiple disease resistant inbreds and hybrids is an important strategy to limit the impact of these diseases. Previously, we determined that some of the candidate QTL that confer partial resistance were significantly associated with resistance across seasons and generations (Asea et al., unpublished). In this study we demonstrated that selection for candidate QTL associated with resistance substantially increased the level of host resistance. Experience obtained in this study also indicates that markers linked

to major resistance loci can facilitate pyramiding of resistant alleles for different diseases by selecting desirable recombination events in addition to bringing unlinked genes together.

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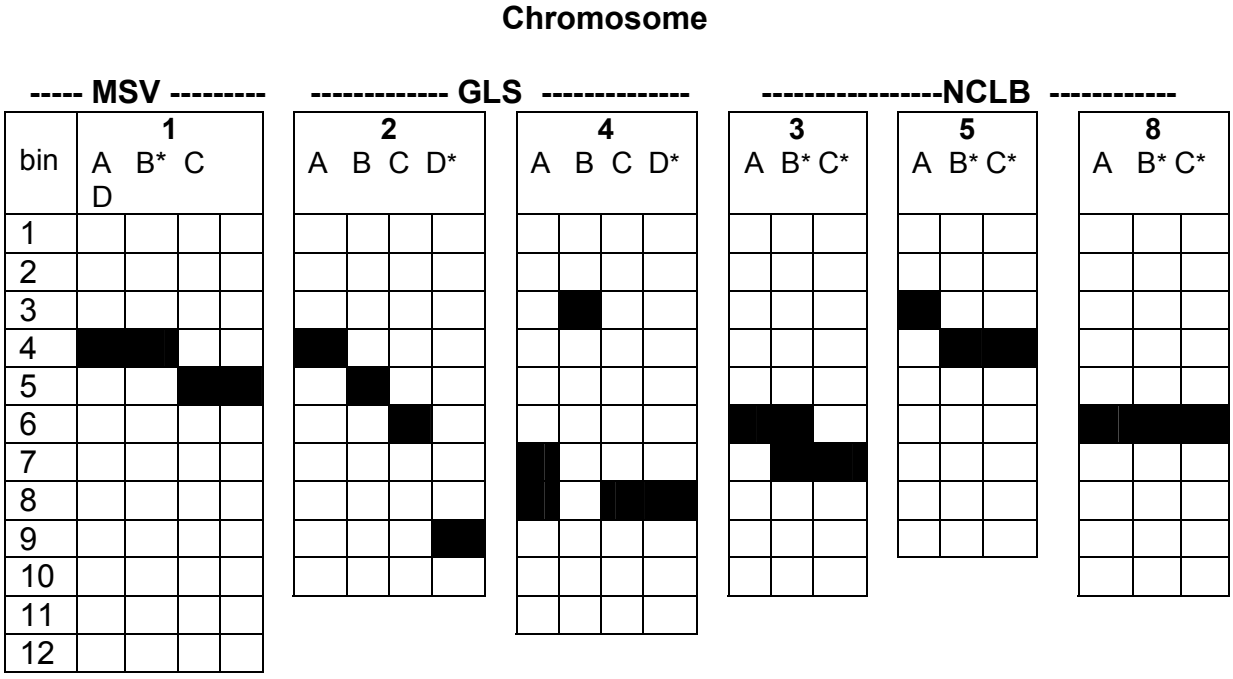

*Study utilized resistant parent in this study. Where consensus QTL did not exist, resistant parent QTL was used.

Figure 3.1. Chromosomal bin positions of the major consensus QTL regions for resistance to maize streak virus (MSV), gray leaf spot (GLS) and northern corn leaf blight (NCLB) in different mapping populations (A, B, C, D). MSV: Kyetere et al., 1999 (A), Welz et al., 1998 (B) Pernet et al., 1999a (C) Pernet et al., 1999b (D), GLS: Bubeck et al., 1993 (A), Clements et al., 2000 (B), Saghai Maroof et al., 1996 (C) and Gordon et al., 2004 (D), NCLB: Freymark et al., 1993 (A), Schechert et al., 1999 (B) and Welz et al., 1999 (C).

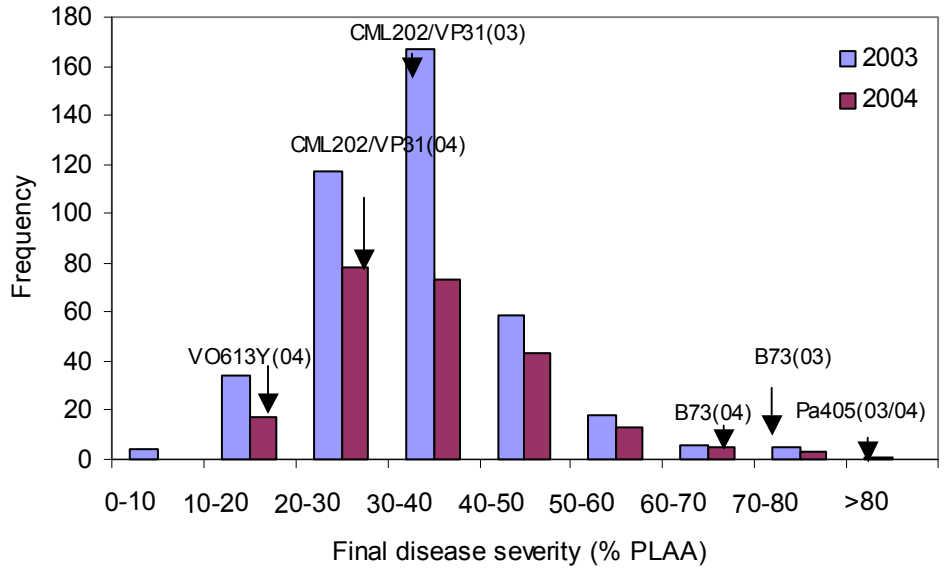

Figure 3.2. Frequency distribution of final disease severity ratings of $F_{2:3}$ and $F_{2:4}$ progenies from CML202 x VP31 cross evaluated for gray leaf spot near Wooster, Ohio during two seasons, respectively.

