HOST RESISTANCE TO GOSS'S WILT OF MAIZE

BY

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THESIS

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ABSTRACT

Since its discovery in 1969, Goss's wilt, a foliar blight and vascular wilt disease caused by the gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), has emerged as one of the top four diseases of maize in the United States and Canada. No source of complete resistance has been described for Goss's wilt and little is known about the genetic and mechanistic basis of host resistance to *Cmn*. The objective of this study was to perform a linkage mapping and genome-wide association study (GWAS) to identify regions of the genome associated with Goss's wilt resistance. Additionally, we sought to use genomic prediction models to evaluate the use of genomic selection in predicting Goss's wilt phenotypes in a panel of diverse maize lines. Within the Intermated B73 x Mo17 (IBM) population and three disease resistant introgression lines (DRIL) populations: B73 x Mo17, Mo17 x B73, and NC344 x Oh7B, we were able to both identify novel QTL and confirm previous findings. In a GWAS of the Goodman maize diversity panel, we were unable to identify any variants significantly associated with Goss's wilt. However, using genomic prediction, we were able to train a model with an accuracy of 0.6971. In addition, when evaluating the accuracy of our prediction model under reduced marker density, it was shown that only 10,000 single nucleotide polymorphisms, or ~20% of our total marker set, was necessary to achieve our control model's prediction accuracy. This is the first report of genomic prediction for a bacterial disease of maize, and these results highlight the potential of genomic selection for disease resistance in maize.

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INTRODUCTION

Goss's wilt, caused by the bacteria *Clavibacter michiganensis* subsp. *nebraskensis*, produces foliar blight lesions and vascular wilt symptoms in susceptible maize lines and is often accompanied by substantial yield losses (Vidaver and Mandel, 1974). The disease was first observed in the cornfields of Dawson County, Nebraska in 1969 (Wysong et al., 1973). By 1979, Goss's wilt was widespread throughout Nebraska, Kansas, Iowa, South Dakota, Wyoming, and Colorado (Jackson et al., 2007;Vidaver et al., 1981). While initially devastating, disease incidence decreased following the initial emergence, due in large part to the development of resistant varieties by plant breeders. In fact, by the early 1980's occurrences were confined to highly susceptible varieties or plants that sustained severe physical injury (Jackson et al., 2007). This period of remission did not last, however. In 2006, farmers in western Nebraska, southeastern Wyoming, and eastern Colorado began reporting symptoms characteristic of Goss's wilt. Submissions of over 50 samples from infected fields to the University of Nebraska-Lincoln Panhandle Plant Disease Diagnostic Lab confirmed a reemergence of the disease (Jackson et al., 2007). In comparison, prior to the 2006 season, only 40 samples total of Goss's wilt had been diagnosed at that same lab since 1998 (Jackson et al., 2007). Since then, the spread of Goss's wilt has been extensive; as of 2013, the disease has been identified in fields stretching from Louisiana to Alberta, Canada (Howard et al., 2015;Jackson et al., 2007;Singh et al., 2015).

To date, little is known about the cause for the reemergence of Goss's wilt, and no permanent solutions exist for combatting its spread. This literature review will examine what is known about the pathogen, host, and management of Goss's wilt in order to evaluate a number of existing hypothesis regarding causes of the current outbreak.

THE PATHOGEN

Basic Anatomy: The pathogen causing Goss's wilt was first isolated by Anne K. Vidaver and Manley Mandel (1974). Thought to be a species of *Corynebacterium*, the bacterium was officially denoted *Corynebacterium nebraskense* (Vidaver and Mandel, 1974). Then, in 1984, Davis et al. (1984) discovered a novel acid within the *Corynebacterium* peptidoglycan cell wall, 2,4-diaminobutryic acid. This warranted the creation of a new genus and the existing species of *Corynebacterium* were divided between *Corynebacterium* and *Clavibacter. Corynebacterium nebraskense* became *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*) (Davis et al., 1984). *Cmn* differs from other subspecies of *Clavibacter* based on its host range and plasmid content. Unlike *Clavibacter michiganensis* subsp. *michiganensis* and subsp. *sepedonicus*, no plasmid is necessary for subsp. *nebraskensis* virulence (Bentley et al., 2008;Vidaver and Mandel, 1974). The three main subspecies of *Clavibacter* are summarized below.

Subspecies	Primary Host	Plasmid for Virulence
michganensis	Tomato	Necessary
sepedonicus	Potato	Necessary
nebraskensis	Corn	Unnecessary

A number of key morphological traits characterize *Cmn*. It is a non-motile, aerobic, gram-positive bacterium with a pleomorphic rod shape (Vidaver and Mandel, 1974). Its primary host is maize (*Zea mays*), but it can also colonize annual ryegrass, johnsongrass, large crabgrass, foxtail, barnyard grass, shattercane, and a number of other weedy grasses (Ikley et al., 2015;Schuster, 1975). *Cmn* is oxidase negative, catalase positive, and is able to digest and produce acids from glucose, sucrose, mannose, xylose, and galactose. It is unable to process arabinose, raffinose, or trehalose. Colonies on culture exhibit an orange or apricot pigmentation and a butyrous, glistening appearance. The optimal temperature for colony growth is between

24°C and 28°C, with the ability to grow in temperatures as low as 10°C but not exceeding 37°C (Vidaver and Mandel, 1974).

Culturing *Cmn* is possible on nutrient broth yeast extract agar, potato dextrose agar, and synthetic mediums supplemented with yeast extract (Vidaver and Mandel, 1974). Once isolated, the culture can be maintained with minimal virulence loss in either a solid or liquid form for up to two years via lyophilization or storage in temperatures less than 6°C (Vidaver, 1977). Other unpublished, first-hand observations have reported maintenance of viable isolates for over 20 years when properly lyophilized. For subsequent testing with isolated *Cmn*, a number of methods have been proposed for artificial inoculation. The most common inoculation method for Goss's wilt is the pin-prick procedure, which involves a foam cushion soaked in inoculum at the terminal end of a needle on a modified tong (Blanco et al., 1977;Calub et al., 1974;Chang et al., 1977). As pressure is applied, the needle penetrates the host leaf and the inoculum is squeezed from the cushion into the newly created wound. This method is successful in causing leaf blight symptoms but rarely causes vascular wilt during testing (Calub et al., 1974).

Lifecycle: Infection by *Cmn* can occur through leaves, stem, or roots (Schuster, 1975). The pathogen overwinters in infected crop residue on the soil surface. Root, stem, and leaf tissue all allow for survival, and the bacteria can persist for up to 10 months before requiring a new host (Jackson et al., 2007). Infection occurs most readily though wounded or damaged tissue. Hail damage or sand-blasting can both cause the type of mechanical injury necessary for infection, but splashing rain is often required to move the pathogen, as *Cmn* is non-motile (Jackson et al., 2007). A study conducted by Mallowa et al. (2016) showed that wounding is not always necessary for plant inoculation. Given high relative humidity, *Cmn* is able to enter the host via natural openings. At 60% relative humidity, the bacteria was able to infect plants 60% of the

time, compared to 37% at ambient humidity and 100% via wounding (Mallowa et al., 2016). This may be attributed to the opening of stomata in high humidity conditions, allowing natural entry points for the bacteria. It should be noted, however, that *Cmn* is non-flagellate and must already be present on the leaf to enter through natural openings. There is no known vector for *Cmn*, however a recent survey conducted by Langemier et al. (2017) reported a relationship between corn rootworm and Goss's wilt outbreaks. This data suggests the possibility of the pathogen entering maize through mechanical damage caused by leaf and root-feeding insects (Langemeier et al., 2017).

Goss's wilt can be seed-borne; however, this mode of entry is the least common for *Cmn*. Biddle et al. (1990) attempted to measure transmission rates of the bacterium from parent to progeny. Using a rifampicin resistant isolate of *Cmn*, the seeds of fertile infected parents were examined to observe if the bacteria was spread to the next generation. They found that only 0.1-0.4% of the seeds successfully inherited the rifampicin tolerant strain, and the exact method mechanism for transmission could not be elucidated (Biddle et al., 1990). Seedling infection, in contrast, is much more common. *Cmn* was recovered at rates ranging from 17-30% both from inoculated seeds (Biddle et al., 1990). As such, infected seeds are able to provide an additional overwintering site for this pathogen.