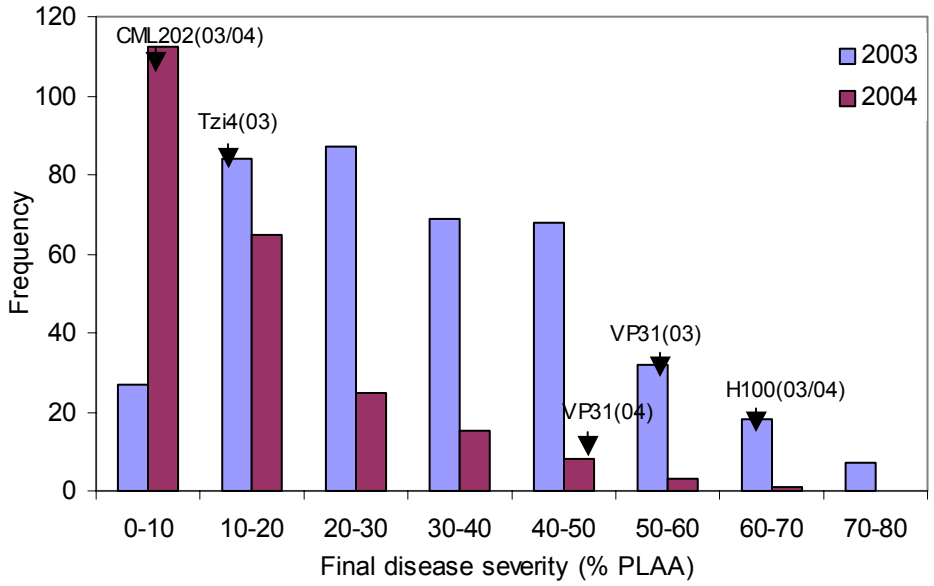

Figure 3.3. Frequency distribution of final disease severity ratings of $F_{2:3}$ and $F_{2:4}$ progenies from CML202 x VP31 cross evaluated for northern corn leaf blight near Namulonge, Uganda (2003) and Wooster, Ohio (2004), respectively.

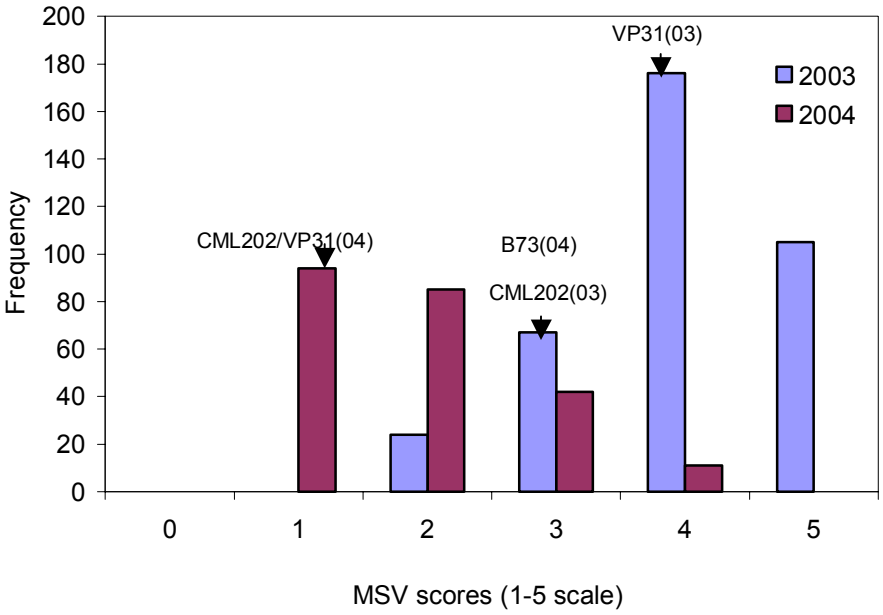

Figure 3.4. Frequency distribution of final disease severity rating of $F_{2:3}$ and $F_{2:4}$ from CML202 x VP31 cross evaluated for maize streak virus at CIMMYT, Zimbabwe (2003 and 2004), respectively.

	Disease ratings			Actual realized genetic gain		
Selection Treatment	Mid epiphytotic	Late epiphytotic	SAUDPC[a]	Mid epiphytotic	Late epiphytotic	SAUDPC
MSV (1-5 scale)				-------- top 10%----------		
Phenotype	1.9±0.2	2.0±0.2	-	-0.3±0.03[b]	-0.4±0.03	-
Genotype	1.7±0.1	1.9±0.1	-	-0.5±0.02	-0.5±0.02	-
Random	2.1±0.1	2.3±0.1	-	-0.1±0.01	-0.1±0.01	-
MAS index	1.7±0.1	1.8±0.2	-	-0.5±0.02	-0.6±0.03	-
$F_{2:4}$ mean	2.2±0.1	2.4±0.1	-			
GLS (0-100% PLAA)						
Phenotype	23.6±1.1	30.5±1.6	23.8±1.2	-3.4±0.2	-5.0±0.2	-3.5±0.2
Genotype	23.3±1.4	30.5±2.1	23.3±1.4	-3.7±0.2	-5.0±0.3	-4.0±0.2
Random	27.8±0.8	35.7±1.1	28.1±0.9	0.8±0.1	0.2±0.1	0.8±0.1
MAS index	21.1±1.1	26.4±1.2	21.0±1.1	-5.9±0.2	-9.1±0.8	-6.3±0.2
MAS (Adjusted)	19.2±1.1	24.2±1.0	19.2±1.0	-7.8±0.2	-11.3±0.1	-8.1±0.1
$F_{2:4}$ mean	27.0±0.5	35.5±0.8	27.3±0.6			

Continued

Table 3.1. Genetic means of $F_{2:4}$ families evaluated at mid and maximum epiphytotic and associated realized genetic gains for different selection procedures for resistance to *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus.

Table 3.1 continued

NCLB (0-100% PLAA)

Phenotype	7.8±1.1	10.3±1.5	6.6±1.0	-2.6±0.2	-3.8±0.2	-2.8±0.2
Genotype	9.4±1.5	11.8±1.6	7.9±1.1	-1.0±0.2	-2.3±0.2	-1.5±0.2
Random	9.7±0.9	13.8±1.3	9.2±0.9	-0.7±0.1	-0.3±0.1	-0.2±0.1
MAS index	8.4±1.4	8.5±1.3	5.4±0.9	-2.0±0.2	-5.6±0.2	-4.0±0.1
MAS (Adjusted)[C]	4.1±1.2	6.1±1.5	3.9±1.1	-6.3±0.2	-4.6±0.2	-5.5±0.2
$F_{2:4}$ mean	10.4±0.6	14.1±0.8	9.4±0.6			

[a]SAUDPC = standardized area under the disease progress curve

[b]Negative realized gains indicate gain in resistance and positive gains indicate loss in resistance compared to overall mean of the population

[c]MAS (adjusted) = MAS for GLS and NCLB calculated using only markers significantly associated with resistance

	GLS			NCLB			MSV
	Sev_{53}[a]	Sev_{62}	SAUDPC[b]	Sev_{54}	Sev_{65}	SAUDPC	
Genetic materials							
$F_{2:3}$	30.04	36.04	30.78	25.89	32.08	23.32	4.17
Mean	33.69	39.62	34.19	33.69	39.62	34.19	4.20
SD	10.08	11.76	9.83	15.27	16.33	13.29	
Genetic parameters ($F_{2:3}$ families)							
σ^2_F	67.60	110.03	67.44	161.3	191.12	126.12	0.22
σ^2_e	53.95	32.96	29.57	29.34	172.47	113.52	0.61
H^2	0.70	0.77	0.69	0.85	0.69	0.60	0.42
ΔG (H^2)	13.58	16.20	11.96	20.66	23.16	18.80	0.67
ΔG (h^2)	6.60	8.21	6.76	1.46	8.39	6.54	0.35

[a]Sev_{53}, Sev_{62}, Sev_{55}, Sev_{65}, = severity assessed 53, 62 days after inoculation for GLS, 54, 65 days after inoculation for NCLB

[b]SAUDPC = standardized area under the disease progress curve

σ^2_F, σ^2_e, H^2 = Estimates of variances between families, residuals and broad-sense heritability

ΔG (H^2), ΔG (h^2) = Predicted genetic gains calculated using broad-sense and narrow-sense heritability, respectively.

Table 3.2. Population mean values, predicted genetic gains and associated genetic parameters of $F_{2:3}$ families evaluated independently for reaction to *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus in Ohio, Uganda and CIMMYT Zimbabwe, respectively.

	V_M/V_A			Efficiency over phenotypic selection: Marker-based selection			Marker-assisted selection		
Trait	Sev_1[a]	Sev_2	SAUDPC	Sev_1	Sev_2	SAUDPC[b]	Sev_1	Sev_2	SAUDPC
GLS	0.65	0.49	0.60	0.91	0.79	0.87	1.04	1.02	1.03
MSV	0.85	0.64	-	0.97	0.85	-	1.02	1.01	-
NCLB	0.05	0.03	0.28	0.29	0.21	0.66	1.02	1.00	1.05

[a]Sev_1 and Sev_2 = mid and late disease severity assessment for GLS, NCLB and MSV

[b]SAUDPC = standardized area under the disease progress curve

CONCLUSIONS

There is a growing interest in understanding and exploiting quantitative (partial) resistance of maize to foliar pathogens. In many breeding programs, quantitative resistance is commonly used for managing damage caused by most of the problematic diseases. In maize, the popularity of the race-specific qualitative form of resistance has declined because the emergence of new races has resulted in “boom-bust” cycles where major genes were deployed and defeated (Leonard, 1993). Traditionally, partial resistance is more difficult to transfer than simply inherited qualitative resistance because of its multigenic nature and strong influence of the environment on its expression.

Some important pathogens such as *Cercospora zeae-maydis*, the casual agent of gray leaf spot, do not have variants differentiated as races. Wang et al. (1998) have identified two sibling types, named as type I and II that vary in aggressiveness. In contrast, E*. turcicum* (causal agent of NCLB) and MSV have several known races and strains, respectively. A high recombination capacity has been detected for both pathogens, implying potential exists for continual development of more virulent races and strains for the pathogens. Several physiological races have been identified for NCLB. The prevalence of the races

varies in different geographical regions. Five races of E. *turcicum* have been reported to overcome specific *Ht* genes in the U.S (Leornard et al., 1989; Windes and Pedersen, 1991). Races 1 and 0 are reported to predominate in the Mid-western Corn Belt (Lipps et al., 1997) and only race 0 has been reported to be the most prevalent race in Uganda (Adipala et al., 1993; Bigirwa et al., 1993). Other races, including 2, 2N, 23 and 23N, do exist but are rare (Lipps et al., 1997). Race O is avirulent on maize lines with any *Ht* genes and Race 1 is virulent against lines with *Ht1* gene (Leornard et al., 1989). For MSV, different subtypes of the virus have been reported in different parts of Africa (Martin et al., 2001). Some subtypes were determined to be more pathogenic in maize than others. These differences are of potential importance in screening and deployment of resistant genotypes in specific geographic regions.

QTL mapping has provided new approaches for understanding and exploiting both qualitative and quantitative resistance factors. There is an increasing amount of information in the literature regarding QTL for many pathosystems in maize. Interestingly, some of the QTL that account for a sizeable proportion of total variance are also associated with major genes such as the *rhm* locus for resistance to southern leaf blight (Cai et al., 2003), the *mdm1* locus for resistance to maize dwarf mosaic virus, wheat streak mosaic virus and sugarcane mosaic virus (McMullen and Louie, 1989, Yuan et al., 2003; Redinbaugh et al., 2004), *msv1* locus for resistance to maize streak (Welz et al., 1998; Kyetere et al., 1999) *and sw1* locus for Stewart's wilt resistance (Ming et al., 1999). We sought to

determine the feasibility of using markers for the goals of improving quantitative (partial) resistance in maize. We also examined the feasibility of using different breeding strategies for combining multiple resistance into maize.

The present results indicated that significantly dominant gene action was associated with major QTL regions, although some additive and recessive genes were noted at other loci. The magnitude of gene action varied with the individual QTL position. For NCLB, the QTL position on chromosomal bin 5.04 had consistently higher significant effects and was associated with resistance across two seasons and one generation. The significant dominant gene action was also expressed throughout the period of disease development starting from about 2 weeks postanthesis to the time of maximum disease severity near leaf senescence. Overall, comparison of genotypic classes for NCLB resistance showed that families homozygous for the CML202 allele in bins 3.06 and 5.04 where significantly more resistant than families homozygous for the VP31 allele. Resistance due to the presence of alleles at these loci was maintained from mid and late season ratings and no epistatic interactions were detected. The QTL position in bin 8.06 showed an unanticipated result – resistance was contributed by the susceptible parent. For GLS, mean severity of genotypic classes homozygous for alleles at bin 4.08 from the resistant parent were more resistant. The QTL in bin 2.09 was associated with susceptibility. The major locus for MSV was consistently associated with segregation distortion.