While the specifics of *Cmn* pathogenesis remain unknown, the general disease cycle can be deduced by examining the other *Clavibacter* subspecies. Both subspecies *michiganensis* and *sepedonicus* invade their hosts through wounding or natural openings and reside as biotrophic pathogens (Benhamou, 1991). Initially, the bacteria multiply via binary fission in the spiral vessels that thicken the xylem cell walls (Wallis, 1977). Upon entering the xylem, the bacteria quickly spread upwards and outwards, degrading primary phloem walls and blocking xylem

vessels. *Clavibacter* is unable to spread through phloem due to the sieve tube morphology (Wallis, 1977).

Some research has been conducted regarding bacterial movement once inside the host. When causing vascular wilt, *Cmn* appears to colonize preferentially, beginning with the xylem annular and spiral rings (Mbofung et al., 2016). This is theorized to allow the bacteria to create a foundational biofilm layer before progressing into the lumen. This type of movement can often be seen in bacteria that employ quorum-sensing as a colonization method (Koutsoudis et al., 2006). Quorum-sensing is a method by which small, diffusible molecules are used to regulate bacterial population density. This allows colonies to synchronize gene systems and only express virulence when their concentration is sufficient to overcome plant defenses (Fuqua et al., 1996). Ten days post inoculation, cell organelles become disorganized and indistinguishable from one another. In resistant varieties, bundle sheath cells are able to remain intact, but in susceptible maize cultivars, lesions are accompanied by an amorphous matrix of bacteria within the xylem (Mbofung et al., 2016).

Symptoms: Goss's wilt produces two distinct types of symptoms; leaf blight and systemic wilt (Schuster, 1975). Foliar blight is more common than systemic wilt, and generally develops on relatively mature plants (Calub et al., 1974). Blight symptoms first appear as pale, water-soaked lesions near the inoculation point. These points spread parallel to the leaf veins and soon coalesce into large, tan lesions (Calub et al., 1974). Along the lesions small, dark green to black, discontinuous, water-soaked spots begin to appear, commonly referred to as "freckles". An orange bacterial exudate can be observed as a shiny gleam on infected leaves when dried (Jackson et al., 2007).

Wilt symptoms are often found on very young plants with severe wounding or as carryover from infected seeds (Jackson et al., 2007). Vascular wilt symptoms can be easily recognized by the internal orange discoloration of the vascular bundles and by the external water-soaked and slimy appearance of the stalk. This type of infection can cause death at any point in the season, but is most common early, before the plant has fully developed (Claflin, 1999;Jackson et al., 2007).

Disease severity is related to the stage of plant development at infection. In a study by Calub et al. (1974), the effect of plant age on Goss's wilt severity was tested. Maize inoculations were performed at two, four, six, and eight weeks to study how maturity influenced disease incidence. They found that maize inoculated two weeks after germination showed the most severe leaf wilting, with symptoms spreading upwards from the point of infection. Symptoms began to appear on the inoculated leaf 9-10 days after inoculation. In contrast, inoculations of maize in the tassel stage (eight weeks) took six to ten weeks to express full disease symptoms (Calub et al., 1974).

Virulence: The mechanisms behind *Cmn* virulence remain largely unknown, and *in vivo* testing is necessary to determine the aggressiveness of any particular strain. In comparison to *Clavibacter michiganensis* subsp. *michiganensis* and subsp. *sepedonicus*, no plasmid is necessary for *Cmn* pathogenicity (Bentley et al., 2008). Instead, all major factors needed for host colonization appear to chromosomally encoded (Bentley et al., 2008;Vidaver and Mandel, 1974). No correlation has been found between colony morphology and plant pathogenicity (Ahmad et al., 2015).

Ahmad et al. (2015) attempted to isolate the causal genes underlying *Cmn* virulence using PCR-RFLP. Unfortunately, they were only able to characterize one out of twelve putative toxin

genes identified. The putative virulence gene is believed to be a chloride anion channel, responsible for forming anions channels between planar lipid bilayers *in vitro*, but its role in maize pathogenicity could not be discerned (Ahmad et al., 2015).

Impact: Host genetics play a large role in determining disease severity. In a replicated, multi-year study of sweet corn, resistant hybrid yield losses ranged from only 0-8%, while in susceptible sweet corn germplasm losses exceeded 27% (Pataky et al., 1988). More recently, yield losses of over 50% have been observed in susceptible fields that develop symptoms early in the season (Jackson et al., 2007). During the period from 2012 to 2015, approximately 501 million bushels were lost in the United States and Canada due to Goss's wilt, making it the fourth most severe disease of maize during this period (Mueller et al., 2016).

CONTROL STRATEGIES

Management: A number of control strategies are currently employed to combat Goss's wilt, with varying degrees of success. No practical chemical control for Goss's wilt currently exists. For *Clavibacter michiganensis* subsp. *michiganensis*, copper hydroxide and citric acid have reportedly been successful in disease control (Hausbeck et al., 2000;Ozdemir, 2009). However, Mehl et al. (2015) showed that these same chemicals did not decrease Goss's wilt occurrence at a statistically significant level (Mehl et al., 2015).

A number of cultural strategies can be employed to limit Goss's wilt occurrence (Jackson et al., 2007). Many weedy grass species serve as alternative hosts for *Cmn*, making proper weed control a necessity in at-risk fields (Ikley et al., 2015;Schuster, 1975). The cover crop annual ryegrass was recently confirmed as a host for *Cmn*, making it the first alternative host with a winter annual growth pattern (Ikley et al., 2015). This could provide the pathogen a continuous

living host for overwintering. Cereal rye, another common cover crop, has not been found to be an alternative host, providing an acceptable substitution for susceptible fields (Ikley et al., 2015). Vascular damage to the plant by *Cmn* may limit water movement. Therefore, ensuring proper irrigation to reduce moisture stress is also recommended (Carson and Wicks, 1991). Additionally, since the bacteria overwinter in infected crop residue on the soil surface, any tillage practices that incorporate residue and encourage decomposition can help reduce pathogen populations at the beginning of the season. Similarly, rotating maize with non-host crops can reduce or eliminate primary inoculum (Jackson et al., 2007).

Host Resistance: Currently, the best control strategy for Goss's wilt remains to plant resistant maize hybrids. Normal pathogen-host interactions follow a "zig-zag" model, as described by Jones and Dangl (2006). In this model, the conflict between pathogen infection and host resistance is described as an evolutionary arms race, with each organism trying to outcompete the other. It starts when pathogen-associated molecular patterns (PAMPs), such as peptidoglycan or lipopolysaccharides, are recognized by pattern recognition receptors (PRRs) within the host membranes, triggering an innate basal defense called PAMP-triggered immunity (PTI). If the pathogen secretes an effector, typically a small molecule capable of selectively bind to and regulating protein activity, capable of overcoming the PTI then the plant is once more susceptible to colonization. This state is called effector-triggered susceptibility (ETS). An effector susceptible host can be colonized by the pathogen and used for nutrients or reproduction. Many plants have ways of combatting ETS. Resistant plants encode resistance genes (R-genes), typically encoding nucleotide binding leucine rich repeats (NB-LRR) domains. Pathogen effectors are recognized by NB-LRR domains and trigger an amplified version of PTI, resulting in localized cell death to limit further infection (hypersensitive response). This utilization of NB-

LRR domains to limit effectors is called effector-triggered immunity (ETI). Finally, if the pathogen is able to secrete another effector, undetected by the NB-LRR, ETS is once again achieved. As long as the pathogen evolves to secrete novel effectors and the plant develops corresponding R-genes, this process can continue indefinitely; hence the "zig-zag" shape between susceptibility and resistance (Jones and Dangl, 2006).