Correlations between disease severity ratings among the three diseases were non-significant, but positive in all stages of disease ratings. Clustering of resistance genes is common in maize (Simcox and McMullen, 1995). It is possible that coupling linkages of multiple resistance alleles of major QTL for one disease and minor QTL for another disease underlie resistance to multiple pathogens in some family lines. This relationship would further be investigated by determining the individual effect of QTL in a near-isogenic line (NIL) background evaluated for resistance to multiple pathogens. Because resistance was negatively correlated with maturity, it is also important to use a large population size to obtain desired recombinants.

We also compared four selection methods including phenotype-based, marker-based, combined phenotype and marker-based selection (MAS index), and random selections, to investigate genetic gains obtained for simultaneous improvement of partial resistance. Results indicated that actual genetic gains tended to vary with the particular disease and selection treatment employed reflecting differences in effectiveness of the treatments. In all cases, MAS index was most effective in reducing mean disease ratings when compared to other selection methods. Genetic gains for improved resistance from genotypic selection based on markers were significantly greater than random selection for all the diseases.

The QTL positions consistently associated with disease resistance in previous mapping studies and in this study can be utilized in the future to gain a better understanding of the genetic effects by development of near-isogenic lines (NIL). NIL differing in QTL for each of the disease may provide valuable materials to isolate and measure the effects of the individual QTL for partial resistance. The NIL in different genetic backgrounds would allow the study of the effects of those backgrounds on the expression of these QTL. The NIL for individual QTL would also help in determination of dosage effects and gene interactions from the crosses involving different NIL and to a susceptible background. By artificially inoculating these lines to increase disease levels, it would also be possible to determine the effect of minor genes that might be involved.

Initially, the top 10% of F_3 families were selected independently for each disease at one standard deviation from the mean for GLS and NCLB, and two standard deviations from the mean for MSV. This resulted into 38 lines for each disease. These lines were grown together and evaluated for each disease. Subsequently, the top 22 lines from F_4 population were selected based on a selection index built on both marker score and phenotype value. Servin et al. (2004) have proposed a gene pyramiding scheme for combining favorable alleles from different lines. They suggested pairwise crosses between selected individuals with complementary favorable alleles. The resultant intermediate genotypes from these crosses would be used as parents in subsequent crosses. They indicated fixing the favorable alleles while maintaining linkage phase by backcrossing to

one of the founding parents since self-pollination would break linkage between favorable alleles.

In addition to validating the effects of QTL, the inbreds developed in this study can be used to develop multiple resistant genotypes. Because CML202 is a confirmed source of resistance to MSV and *E. turcicum* there is opportunity to develop multiple disease resistance in CML202 derived lines with acceptable agronomic traits for adaptation in both temperate and tropical environments. There is also opportunity to backcross elite lines derived from the population to develop multiple resistant, earlier maturing derivative lines of CML202.

There is also a growing interest in exploiting exotic germplasm for introgressing resistance into local cultivars. Improving the levels of multiple disease resistance is also considered a high priority for important varieties such as the high nutritional Quality Protein Maize (QPM) developed by CIMMYT that is susceptible to most important foliar diseases. Reid Yellow Dent and its derivatives plus Stiff-Stalk Synthetic germplasm form >50% of the background of US hybrids (Troyer, 1999). Introgression of exotic germplasm into temperate adapted maize has been widely emphasized as a method to expand genetic diversity of maize germplasm (Goodman, 2004). Major reasons for underutilization of exotic germplasm, are photoperiod sensitivity, late maturity, excessive lodging, poor standability, and low grain yield in comparison with temperate adapted germplasm (Lewis and Goodman, 2004). In addition to

selecting for disease resistance, selection was made for desirable agronomic traits. The broadly adapted lines produced from this study provide resource materials for multiple diseases that can be introgressed into QPM lines in collaboration with CIMMYT. This would complement efforts taking place in the breeding programs by strengthening effective disease management through developing resistant genotypes with broader host resistance to the destructive foliar pathogens.

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APPENDIX

Table 1a. Summary of disease severity scores and agronomic traits of $F_{2:4}$ progeny lines evaluated for resistance to *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus at Wooster, Ohio (GLS and NCLB) and CIMMYT Zimbabwe (MSV).