Cmn differs from this model, however. The bacterium lacks the type III secretory system (T3SS), one of the main delivery methods of effectors into the host plant (Mbofung et al., 2016). Other subspecies of *Clavibacter* also lack the T3SS and are still able to utilize effectors. A well-known example is the use of tomatinase by *Clavibacter michiganensis* subsp. *michiganensis* to degrade α-tomatine, which has antimicrobial properties, in tomatoes (Kaup et al., 2005).

Quantitative Resistance: Disease resistance in plants can be classified as either qualitative or quantitative. Qualitative R-genes confer complete resistance to a disease. To date, no R-genes for Goss's wilt have been discovered and complete immunity does not exist (Jackson et al., 2007). In contrast, quantitative disease resistance varies in a continuous fashion across a population (Jamann et al., 2015;Poland et al., 2009). While quantitative resistance is generally conferred by a single R-gene, quantitative resistance is composed of many quantitative resistance loci (QRL). QRL are regions of the genome that are significantly associated with disease resistance (Jamann et al., 2015;Stuber, 1995). R-genes impart a large selection pressure on pathogens and quickly break down, whereas QRL exert a smaller effect and are therefore more durable (Lindhout, 2002;Parlevliet, 2002).

There are two methods commonly used to map quantitative trait loci (QTL). Linkage mapping uses populations derived from controlled crosses. A number of different population structures have been developed for linkage mapping. An advanced intercross line (AIL)

population increases resolution through multiple generations of intermating. An AIL population is created by crossing the parents and self-pollinating the F_1 generation, after which each plant is used as either a male or a female in a cross with another line. A single kernel is then taken from each ear, bulked together with the other seeds collected, and randomly intermated. This process is repeated for five generations, resulting in a four-fold increase in the number of recombination events captured compared to a conventional breeding methods (Lee et al., 2002). In contrast, near isogenic lines (NIL) populations have a uniform genetic background with introgressions from a donor parent. NIL populations are created by repeatedly crossing a donor line to a recurrent parent for four generations, and then selfing five times with single seed descent to generate a population of $BC_3F_{4:5}$ lines (Jamann et al., 2015). While NILs lack the fine resolution offered by AILs, their uniform backgrounds allow for the confirmation of QTL and the detailed dissection of individual QTL.

The second method of QTL mapping is association mapping. Genome-wide association studies (GWAS) take advantage of historical recombination to identify single nucleotide polymorphisms (SNPs) in linkage disequilibrium with a trait of interest (Jamann et al., 2015). Advantages of association mapping include elimination of the need to develop biparental crosses, increased allelic diversity, and higher resolution (Jamann et al., 2015). One disadvantage of association mapping is the need to eliminate confounding effects, such as population structure. Within an association panel, sub-populations may contain different allele frequencies for a SNP. As such, SNPs in a population with a higher mean trait value may be detected as significant, even if they are not linked to a causal variant (Jamann et al., 2015;Lipka et al., 2015). Relatedness, or the chances of two alleles being identical by state or identical by descent, can also lead to spurious associations (Lipka et al., 2015). To account for relatedness and population

structure, a Q+K mixed linear model must be employed when performing GWAS (Price et al., 2006).

Resistance Mechanisms: Very little is known about the vascular or foliar resistance mechanisms employed by maize to combat Goss's wilt. Mbofung et al. (2016) compared resistant and susceptible maize hybrids and found that the resistant plants were able to reduce disease impact by limiting *Cmn* movement within the internal xylem vessels. This suggests some form of pattern recognition receptors (PRR) in the host cell membrane that are able to detect conserved structures or peptides on the *Clavibacter* bacteria (Mbofung et al., 2016). In some instances of fungal infection, for example, maize PRRs are able to mount defense responses to pathogen colonization by quickly oxidizing their phenolic compounds in order to lignify their cell walls (Beckman, 2000). This causes localized cell death, which contains the current infection and blocks further pathogen movement through the vasculature. Alternatively, structural adaptations in resistant maize may keep *Cmn* below the quorum sensing density threshold necessary for disease (Mbofung et al., 2016).

Another bacterial maize pathogen that infects through the vasculature is *Pantoea stewartii*, the causal organism of Stewart's wilt. *Pantoea stewartii* and *Cmn* cause many of the similar symptoms, including water-soaking and similar shaped lesions. In addition, resistance between the two diseases is correlated, despite *P. stewartii* being a gram-negative bacterium while *C. michiganensis subsp. nebraskensis* is gram-positive (Pataky, 1985;Pataky et al., 1988;Suparyono and Pataky, 1989). This suggests that multiple disease resistance (MDR) effective against both diseases may be present. Multiple disease resistance is defined as "host plant resistance to two or more diseases", although it does not necessarily imply a shared causal locus (Nene, 1988;Wiesner-Hanks and Nelson, 2016). Multiple disease resistance may be

achieved through pleiotropy, where one gene imparts two, unrelated effects, or through colocalization, where genetic resistance for one disease is in linkage disequilibrium with resistance to another disease and, as such, the two are inherited together.

Breeding for Disease Resistance: Quantitative trait locus mapping identifies SNPs in linkage disequilibrium with a trait of interest. These molecular markers can then be used to select for lines carrying elite agronomic traits, even when the genes underlying those properties are unknown. This process of breeding for markers rather than genes is called marker assisted selection (MAS) and can be useful for pyramiding complimentary QTL together by selecting for lines with the associated SNPs (Ribaut and Hoisington, 1998). Unfortunately, for MAS to be successful the trait of interest must not only be in strong linkage disequilibrium with an identifiable marker, but must also have a large enough effect size to be detected by QTL mapping. In order to account for the genotypic effects too small to detect using classical linkage or association mapping, breeders have turned to a new method called genomic selection.

Genomic selection uses genome-wide molecular makers to predict the phenotypic value of an individual based off all genotypic effects, large and small (Meuwissen et al., 2001). In 1918, R.A. Fisher proposed the “infinitesimal model”, which states that as the number of genes affecting a trait increases, the effect size of each gene decreases (Fisher, 1918). Genomic selection is based on the theory that quantitative traits are highly polygenic and thus their variation is better captured by modeling of all genome-wide markers. If all genetic variance can be explained by the available marker data, then it becomes possible to quantify the additive contribution of countless, small effect loci to the phenotypic variation (Goddard and Hayes, 2007).

Genomic selection is performed by dividing a population into a "training" and a "prediction" population. The "training" population consists of both phenotypic and genotypic data (Meuwissen et al., 2001). Based off the phenotypic values, a prediction model is calculated that assigns an additive effect to each marker in the genotypic dataset. This prediction model is then used to calculate a genomic estimated breeding value (GEBV) for the "prediction" population using only genotypic data (Meuwissen et al., 2001). If the prediction model has high accuracy, elite lines can be selected based on the GEBV rather than relying on time-consuming and costly phenotyping. Genomic selection increases gains per unit time and immediately expedites the entire breeding cycle (Wong and Bernardo, 2008).

HYPOTHESES FOR REEMERGENCE

Genome Shift: Many theories have been proposed regarding the reemergence of Goss's wilt, but to date no single cause has been confirmed. One possible hypothesis is that a shift in the pathogen genome has afforded *Cmn* an advantage in its evolutionary arms race with maize. Evidence for this theory can be seen by comparing two existing studies, one performed by Vidaver et al in 1981 and one more recently by Agarkova et al. in 2011.

In the Vidaver et al. (1981) study, the *Cmn* genome was found to be relatively stable with no discernable patterns across years or even geographic location. However, Agarkova et al. (2011) revealed that, in the years following 1981, there was a significant shift in the pathogen. Using AFLP and BOX-PCR to perform cluster analysis, *Cmn* was split into two distinct groups, subtypes A and B. Population structure can arise when allele frequencies begin to differ within sub-populations due to factors such as selection or genetic drift. In the case of Goss's wilt, subtype A was the stable isolate of *Cmn* seen by Vidaver in 1981. Subtype B, however, appears to have only formed after 1999 and has increased genetic diversity (Agarkova et al., 2011).

These studies are based on population structure and phylogeny, so it is so far unclear if and how this genetic shift impacts *Cmn* virulence. In addition, the proportion of strains in Subtype B only accounted for 18.8% of the total isolates examined in this experiment. This does not make up a significant enough proportion of the pathogen population to account for its unprecedented recent outbreak.