ENTRY	GLS1	GLS2	GLS3	NLB1	NLB2	NLB3	MSV1	MSV2	PHT	EHT	Stand	%lodged
4001	22.5	30	40	4.0	17.5	17.5	*	*	169	89	10	0.17
4002	22.5	22.5	27.5	1.0	17.5	12.5	2.8	2.8	140	74	12	0.13
4013	25	30	40	5.0	15.0	10.0	3.8	2.3	141	77	8	0.20
4014	22.5	27.5	32.5	3.5	14.0	22.5	3.3	3.0	153	82	10	0.15
4015	10	12.5	21.5	6.0	10.0	37.5	1.3	1.0	182	100	13	0.11
4017	25	30	35	10.0	7.5	22.5	2.5	1.5	150	70	19	0.08
4018	32.5	40	50	0.0	5.0	7.5	1.5	1.5	156	83	12	0.14
4019	27.5	27.5	30	5.0	7.5	7.5	1.8	2.0	147	67	17	0.09
4020	25	27.5	37.5	0.0	15.0	2.5	2.0	2.0	164	82	8	0.21
4021	32.5	37.5	50	0.0	2.5	2.5	3.5	3.0	176	107	16	0.09
4023	15	18.5	25	1.0	7.5	12.5	3.3	2.3	165	89	15	0.12
4024	17.5	25	52.5	4.0	12.5	20.0	1.5	1.0	177	103	17	0.10
4025	20	25	32.5	1.0	10.0	15.0	4.0	2.3	175	80	11	0.15
4027	17.5	20	27.5	2.5	20.0	7.5	4.0	2.3	178	100	13	0.12
4028	27.5	35	45	0.0	10.0	7.5	3.0	3.5	134	69	9	0.17
4029	8.5	18.5	25	4.0	37.5	27.5	1.0	1.0	192	115	10	0.16
4030	22.5	27.5	32.5	7.5	27.5	45.0	2.0	2.0	163	81	10	0.15
4032	20	25	30	3.5	13.5	15.0	1.5	1.0	166	70	17	0.09
4034	22.5	30	32.5	12.5	2.5	35.0	2.3	2.0	160	81	14	0.12
4035	32.5	35	40	2.5	12.5	15.0	1.8	2.5	142	60	15	0.10
4036	27.5	32.5	50	9.0	15.0	35.0	2.8	3.3	183	92	13	0.11
4037	27.5	30	45	0.0	10.0	10.0	2.3	2.5	155	70	14	0.11
4038	17.5	27.5	32.5	2.5	2.5	12.5	1.5	1.0	158	80	11	0.15
4040	32.5	42.5	45	0.0	0.0	2.5	3.3	2.0	160	81	13	0.12
4041	22.5	25	30	0.0	15.0	12.5	1.3	1.0	149	68	13	0.12
4042	12.5	25	30	1.0	1.0	5.0	2.3	3.0	210	95	11	0.15
4043	35	35	45	0.0	10.0	10.0	3.5	4.0	197	129	12	0.13
4045	16	21.5	26	5.0	12.5	20.0	2.3	2.5	162	107	7	0.24
4046	25	32.5	32.5	0.0	10.0	7.5	2.0	1.8	164	86	16	0.09
4047	20	23.5	32.5	7.5	17.5	37.5	2.0	2.0	150	80	12	0.13
4052	32.5	32.5	47.5	0.0	2.5	22.5	1.5	1.0	175	88	15	0.10
4053	17.5	25	30	2.5	30.0	30.0	2.3	2.3	154	80	11	0.13
4054	37.5	42.5	62.5	0.0	7.5	7.5	4.5	4.5	164	86	17	0.09
4055	32.5	35	45	1.0	5.0	7.5	4.0	2.8	215	110	18	0.09
4057	30	32.5	37.5	2.5	7.5	20.0	2.0	2.3	140	61	10	0.16
4060	25	30	47.5	0.0	15.0	15.0	2.5	1.8	202	110	16	0.10
4061	16.5	22.5	30	0.0	20.0	7.5	3.8	2.3	169	75	7	0.24
4062	17.5	22.5	27.5	1.0	5.0	12.5	1.3	1.0	158	93	16	0.10
4063	35	40	55	2.5	17.5	15.0	3.5	3.8	147	77	8	0.20

4067	15	21.5	27.5	2.5	7.5	20.0	2.0	1.5	165	90	16	0.10
4069	20	25	35	0.0	2.5	2.5	1.0	1.0	189	102	14	0.10
4070	30	32.5	40	16.0	20.0	45.0	1.3	2.0	150	90	12	0.13
4071	20	22.5	25	3.5	17.5	15.0	1.3	1.3	177	92	11	0.15
4074	20	25	32.5	0.0	7.5	7.5	4.3	4.3	172	98	13	0.12
4077	15	25	27.5	5.0	7.5	32.5	3.8	2.8	161	93	12	0.13
4078	22.5	25	32.5	0.0	2.5	2.5	2.0	2.3	125	75	13	0.12
4080	10	15	19	1.0	12.5	12.5	2.5	2.0	172	97	13	0.12
4081	12.5	17.5	22.5	2.5	5.0	7.5	1.0	1.0	172	90	6	0.29
4083	20	25	27.5	2.5	20.0	5.0	1.8	1.5	142	71	14	0.11
4089	27.5	30	40	5.0	5.0	17.5	2.5	1.5	158	66	8	0.22
4090	25	35	40	0.0	2.5	2.5	2.8	2.5	153	76	14	0.11
4091	25	30	37.5	2.5	12.5	21.5	3.5	3.8	159	82	12	0.12
4092	10	17.5	27.5	0.0	17.5	5.0	2.3	1.5	150	97	2	0.75
4093	10	18	22.5	2.5	12.5	22.5	1.0	1.0	146	87	11	0.15
4095	35	40	55	5.5	7.5	20.0	4.0	2.0	137	65	10	0.15
4096	22.5	31	40	3.5	5.0	27.5	3.0	3.0	187	80	16	0.10
4098	17.5	24	30	0.0	5.0	2.5	3.5	3.3	186	115	11	0.13
4101	17.5	25	27.5	1.0	5.0	12.5	2.8	2.8	198	97	14	0.12
4104	30	35	42.5	2.5	27.5	15.0	3.5	3.0	167	70	12	0.16
4107	20	21	25	2.5	7.5	12.5	2.5	1.5	171	105	10	0.15
4110	25	30	35	0.0	0.0	2.5	2.8	2.5	173	103	10	0.16
4112	19	25	27.5	8.0	10.0	37.5	1.5	1.5	180	102	17	0.09
4114	17.5	20	25	2.5	15.0	10.0	3.3	2.8	188	92	5	0.35
4115	8	12	19	0.0	0.0	7.5	1.5	1.0	176	104	13	0.12
4117	22.5	27.5	35	0.0	15.0	15.0	2.0	1.8	163	100	11	0.15
4121	10	20	27.5	0.0	0.0	15.0	1.5	1.3	156	66	11	0.16
4122	11.5	20	25	0.0	17.5	5.0	2.3	2.0	153	80	11	0.14
4124	17.5	22.5	27.5	11.5	22.5	40.0	1.8	2.0	186	122	12	0.13
4125	27.5	30	37.5	0.0	5.0	5.0	1.5	1.8	172	92	11	0.13
4127	10	10	20	7.5	20.0	52.5	1.3	1.0	169	88	15	0.10
4128	25	27.5	32.5	0.0	2.5	2.5	1.8	1.0	168	95	10	0.15
4129	10	10	20	3.5	10.0	17.5	1.8	1.5	156	73	9	0.17
4131	17.5	17.5	22.5	0.0	7.5	10.0	3.8	3.3	152	71	13	0.12
4133	30	32.5	45	0.0	10.0	10.0	2.3	1.0	134	76	17	0.09
4134	17.5	19	25	0.0	5.0	10.0	3.5	2.5	171	86	13	0.12
4136	32.5	35	40	0.0	7.5	5.0	1.5	1.8	152	77	14	0.11
4137	10	15	20	8.0	15.0	27.5	1.0	1.0	168	102	14	0.11
4138	25	30	37.5	0.0	2.5	10.0	3.8	3.8	156	78	12	0.13
4139	20	22.5	30	5.0	10.0	22.5	2.8	2.5	180	90	9	0.16
4140	42.5	55	77.5	2.5	7.5	10.0	3.3	2.3	155	83	14	0.11
4141	30	32.5	35	0.0	2.5	7.5	3.3	1.8	163	80	12	0.13
4145	16.5	22.5	32.5	0.0	2.5	2.5	3.0	2.3	159	85	13	0.12
4146	22.5	25	30	0.0	5.0	12.5	1.0	1.0	180	110	11	0.14
4147	25	32.5	42.5	0.0	2.5	2.5	1.3	1.3	142	60	17	0.09
4148	27.5	32.5	57.5	0.0	5.0	2.5	3.0	3.3	144	78	17	0.09