Cultural Practices: Many researchers accredit the increase in Goss's wilt with changes in cultural practices. Recent years have seen a shift in both continuous cornfields and reduced or no-till systems (Borchers et al., 2014;Wade et al., 2015). Because *Cmn* overwinters in infested maize residue along the soil surface, no-till continuous cornfields are predisposed towards Goss's wilt outbreak (Johal and Huber, 2009). Another cultural trend worth noting is the decrease in spacing between maize rows since the 1950's (Lauer, 1996). This increase in plant density has been associated with an increase in *Cmn* outbreaks. In a survey of over 486 infected fields, locations planted with susceptible hybrids at >67,500 seeds/ha displayed Goss's wilt symptoms 88% of the time (Langemeier et al., 2017). This may be due to infected leaves being in closer physical proximity to uninfected leaves or a more humid microclimate within the foliage.

Some speculate that glyphosate resistance in maize may be to blame for the increase of *Cmn* in recent years. Survey results from 486 locations across nine states including Nebraska, Iowa, Colorado, Indiana, Kansas, Minnesota, South Dakota, and North Dakota showed that applications of glyphosate or foliar fungicides were associated with higher levels of disease incidence (Langemeier et al., 2017). It should be noted that this paper only notes a correlation, and does not attempt to prove any causation between Goss's wilt and glyphosate application. Williams et al. (2015) found no correlation between glyphosate resistance and Goss's wilt. When

maize plants were inoculated both prior to and following glyphosate applications, not only were no significant correlations found between Goss's wilt yield loss and glyphosate resistance, but transgenic glyphosate was actually seen to improve yield traits when compared to herbicide free-treatments (Williams et al., 2015).

Another factor that may play a role in the spread of *Cmn* is the increase of severe weather events due to climate change (EPA, 2017). Extreme storms are more likely to produce the type of strong winds, rain, and hail that cause physical damage to corn fields. Wounding is the primary entry method for *Cmn* and strong rains are needed to splash the non-motile bacteria onto its compromised host (Jackson et al., 2007). With climate change showing no signs of abating, Goss's wilt incidence may only get worse.

CONCLUSIONS

Goss's wilt, caused by *Cmn*, causes foliar blight lesions and vascular wilt on maize and poses a serious threat to our continued corn production abilities. Although Goss's wilt has emerged as one of the leading causes of yield loss in maize over the last decade, this review has demonstrated that very little is known about the bacteria's virulence. Even less is known about host resistance mechanisms. Future research should be directed at determining the cause behind Goss's wilt reemergence and characterizing host resistance and bacterial virulence.

This research intends to identify regions of the host genome associated with Goss's wilt resistance and to evaluate the efficacy of genomic selection in predicting the Goss's wilt phenotype. The insights we gain through these experiments will have implications for other vascular diseases of maize. Through this work, we hope to provide a substantial contribution towards research across the country to combat this devastating disease.

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CHAPTER 2: IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR GOSS'S WILT IN THE INTERMATED B73 X MO17 AND RELATED NILS

ABSTRACT

Since its discovery in 1969, Goss's wilt, a foliar blight and vascular wilt disease caused by the gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), has emerged as one of the top four diseases of maize in the United States and Canada. No source of complete resistance has been described for Goss's wilt, and little is known about the genetic and mechanistic basis of host resistance to *Cmn*. Our objective was to perform linkage mapping on three populations to uncover the genomic regions associated with Goss's wilt resistance. We evaluated the Intermated B73 x Mo17 (IBM) population and two corresponding disease resistant introgression lines (DRIL) populations: B73(4) x Mo17 and Mo17(4) x B73. We identified putative QTL in bins 1.05-1.06, 2.06, 7.01-7.02, 8.05, and 10.04, both confirming previous findings and identifying novel resistance QTL. The QTL on chromosome 1, designated *qGW1.06*, was identified in multiple environments and overlaps with a known multiple disease resistance locus. The QTL in bin 8.05 represents a novel region associated with Goss's wilt. Using the data from this study and previous studies, we found that Goss's wilt resistance was correlated with northern leaf blight, but not gray leaf spot or southern leaf blight. These results offer a deeper understanding of the genetic basis of resistance to Goss's wilt in maize that may facilitate breeding for resistance and *qGW1.06* is a strong candidate for further characterization and use.

INTRODUCTION

Little is known about resistance to the recently emerged disease Goss's wilt of maize (Jackson et al., 2007). Goss's wilt is caused by the gram positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), which produces foliar blight lesions and vascular wilt symptoms in susceptible maize varieties (Vidaver and Mandel, 1974). The disease was first observed in Nebraska in 1969 and over the next ten years was observed in Kansas, Iowa, South Dakota, Wyoming, and Colorado (Jackson et al., 2007; Vidaver et al., 1981). While initially devastating, disease incidence decreased following the initial emergence, due in large part to the development of resistant varieties by plant breeders, and by the early 1980's occurrences were confined to highly susceptible varieties or plants that sustained severe physical injury (Jackson et al., 2007). This period of remission did not last, however. In 2006, farmers in western Nebraska, southeastern Wyoming, and eastern Colorado began reporting symptoms characteristic of Goss's wilt (Jackson et al., 2007). Since then, the spread of Goss's wilt has been extensive; as of 2013, the disease has been identified in fields stretching from Louisiana to Alberta, Canada (Howard et al., 2015; Jackson et al., 2007; Singh et al., 2015). To date, little is known about the cause of this sudden reemergence of Goss's wilt; hypotheses range from pathogen genome shifts (Agarkova et al., 2011) to the recent trends towards no-till agriculture practices (Jackson et al., 2007).

Cmn overwinters in infected crop residue on the soil surface (Schuster, 1975). Infection occurs most readily through wounded or damaged tissue; however, disease development can occur in unwounded plants subjected to high humidity conditions (Mallowa et al., 2016). Hail damage and sand-blasting both cause the type of mechanical injury necessary for infection, but

splashing water is often necessary to move the pathogen, as *Cmn* is non-motile (Jackson et al., 2007; Vidaver and Mandel, 1974).

Goss's wilt is well known for having two distinct types of symptoms - systemic wilt and leaf blight. Severe wilt symptoms are often found in young plants, with 2-week-old seedlings being the most susceptible (Calub et al., 1974). In contrast, 8-week-old plants may take an additional 6-10 weeks to display symptoms (Calub et al., 1974). Seed transmission from infected parents is extremely rare, only occurring in 0.04-0.10% of cases (Biddle et al., 1990). Vascular wilt symptoms are easily recognizable by the internal orange discoloration of the vascular bundles and by the external water-soaked and slimy appearance of the stalk (Jackson et al., 2007). Mature maize plants are more likely to develop foliar blight lesions rather than vascular wilt (Calub et al., 1974). Blight symptoms first appear as pale, water-soaked areas near the inoculation point. Necrotic areas develop parallel to the leaf veins and soon coalesce into large, tan lesions (Calub et al., 1974). Along the lesions, small, dark green to black, discontinuous, water-soaked spots begin to appear, commonly referred to as "freckles" (Jackson et al., 2007).

Yield losses from Goss's wilt vary depending on the susceptibility of the maize hybrids tested. In a replicated, multi-year study of sweet corn, resistant hybrid yield losses ranged from only 0-8%, however, in susceptible sweet corn germplasm losses exceeded 27% (Pataky et al., 1988). In inoculated trials with field corn germplasm, yield losses in susceptible maize hybrids was >40% (Carson and Wicks, 1991). During the period from 2012 to 2015, approximately 501 million bushels were lost in the United States and Canada due to Goss's wilt, making it the fourth most severe disease of maize during this period (Mueller et al., 2016).

Cultural, chemical, and genetic control strategies have been investigated to combat Goss's wilt, with varying degrees of success. No chemical control methods have been shown to decrease Goss's wilt occurrence at a statistically significant level (Mehl et al., 2015). Because Goss's wilt is known to overwinter in infected residue, cultural practices, such as crop rotation and conventional tillage systems, have been employed to reduce initial disease incidence (Jackson et al., 2007). High plant density has been correlated with increased disease pressure. This may be due to increased physical contact between symptomatic and healthy leaves or increased humidity within the row canopy (Langemeier et al., 2017). Therefore, fields planted at lower densities face a lower risk of a Goss's wilt outbreak.

One of the best strategies for mitigating the effects of Goss's wilt remains to plant resistant maize hybrids; however only two previous studies have examined genetic resistance in maize and identified genomic regions involved with resistance to Goss's wilt (Schaefer and Bernardo, 2013; Singh et al., 2016). Resistance to Goss's wilt is quantitative (Treat and Tracy, 1990), consisting primarily of small, additive effects (Singh et al., 2016). To date, no R-genes for Goss's wilt have been discovered, and complete immunity does not exist (Jackson et al., 2007). Several regions of the maize genome have been associated with quantitative resistance to Goss's wilt. Linkage mapping using three recombinant inbred line (RIL) populations, B73 x Oh43, B73 x HP301, and B73 x P39, identified 19 putative resistance quantitative trait loci (QTL) (Singh et al., 2016). The effect size of each QTL was small, and none contributed more than 6% of the total observed phenotypic variation. In this study, heritability was high, ranging from 0.60-0.62, across all three populations (Singh et al., 2016). Genome-wide association mapping for Goss's wilt was conducted on a panel of historically important maize lines from Minnesota (Schaefer and Bernardo, 2013). This study identified 8 putative QTL, located on chromosomes 1, 4, 5, 7,

and 9, however this study was conducted in only one environment and requires further validation (Schaefer and Bernardo, 2013).

Populations with previously unstudied allelic diversity are needed to identify novel regions associated with Goss's wilt resistance and to confirm previous findings. The intermated B73 x Mo17 (IBM) population, an advanced intercross line (AIL) population, has been used in numerous mapping studies of complex quantitative traits and offers high-resolution mapping (Lee et al., 2002). The IBM population was generated by self-pollinating the F_1 generation, after which each plant was used once, either as a male or a female, in a cross with another plant for a total of 250 new crosses. A single kernel was then taken from each ear, bulked together with the other seeds collected, and randomly intermated. This process was repeated for a total of five generations, resulting in a four-fold increase in the number of recombination events captured compared to a conventional recombinant inbred line (RIL) population (Lee et al., 2002). Compared with a conventional RIL population with the same parents, between 5 and 50 times greater mapping resolution was observed in the IBM (Balint-Kurti et al., 2007).

Two disease resistance introgression line (DRIL) populations complimentary to the IBM population have been developed, one with B73 as the recurrent parent and one with Mo17 as the recurrent parent (Lopez Zuniga, 2016). The DRIL populations were created by repeatedly crossing the donor line to the recurrent parent for four generations and selfing five times with single seed descent to generate a population of $BC_3F_{4:5}$ lines (Lopez Zuniga, 2016). While these two populations lack the fine resolution of the IBM, their uniform backgrounds allow for the confirmation of QTL and the detailed dissection of individual QTL.

The objective of this study was to use linkage mapping to identify regions of the genome associated with Goss's wilt resistance, confirm these putative QTLs in the B73(4) x Mo17 and Mo17(4) x B73 DRIL populations, and compare these results to previous studies to assess whether multiple disease resistance effective against Goss's wilt and other foliar diseases exists in the IBM population.

MATERIALS AND METHODS

Germplasm: Three mapping populations were used to identify QTL for Goss's wilt resistance in maize, including the IBM population (Lee et al., 2002) and two related DRIL populations: B73 (donor) x Mo17 (recurrent) introgression lines (DRIL14) and Mo17 (donor) x B73 (recurrent) introgression lines (DRIL41) (Lopez Zuniga, 2016). The DRIL41 and DRIL14 populations were derived from a reciprocal cross between B73 and Mo17 and three generations of backcrosses, followed by four consecutive generations of self-pollinating via single seed descent (Lopez Zuniga, 2016). The seed for the IBM population was obtained from Dr. Steve Moose at the University of Illinois at Urbana-Champaign.

Field Design: Populations were grown as single-row plots in the 2016 and 2017 summer field season at the Crop Science Research and Education Center in Urbana, IL. Plots measured 3.2-meters with 0.76-meter alleys and had a row spacing of 0.762 meters. Plots were machine-planted at a density of 20 kernels/row. Standard agronomic practices for central Illinois were used. In 2016, 287 IBM lines were evaluated for Goss's wilt with three replications, and, in 2017, 234 IBM lines were tested over two replications. The difference in the number of lines tested in 2016 and 2017 was due to seed availability. An incomplete block design was created

using the *agricolae* package (Mendiburu, 2017) in R statistical software version 3.3.2 (R Core Team, 2016) with B73 and Mo17 included as checks in each incomplete block.

To confirm findings from the IBM population, the DRIL14 and DRIL41 populations were evaluated for Goss's wilt. In 2016, two replications of 48 lines of the DRIL14 population were evaluated, followed by two replications of 45 lines in 2017. Additionally, one replication of 53 lines of the DRIL41 population was evaluated in 2016, and three replications of 47 lines were screened in 2017. Different numbers of replications were used in different years due to seed availability and space constraints. Due to the small number of total lines, the DRIL experiments were designed as randomized complete blocks using the *agricolae* package (Mendiburu, 2017) in R statistical software version 3.3.2 (R Core Team, 2016). One plot of each of the parents (B73 and Mo17) was included in each replication.

Inoculation and Disease Rating: *Cmn* strain *16Cmn001* was previously isolated from diseased leaf material from Illinois and maintained in glycerol stocks stored at -80°C for use in the 2016 and 2017 field seasons. Single colonies were grown in nutrient broth yeast extract (NBY) on a shaker for two to three days. The final bacterial cell concentration was adjusted to 10^7 colony forming units per ml using a spectrophotometer (OD_{600}=0.05) (Pataky, 1985). Inoculations were performed twice on all plants within the plot, one week apart, between the V4 and V7 stages using a pinprick inoculation method to simulate mechanical damage (Blanco et al., 1977; Chang et al., 1977).

Disease ratings were performed twice, approximately every two weeks after initial inoculation. Inbreds were scored on a per-plot basis using a 0-100% scale with 5% intervals (Figure A.1). Ratings represented the total percent of infected leaf area, with 0% representing no

symptoms and 100% denoting complete plant death (Poland and Nelson, 2011). The area under disease progress curve (AUDPC) was calculated for each plot to represent disease progression throughout the season based on the equation $AUDPC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})(t_{i+1} - t_i)}{2}$ where y_i is the disease severity rating at time *i*; $t_{i+1} - t_i$is the day interval between two ratings; and *n* is the number of ratings (Wilcoxson et al., 1974) (Figure 1.1).

Phenotypic Data Analysis: Linear models were run using the lme4 package (Bates et al., 2015) in R statistical software version 3.3.2 (R Core Team, 2016). Best linear unbiased predictors (BLUPs) were predicted for each of the populations across all years, as well as for each year individually. All factors were fit as random effects. For the IBM population genotype, year, replication nested within year, and block nested within replication nested within year were included in the final combined year model (Table 1.1). The genotype-by-environment interaction was not found to be significant and excluded from the final model. The final model for the DRIL14 population included genotype and year, as replication and the genotype-by-environment interaction were not significant. The final model for the DRIL41 population included genotype, year, and the genotype-by-year interaction (Table 1.1), as replication was not significant. BLUPs, including the intercept, were calculated for each genotype (Figure 1.1). Individual years for the IBM population were analyzed using a linear model where AUDPC ~ genotype, replication, and block nested in replication. For the DRIL14 and DRIL41 populations, models for individual years included genotype and, in the case of DRIL14 in 2016, a factor for the field coordinate range was also included.