4149	30	35	55	2.5	25.0	20.0	2.5	2.5	172	82	17	0.09
4150	15	24	26.5	4.0	17.5	27.5	1.8	2.0	172	106	8	0.21
4153	11.5	20	30	1.0	12.5	12.5	2.0	2.3	190	96	18	0.08
4154	27.5	35	45	3.5	10.0	15.0	2.0	1.3	172	97	13	0.11
4155	12.5	17.5	27.5	1.0	2.5	2.5	3.3	3.0	154	76	17	0.09
4156	15	22.5	30	0.0	10.0	20.0	1.5	2.8	170	93	14	0.11
4158	12.5	12.5	17.5	2.0	12.5	20.0	3.0	3.3	162	84	11	0.14
4159	12.5	20	27.5	0.0	15.0	15.0	2.0	2.3	147	82	16	0.09
4161	30	30	45	2.5	0.0	5.0	2.5	2.8	175	103	14	0.10
4162	22.5	31	40	6.5	15.0	30.0	2.3	2.3	160	84	18	0.09
4163	17.5	25	32.5	0.0	0.0	5.0	2.0	1.8	171	102	15	0.10
4164	30	35	67.5	0.0	0.0	0.0	1.5	1.0	165	71	12	0.13
4165	17.5	27.5	35	0.0	16.0	12.5	4.0	4.0	182	90	12	0.13
4170	15	20	30	0.0	37.5	2.5	1.0	1.0	222	131	9	0.21
4171	27.5	27.5	32.5	0.0	12.5	14.0	1.0	1.0	186	100	7	0.24
4173	17.5	25	32.5	3.5	16.0	27.5	2.8	3.5	136	74	14	0.11
4174	17.5	25	35	20.0	30.0	75.0	3.5	3.3	140	55	12	0.14
4178	20	30	35	3.5	2.5	12.5	2.3	1.5	166	87	19	0.08
4179	20	25	30	0.0	7.5	22.5	3.5	2.5	167	85	7	0.21
4180	17.5	22.5	30	0.0	5.0	10.0	1.3	1.3	174	86	18	0.08
4181	22.5	27.5	32.5	0.0	0.0	0.0	1.8	1.5	182	100	12	0.13
4182	17.5	21.5	28.5	4.0	12.5	25.0	2.5	2.3	200	112	13	0.12
4184	5	8	12.5	1.0	17.5	12.5	*	*	188	100	8	0.20
4185	5	10	17.5	0.0	0.0	0.0	4.0	2.5	173	105	13	0.12
4190	30	35	52.5	0.0	0.0	5.0	2.0	2.0	166	93	13	0.12
4192	10	22.5	30	9.0	22.5	42.5	3.8	3.5	154	66	14	0.13
4194	17.5	27.5	32.5	1.0	20.0	5.0	3.0	3.0	128	78	11	0.14
4195	15	22.5	27.5	0.0	5.0	5.0	1.5	1.8	168	98	12	0.13
4198	27.5	27.5	35	0.0	2.5	10.0	2.8	3.0	157	88	12	0.13
4202	25	27.5	47.5	11.5	22.5	42.5	3.0	2.0	150	78	11	0.14
4203	32.5	35	40	1.0	2.5	10.0	1.5	1.8	167	83	10	0.23
4204	17.5	22.5	30	0.0	5.0	10.0	*	*	165	87	11	0.14
4206	12.5	21	29	4.0	20.0	17.5	1.0	1.0	158	83	8	0.25
4208	25	35	40	0.0	15.0	15.0	3.8	2.3	163	72	12	0.13
4209	37.5	40	47.5	0.0	15.0	15.0	*	*	177	110	14	0.11
4211	7.5	12.5	16.5	2.5	2.5	20.0	1.8	1.0	182	106	10	0.15
4212	5	10	15	1.0	5.0	17.5	1.5	1.5	151	80	10	0.16
4213	11.5	17.5	20	2.5	10.0	30.0	2.0	2.0	164	82	11	0.14
4214	22.5	25	30	0.0	15.0	15.0	2.5	1.5	157	98	11	0.14
4215	19	27.5	32.5	0.0	15.0	15.0	1.0	1.0	187	86	12	0.13
4216	32.5	40	55	2.5	12.5	15.0	2.5	1.8	172	80	18	0.08
4217	12.5	20	25	0.0	0.0	7.5	1.3	1.5	156	79	5	0.38
4218	25	27.5	40	5.0	30.0	25.0	2.0	2.5	191	100	16	0.10
4219	30	35	47.5	5.0	20.0	25.0	2.0	2.5	178	83	12	0.13
4220	12.5	22.5	32.5	0.0	2.5	5.0	1.3	1.0	173	100	14	0.11
4224	12.5	20	27.5	10.0	27.5	40.0	1.5	1.8	185	102	12	0.13