Heritability was calculated using the PROC MIXED procedure of SAS software (SAS ver. 9.4, SAS Institute, Cary NC), as described by Holland et al. (2003). A Pearson's correlation

coefficient was obtained between Goss's wilt BLUPs across years for each population. Pearson's correlation coefficient was also obtained between BLUPs for Goss's wilt, northern leaf blight (NLB), southern leaf blight (SLB), and gray leaf spot (GLS) (Balint-Kurti et al., 2008; Balint-Kurti et al., 2010; Balint-Kurti et al., 2007) using the PROC CORR procedure of SAS software (SAS version 9.4, SAS Institute). NLB and GLS scores were inverted so that scales were consistent between diseases with low values indicating resistance and high values indicating susceptibility.

Linkage Mapping: For the IBM population we utilized 1,324 genotyped single nucleotide polymorphism (SNP) markers for QTL analysis (http://maizegdb.org/data_center/qtl-data). A total of 337 genotyped SNPs were used for the DRIL41 DRIL population and 323 genotyped SNPs were used for the DRIL14 DRIL population (Lopez Zuniga, 2016). QTL mapping was performed on the IBM using R/qtl version 1.41-6 (Broman et al., 2003) and the DRILs using ICIMapping version 4.0.6.0 (Meng et al., 2015). Different programs were used due to the different population types and program optimization for these population types. In R/qtl, genotypes were first imputed using the function "sim.geno" in order to estimate missing genotypes based on the observed marker data. A total of 128 imputations were simulated, with a step size of two and a genotyping error rate of 0.001. To determine the LOD threshold representing an experiment-wide error rate of 0.10 for each population, we performed 1,000 permutations using the multiple imputation method algorithm (Chen and Kendziorski, 2007; Meng et al., 2015). Peak markers with LOD scores above the permuted threshold were identified using the multiple imputation "imp" method of the "scanone" function, with a window size of 3 for each population. A 2-LOD support interval and the percent of phenotypic variance associated with each marker were calculated using the R/qtl functions "lodint" and "fitqtl," respectively.

QTL positions are reported by bin number (Davis et al., 1999). For ICIMapping, the Chromosome Segment Substitution option was chosen (Meng et al., 2015). Chromosome Segment Substitution allows the comparison of trait performance between unique chromosome segments and the recurrent parent. A likelihood ratio is then used to identify the segments statistically associated with the trait of interest. The step-wise regression likelihood ratio test was performed using the RSTEP-LRT-ADD function and QTL with a LOD score greater than 3.00 were identified.

RESULTS AND DISCUSSION

Characterization of Germplasm: The IBM is an AIL population derived from B73 and Mo17 that offers enhanced genetic resolution compared to other biparental populations. While the identified QTL may not be easily used in breeding programs due to the interheterotic group nature of the population, there are certain advantages associated with the IBM. It is a powerful tool for QTL discovery and is readily available. There are two corresponding DRIL populations associated with the IBM; B73 as a donor and Mo17 as the recurrent parent (DRIL14) and Mo17 as the donor and B73 and the recurrent parent (DRIL41). The DRIL populations can be used to corroborate findings and further dissect the QTL mapped in the IBM. Together, these three populations can be used to identify the genes underlying resistance to Goss's wilt.

Substantial transgressive segregation was observed in the IBM population (Figure 1.1). The IBM population had a wide range of disease scores, from 0-95% infected leaf area for individual disease ratings. In comparison to the IBM, the DRIL14 and DRIL41 populations never reached a disease rating >35% for an individual diseased leaf area rating in either year and had lower population means than the IBM population. This is reflected in the BLUP values that

were calculated based on the AUDPC scores, where the highest BLUP within the IBM population was 335.59, and the highest BLUP in the DRIL14 and DRIL41 populations were 219.21 and 256.25, respectively. The DRIL14 BLUPs were skewed towards resistance because of the relatively resistant phenotype of the recurrent Mo17 and more susceptible phenotype of the donor B73. In contrast, the DRIL41 BLUPs were concentrated around the recurrent parent B73 and showed susceptible transgressive segregation with lines more susceptible than either the recurrent or donor parent. Less disease was observed in the DRIL41 population than in the IBM, despite the susceptible parent (B73) being the recurrent parent in the DRIL41 population. We believe this is because less disease was observed in 2017 than 2016, and the DRIL41 population had 1 replication in 2016 and three replications in 2017, while the IBM had three replications in 2016 and two replications in 2017.

Genotype, year, replication, block (for the IBM), and genotype-by-year were included in the analysis to determine which factors to include in the BLUP calculations. For the IBM, genotype, year, replication nested within year, and block nested within replication nested within year were significant in an ANOVA and included in BLUP calculations (Table 1.1). The DRIL14 population analysis included genotype and year, while the DRIL41 linear model included genotype, year, and the genotype-by-year interaction (Table 1.1). In each population, year accounted for the most variance. Unlike previous studies, which found highly significant genotype-by-year interactions (Ngong-Nassah et al., 1992; Treat and Tracy, 1990), the genotype-by-year effect was only significant for one of the three populations. Our findings are consistent with Singh et al.'s (2016) findings which did not find significant variation caused by genotype-by-year in some B73-derived populations.

Pearson's correlation coefficients were calculated for each population across years. Between 2016 and 2017, the IBM had a correlation of 0.3465 (P <0.0001), DRIL14 of 0.3741 (P <0.0001), and DRIL41 of 0.42272 (P <0.0001). Hot and dry weather conditions during inoculations in 2017 may have accounted for some of the differences observed between years; however, *p*-values were significant for all populations. Because of these environmental conditions, less disease was observed in 2017 than in 2016.

Heritability was calculated for each population using the method described by Holland et al. (2003). Heritabilities were consistent across populations, with plot heritability values ranging from 0.24 to 0.35 and family-mean heritability values falling between 0.53 and 0.63 (Table 1.2). In comparison, Singh et al. (2016) reported family-mean heritabilities ranging from 0.60 to 0.62. A study by Ngong-Nassah et al. (1992) crossed four resistant x susceptible and two moderately resistant x susceptible inbreds to create six populations to study the inheritance of Goss's wilt resistance in maize populations, and broad-sense heritabilities ranged from 0.21 to 0.80. Heritability was highest in crosses between extremely resistant and extremely susceptible lines. Heritability was lower in populations with parents of more similar phenotypes, as was the case with our B73 x Mo17 derived populations.

Linkage Mapping: We identified seven QTL for Goss's wilt resistance using linkage mapping (Table 1.3 and Figure 1.2). Five QTL were found in the IBM population and were located in bins 1.05-1.06, 2.06-2.07, 7.01-7.02, 8.05, and 10.04. Two additional QTL were found in the DRIL14 population in bins 5.09 and 7.00. No significant QTL were identified in the DRIL41 population. In the DRIL 41 population, we expected to observe lines that were more resistant than B73, as Mo17 is the donor and is more resistant, but were unable to do so (Figure 1.1). Thus, the lack of

QTL in this population may have been the result of our inability to accurately distinguish phenotypic differences. In addition to the mapping on the combined dataset, linkage mapping was performed for individual years. In the IBM, QTL corresponding to those found in the combined dataset were identified, with QTL that mapped to 1.05-1.06 and 8.05 in 2017 and 1.05-1.06, 2.06, and 10.04 in 2016 (Figure 1.2). In the DRIL14 population, the putative QTL at 7.00 was identified in 2016, however no significant QTL were observed in 2017. It is possible we did not identify the chromosome 1 QTL in this population because the population size is small and only three lines had introgressions spanning the 1.06 region.

One of our goals was to confirm IBM QTL in related, yet independent, populations. There were no overlapping QTL between the three populations examined. It is possible that the chromosome 7 QTL identified in the DRIL14 population and the chromosome 7 IBM QTL are the same QTL with slightly shifted positions in the two populations. The lack of correspondence in QTL between the three populations may be attributed to a number of possible factors. Small population sizes in the DRIL populations reduced the power to detect QTL, particularly the power to detect QTL with small effect sizes. The QTL detected in the IBM accounted for a small percentage of the total variance (R^2 = 3.4 - 7.5%) and had small effect sizes (A= -13.7-9.67) (Table 1.3). Phenotyping of the DRIL populations was problematic, as both parents were relatively resistant and differences due to the introgressions were difficult to distinguish. While we found significant differences between Mo17 and B73 (P-value=4.9×10^{-8}), both parents are relatively resistant to Goss's wilt. Parents with a stronger phenotypic difference would allow for more accurate phenotyping and improved QTL detection.