4226	15	20	25	0.0	5.0	10.0	3.8	4.0	168	79	15	0.11
4228	27.5	35	40	0.0	2.5	2.5	1.0	1.0	151	82	15	0.10
4229	7.5	17.5	27.5	2.5	20.0	22.5	4.5	4.5	152	82	14	0.14
4230	30	35	42.5	0.0	2.5	12.5	1.8	2.0	158	60	12	0.13
4231	17.5	17.5	25	0.0	7.5	5.0	2.3	1.3	167	86	14	0.11
4232	27.5	30	45	10.0	20.0	42.5	1.0	1.0	155	74	14	0.12
4233	15	17.5	25	0.0	0.0	5.0	2.0	2.0	170	102	11	0.14
4234	17.5	25	35	3.5	10.0	17.5	3.3	3.0	160	82	15	0.10
4237	35	37.5	50	1.0	10.0	10.0	3.3	3.0	147	72	10	0.16
4238	22.5	25	32.5	0.0	2.5	12.5	3.3	2.5	180	86	16	0.09
4239	15	17.5	22.5	0.0	7.5	2.5	1.0	1.0	157	74	11	0.13
4240	27.5	30	37.5	11.5	12.5	22.5	1.5	1.3	164	91	11	0.14
4246	7.5	18.5	25	0.0	12.5	15.0	1.3	1.0	204	120	8	0.18
4248	27.5	35	52.5	1.0	2.5	7.5	2.3	2.0	170	93	12	0.12
4249	20	23.5	31	5.0	8.0	10.0	3.3	3.5	187	100	16	0.10
4251	20	25	35	2.0	7.5	15.0	3.0	2.0	182	102	14	0.11
4253	10	19	26	5.0	35.0	20.0	1.8	1.8	206	112	8	0.22
4256	15	19	23.5	0.0	10.0	10.0	2.0	1.8	188	90	13	0.12
4257	27.5	27.5	42.5	1.0	2.5	7.5	2.5	2.3	159	100	15	0.10
4262	32.5	37.5	47.5	4.0	0.0	20.0	1.5	1.0	170	93	15	0.10
4264	22.5	32.5	35	7.5	12.5	27.5	2.3	1.3	203	119	16	0.10
4267	37.5	40	52.5	0.0	20.0	2.5	4.5	4.0	160	73	13	0.11
4270	27.5	30	32.5	0.0	2.5	5.0	2.6	3.0	155	102	13	0.12
4272	27.5	30	35	0.0	10.0	12.5	3.0	3.0	170	85	14	0.11
4275	12.5	17.5	25	5.0	25.0	45.0	3.3	3.3	170	82	18	0.09
4276	27.5	35	47.5	0.0	20.0	20.0	4.5	4.5	160	78	16	0.09
4278	25	40	45	2.5	2.5	17.5	2.8	2.5	163	80	10	0.16
4279	16.5	20	30	7.5	17.5	22.5	3.3	3.5	154	82	11	0.16
4280	22.5	25	30	3.0	2.5	20.0	3.3	2.3	147	86	7	0.21
4281	14	17.5	24	0.0	0.0	5.0	1.8	2.0	158	77	10	0.16
4287	35	47.5	60	2.5	10.0	12.5	2.0	1.0	160	90	7	0.21
4290	35	35	45	0.0	12.5	7.5	3.3	3.3	173	92	14	0.11
4294	32.5	35	60	0.0	22.5	22.5	2.8	3.0	150	72	9	0.17
4302	17.5	25	32.5	1.0	5.0	17.5	1.5	1.3	193	112	15	0.10
4303	25	30	37.5	2.5	15.0	32.5	2.8	2.5	150	66	19	0.08
4309	20	22.5	30	2.5	5.0	17.5	4.3	4.0	170	95	11	0.16
4310	27.5	30	35	2.5	10.0	10.0	1.5	1.8	165	85	10	0.15
4311	32.5	40	50	2.5	7.5	12.5	2.0	1.5	155	87	18	0.09
4314	22.5	27.5	32.5	2.5	7.5	14.0	3.8	3.8	172	87	17	0.09
4315	30	32.5	42.5	0.0	0.0	5.0	3.8	3.0	144	68	13	0.12
4317	15	21.5	25	1.0	17.5	5.0	2.0	1.8	159	72	13	0.12
4320	12.5	25	32.5	3.5	35.0	35.0	1.8	2.0	154	92	12	0.13
4328	15	23.5	29	0.0	0.0	2.5	3.3	2.5	157	80	13	0.11
4330	10	17.5	22.5	7.5	12.5	32.5	2.8	2.5	182	97	12	0.14
4334	30	35	42.5	7.5	5.0	17.5	2.0	2.0	146	90	13	0.12
4341	17.5	27.5	27.5	0.0	5.0	5.0	1.8	1.8	172	110	14	0.11