Only two other studies have mapped resistance to Goss's wilt. Singh et al. (2016) reported Goss's wilt resistance QTL in B73-derived populations, including B73 x HP301, B73 x Oh43, and B73 x P39. Their study identified putative QTL on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, and 10. Schaefer and Bernardo (2013) examined a collection of Minnesotan inbred lines and identified significant associations in bins 1.06, 1.10, 4.05, 4.09, 5.04, 5.05, 7.02, 9.02, and 9.06. The QTL we found in 1.05-1.06 colocalizes with the QTL described by Singh et al. (2016) and Schaefer and Bernardo (2013). In addition, the QTL we found in 2.06 overlaps with the QTL identified by Singh et al. (2016), and the QTL we identified in bins 7.01-7.02 colocalizes with the significant association identified by Schaefer and Bernardo (2013). The results of our study confirm the previous finding of potential resistance loci on chromosomes 1, 2, and 7, while the putative QTL we report in bin 8.05 is the first description of a Goss's wilt resistance QTL on that chromosome.

Multiple Disease Resistance: Multiple disease resistance refers to either host plant resistance to more than one disease or a gene or allele that confers resistance to more than one disease (Wiesner-Hanks and Nelson, 2016). We wanted to test the hypothesis that resistance to Goss's wilt is related to resistance to other diseases. Previous studies have reported a correlation between sweet corn hybrids evaluated for Goss's wilt, Stewart's wilt, and NLB (Pataky, 1985; Pataky et al., 1988; Suparyono and Pataky, 1989). Our goal was to test whether there was a correlation between Goss's wilt and other foliar diseases. In addition to Goss's wilt, the IBM population has been evaluated for other diseases including NLB, SLB, and GLS (Balint-Kurti et al., 2010; Balint-Kurti et al., 2007; Benson et al., 2015). Pearson's correlation coefficients were calculated for Goss's wilt, NLB, GLS, and SLB in the IBM population. Goss's wilt was significantly correlated with NLB, but not GLS and SLB (Table 1.4). These results indicate that

an IBM line that is more resistant to NLB has an increased probability of being resistant to Goss's wilt. There may be some mechanistic basis for this observation, as the vascular nature of pathogenesis by *Setosphaeria turcica*, causal agent of NLB, is comparable to the vascular nature of infection by *Cmn* (Chung et al., 2010; Jennings and Ullstrup, 1957; Mbofung et al., 2016).

In order to identify regions that may be implicated in resistance to multiple diseases including Goss's wilt, we compared QTL found in the IBM for the other diseases to those identified for Goss's wilt. The 1.06 region that has been previously associated with resistance to GLS (Benson et al., 2015) and NLB (Balint-Kurti et al., 2010) falls within the 1.05-1.06 interval associated with Goss's wilt in our study. Additionally, this QTL falls within the same region on chromosome 1 as *qMDR1.06*, a multiple disease resistance locus identified by Wisser et al. (2006). *qMDR1.06* has been implicated in resistance to Stewart's wilt, NLB, SLB, and a number of other maize diseases (Chung et al., 2010; Jamann et al., 2014; Wisser et al., 2006). The Goss's wilt resistance QTL at 1.05-1.06, which we will refer to as *qGW1.06*, was found in 2016, 2017, and in the combined dataset. *qGW1.06* accounted for the most variance of all QTL detected in the IBM, had one of the largest effect sizes, and its presence in multiple years demonstrates that it is active across different environments. The effect size of *qMDR1.06* varies by disease. While it confers a large effect for Stewart's wilt, it has a smaller effect on NLB (Jamann et al., 2014). The *pan1* gene which contributes to asymmetric cell division (Cartwright et al., 2009) is located within the *qGW1.06* interval. Two independent lines homozygous for independent mutations in *pan1* have increased resistance to the vascular diseases NLB and Stewart's wilt, implying that *pan1* is a multiple disease susceptibility gene (Jamann et al., 2014). Further work is needed to determine whether *pan1* is involved in resistance to Goss's wilt.

CONCLUSIONS

We have identified several QTL in the IBM and DRIL14 populations associated with Goss's wilt. Quantitative trait loci in bins 1.05-1.06, 2.06-2.07, 7.01-7.02, 8.05, and 10.04 confirm putative QTL found in previous studies and correspond to known regions associated with multiple disease resistance (Balint-Kurti et al., 2010; Benson et al., 2015; Schaefer and Bernardo, 2013; Singh et al., 2016; Wisser et al., 2006). In particular, we have identified a QTL in bins 1.05-1.06 that overlaps with a known multiple disease resistance locus (Wisser et al., 2006) and the NLB and Stewart's wilt susceptibility gene *pan1* (Jamann et al., 2014). Additionally, we have discovered a novel QTL for Goss's wilt resistance at 8.05. Future work will confirm the QTL identified in this study, examine the role of the *pan1* gene with respect to Goss's wilt, and attempt to isolate the casual mechanisms underlying the identified QTL. Because so little is known about *Cmn* pathogenesis, dissection of these QTL may offer insight regarding the pathogen's virulence and the corresponding plant defense mechanisms. In order to breed for resistance to Goss's wilt, future work includes testing the stacking of QTL within the DRIL populations and studying hybrid efficacy by testcrossing the best DRILs and recurrent parents. These studies would inform the improvement of Goss's wilt within breeding populations. In conclusion, the QTL we have identified improve the understanding of the genetic architecture of resistance to Goss's wilt, and we have identified regions for follow up for resistance breeding and to dissect disease resistance mechanisms effective against *Cmn*.

TABLES AND FIGURES

Table 1.1. Variance component estimates and their standard errors for factors included in the analysis for the IBM, DRIL14 (B73(4) x Mo17), and DRIL41 (Mo17(4) x B73) populations. All tabled variance component estimates were found to be significantly different than zero (P < 0.05).

	Variance	Standard Error
IBM		
Genotype	2672.3	51.69
Year	15043.2	122.65
Replication(Year)	403.9	20.1
Block(Replication(Year))	925.8	30.43
Error	5295.5	72.77
DRIL14		
Genotype	1758	41.93
Year	2809	53
Error	3581	59.84
DRIL41		
Genotype	1418.3	37.66
Year	9960.1	99.8
Genotype * Year	687.4	26.22
Error	1537.6	39.21

Table 1.2. Heritabilities on a plot and family-mean basis with corresponding standard errors (SE) for the IBM, DRIL14 (B73(4) x Mo17), and DRIL41 (Mo17(4) x B73) populations.

Population	Heritability (Plot basis) (SE†)	Heritability (Family-mean basis) (SE†)
IBM	0.312(0.038)	0.630(0.051)
B73(4) x Mo17 DRIL	0.235(0.104)	0.525(0.162)
Mo17(4) x B73 DRIL	0.348(0.109)	0.565(0.128)

† Standard error

Table 1.3. Significant genetic markers for the IBM and DRIL14 (B73(4) x Mo17 Introgression Line) populations in the combined 2016&17 dataset, as well as individual years.