4344	35	40	45	0.0	0.0	0.0	2.8	3.3	141	*	6	0.28
4345	25	27.5	32.5	2.5	5.0	15.0	4.0	3.5	168	87	13	0.13
4347	25	27.5	30	1.0	5.0	5.0	2.5	2.0	147	85	10	0.15
4349	12.5	17.5	30	0.0	7.5	5.0	1.0	1.0	183	98	9	0.18
4355	27.5	40	47.5	0.0	5.0	5.0	3.8	2.8	152	82	15	0.10
4356	25	30	40	2.5	12.5	10.0	2.8	2.5	172	110	13	0.11
4358	40	50	75	0.0	4.0	10.0	2.0	2.3	140	57	12	0.14
4359	22.5	27.5	37.5	0.0	5.0	8.5	2.3	2.0	190	90	17	0.10
4360	21.5	23.5	30	3.5	12.5	15.0	1.5	1.3	195	92	15	0.11
4361	7.5	11.5	15	0.0	2.5	5.0	3.3	2.8	173	94	13	0.12
4363	6.5	10	14	0.0	10.0	10.0	2.0	2.3	194	88	10	0.19
4364	27.5	32.5	45	2.5	2.5	20.0	1.5	2.0	168	76	14	0.11
4366	25	32.5	40	0.0	2.5	2.5	4.0	4.0	178	86	15	0.10
4367	31.5	40	47.5	2.5	10.0	10.0	2.3	1.8	162	68	13	0.12
4368	27.5	32.5	40	3.5	20.0	17.5	1.0	1.0	190	110	17	0.09
4370	32.5	37.5	65	2.5	20.0	15.0	1.8	2.0	154	84	6	0.27
4371	5	7.5	15	13.0	30.0	37.5	2.5	2.0	162	77	9	0.19
4372	20	30	40	0.0	0.0	10.0	1.0	1.0	160	93	8	0.18
4373	10	15	27.5	4.0	2.5	15.0	3.0	2.3	158	93	14	0.11
4374	30	37.5	55	6.5	10.0	30.0	2.0	2.0	152	74	13	0.12
4375	18.5	25	30	1.0	12.5	7.5	2.8	2.8	147	77	10	0.15
4377	22.5	22.5	25	2.0	17.5	10.0	1.5	1.3	143	58	11	0.14
4378	20	25	32.5	0.0	2.5	2.5	3.8	4.0	160	85	12	0.13
4379	22.5	30	40	3.0	5.0	30.0	4.0	3.3	145	76	16	0.10
4380	32.5	35	50	2.5	5.0	15.0	3.3	2.5	164	100	13	0.11
4382	28.5	32.5	42.5	2.5	7.5	2.5	3.5	2.5	127	52	9	0.19
4383	25	30	37.5	0.0	20.0	20.0	2.3	2.5	164	79	11	0.14
4385	13.5	25	32.5	0.0	17.5	10.0	1.0	1.0	144	80	13	0.12
4386	32.5	37.5	45	0.0	5.0	20.0	4.0	3.8	150	82	14	0.11
4387	37.5	45	70	8.5	12.5	40.0	1.8	1.8	140	50	14	0.11
4388	15	25	32.5	4.0	7.5	42.5	4.0	3.3	192	107	16	0.09
4389	27.5	35	45	0.0	0.0	2.5	2.8	2.0	167	87	17	0.09
4390	35	35	47.5	0.0	2.5	5.0	3.5	3.0	173	92	13	0.12
4391	12.5	17.5	21.5	0.0	7.5	5.0	3.5	3.5	175	98	11	0.13
4392	32.5	32.5	40	1.0	2.5	9.0	2.8	3.0	140	57	11	0.14
4393	32.5	32.5	45	1.0	10.0	10.0	1.0	1.0	196	114	11	0.13
4395	30	30	47.5	0.0	5.0	7.5	3.5	3.8	162	92	14	0.11
4396	20	22.5	30	0.0	10.0	5.0	1.0	1.0	150	106	11	0.13
4398	17.5	20	25	4.5	22.5	25.0	1.0	1.0	162	84	8	0.25
4399	11.5	20	27.5	1.0	2.5	5.0	1.3	1.0	190	96	17	0.09
4400	22.5	32.5	42.5	2.5	12.5	15.0	4.0	3.8	162	80	19	0.08
4401	27.5	30	40	0.0	2.5	5.0	2.3	1.5	180	90	14	0.11
4403	20	27.5	32.5	2.5	2.5	2.5	1.8	1.5	146	70	13	0.11
4405	17.5	22.5	25	0.0	15.0	20.0	1.3	1.0	182	95	15	0.10
4406	11.5	25	45	5.0	10.0	17.5	2.3	1.8	120	50	11	0.15
4407	32.5	40	57.5	0.0	0.0	2.5	1.3	1.0	196	104	15	0.10

4409	27.5	35	45	0.0	27.5	27.5	1.5	1.5	172	95	19	0.08
4410	22.5	27.5	32.5	0.0	32.5	32.5	1.8	1.0	155	84	14	0.11
B73	30	39.4	65	12.0	38.3	40.0	3.0	2.2	176	78	14	0.11
CML202	22.5	25	29	0.0	20.0	20.0	1.3	1.0	187	102	11	0.15
Pa405	60	87.5	95	*	*	*	4.0	2.8	*	*	17	0.06
V0613Y	3.5	5.0	15.0	*	*	*	2.5	1.8	*	*	*	*
VP31	17.5	25	30	10.0	30.0	47.5	1.5	1.8	143	68	13	0.11
H100	*	*	*	27.5	67.5	72.5	*	*	*	*	*	*

GLS1, GLS2, GLS3 = Gray leaf spot severity ratings (0-100% PLAA) at 46, 53 and 62 days after inoculation

NLB1, NLB2, NLB3 = Northern corn leaf blight severity ratings (0-100% PLAA) at 43, 50 and 60 days after inoculation

MSV1, MSV2 = Maize streak severity ratings (1-5 scale) at 41 and 64 days after inoculation

PHT = Plant height (cm)

EHT = Ear height (cm)

Stand = number of plant in a family row

% lodged = Percentage of plants lodged

Table 2a: Field costs for maize plantings and disease evaluation at Ohio-OARDC (USD)

Chemicals	**Cost/acre/annum**	**Total cost**	**Annual adjust.**	**Total adjust**
Fertilizer	63.75	76.5	2.05	78.55
Herbicide	22.04	26.45	0.71	27.16
Seed Treatment	7.05	8.46	0.23	8.69
Fuel (diesel)	1.66	2.00	0.05	2.05
Overhead				
Chisel plow	12.00	14.40	0.39	14.79
Disk	9.00	10.80	0.29	11.09
Field cultivator	9.00	10.80	0.29	11.09
Spray	5.00	6.0	0.16	6.16
Supplies				
Envelopes	40.80	48.96	1.31	50.27
Bags	31.50	37.80	1.01	38.81
Other supplies	20.00	24.00	0.64	24.64
Labor				
Technical assistant	2300.00	2760.00	74.00	2834.00
Temporary labor	1200.00	1440.00	38.60	1478.60
Inoculum				
Sorghum seeds	64.50	77.40	2.07	79.47
Qorpak, bottles	312.50	468.75	12.56	481.31
Petri dishes	32.50	48.75	1.31	50.06
Plastic trays	22.00	33.00	0.88	33.88
Zip-pak track bags	53.20	63.84	1.71	65.55
Total	4206.50	5157.91	138.26	5296.17

Table 3a: Cost of genotyping maize population for selection of resistance to three maize diseases (USD)

Reagents	**Cost**	**Ann. Adjust.**	**Total Adjust.**
Taq polymerase	236	6.32	242.32
dNTPs	336	9.00	345.00
Other reagents	45	1.20	45.80
Tris Base	99	2.65	101.65
Boric acid	76	2.04	78.04
DNA ladder	90	2.41	92.41
Buffers	280	7.50	287.50
MgCl2	102	2.73	104.73
Agarose	370	9.92	379.92
Oligo primers	603	16.16	619.16
Supplies			
Pipette tips	1652.3	44.28	1696.58
Eppendorf tubes	340	9.11	349.11
PCR plates	1412.76	37.86	1450.62
Gloves	216.89	5.81	222.70
Snap tubes	175.61	4.71	180.32
Thermal sealing film	172.95	4.64	177.59
Other	65.03	1.74	66.77
Labor			
Research Associate	3747	100.42	3847.42
Total	**10019.14**	**268.51**	**10287.65**