Dataset[†]	Chr.[‡]	Bin[§]	Peak Marker	2-LOD interval[¶]	LOD[#]	Threshold[††]	$R^{2‡‡}$	A[§§]
IBM-2016&17	1	1.05-1.06	asg58	168.0-187.8	6.54	3.44	7.60	-11.8
IBM-2016&17	2	2.06-2.07	uaz194a	184.0-189.4	4.14	3.44	5.24	9.67
IBM-2016&17	7	7.01-7.02	bnlg2203	8.837-89.907	3.22	3.44	4.10	8.77
IBM-2016&17	10	10.04	isu058b	84.172-124.4	3.72	3.44	3.43	7.96
IBM-2016	1	1.05-1.06	c1.loc482	155.7-188.1	4.84	3.19	5.77	-9.85
IBM-2016	2	2.06-2.07	uaz194a	175.1-189.4	3.69	3.19	4.35	8.69
IBM-2016	10	10.04	c10.loc260	84.172-124.4	3.77	3.19	5.07	2.35
IBM-2017	1	1.05-1.06	asg58	156.4-177.1	3.78	3.09	6.37	-11.5
IBM-2017	8	8.05	c8.loc346	110.3-137.7	4.02	3.09	6.55	-13.7
DRIL14-2016&17	5	5.09	PHM13639.13	215.8	3.29	3.00	22.32	35.80
DRIL14-2016&17	7	7.00	PZA02035.5	2.584	3.65	3.00	23.45	35.9
DRIL14- 2016	7	7.00	PZA02035.5	2.584	3.16	3.00	27.30	49.82

Dataset[†]: Population followed by year(s).
Chr.[‡]: Chromosome.
Bin[§]: Chromosome bin location of each QTL (Davis et al., 1999).
2-LOD interval[¶]: Physical map positions in Mb (RefGen_v3); For DRIL14 population only the physical position of peak marker is given (RefGen_v3).
LOD[#]: The log of odds (LOD) value at the position of the peak likelihood of the QTL.
Threshold[††]: LOD threshold to identify significant QTL corresponding to an empirical Type I error value of 0.10 produced from 1000 imputations. A LOD score of 3 was used to detect significant QTL in the DRIL14 population.
$R^{2‡‡}$: Proportion of phenotypic variance explained by the detected QTL.
A[§§]: Additive effect estimates of the detected QTL. Effects are in terms of the disease rating scale employed. A negative value indicates that the Mo17 allele is the more resistant allele, while a positive value indicates that the B73 allele is the resistant allele.

Table 1.4. Pearson's correlations for multiple disease resistance in the IBM population. A correlation coefficient and *p*-value is included for each comparison. Correlations were found by comparing BLUPs of AUDPC scores for each of the diseases. Data were derived from this and three previous studies (Balint-Kurti et al., 2008; Balint-Kurti et al., 2010; Balint-Kurti et al., 2007).

	Northern Leaf Blight	Gray Leaf Spot	Southern Leaf Blight
Goss's wilt	0.384***	0.025	0.117
Northern Leaf Blight		0.291***	0.160*
Gray Leaf Spot			0.407***

*Significant at $P < 0.05$
** Significant at $P < 0.001$
***Significant at $P < 0.0001$

Figure 1.1. Phenotypic distribution of Goss's wilt in the IBM (Intermated B73 x Mo17), DRIL14 (B73(4) x Mo17 Introgression Line), and DRIL41 (Mo17(4) x B73 Introgression Line) populations. Lines were assessed visually using a 0-100% scale, and the area under the disease progress curve values were calculated based on these visual scores. The phenotypic data shown is expressed as best linear unbiased predictors of the area under disease progress curve including the intercept.

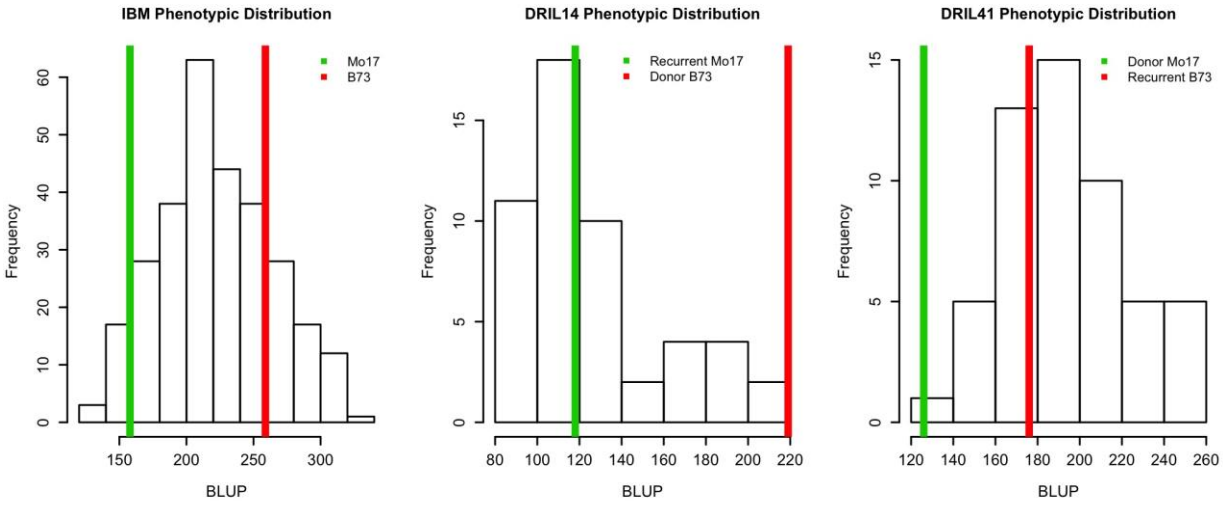
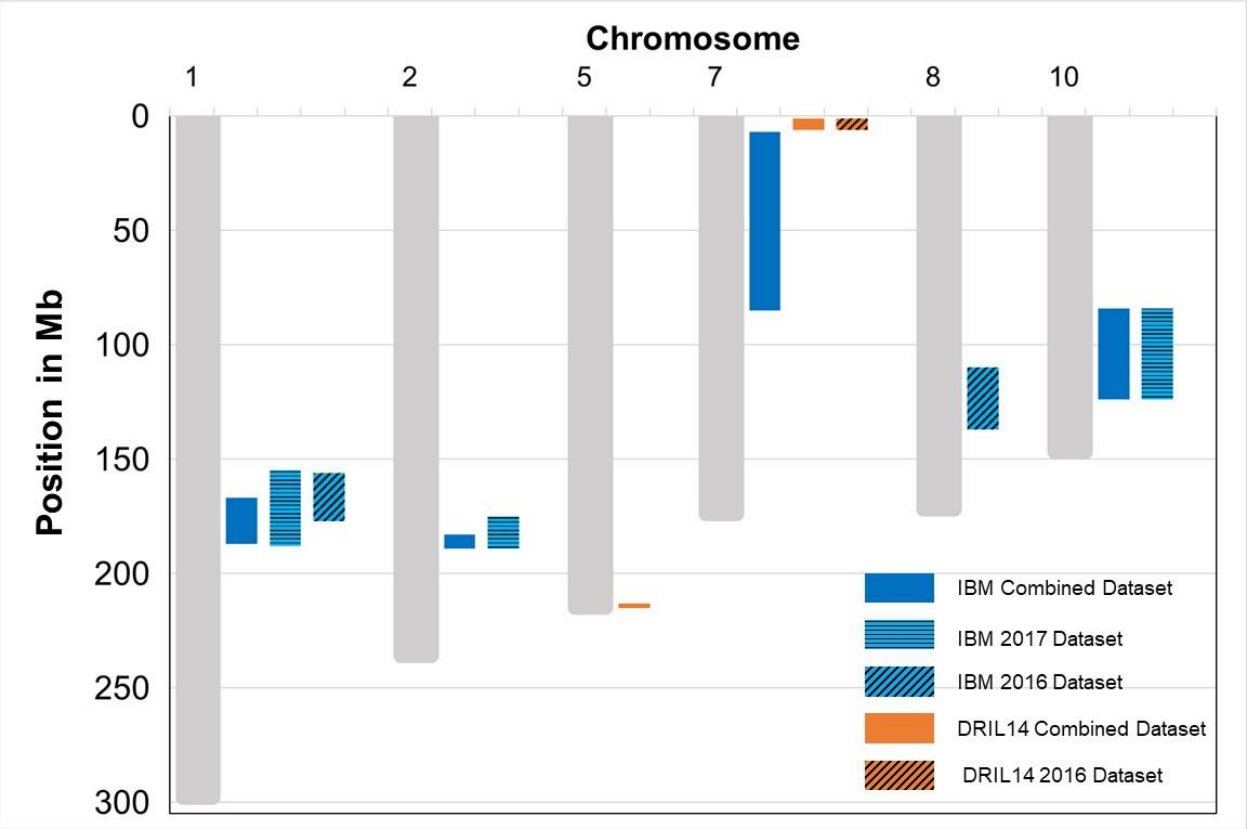


Figure 1.2. Peak SNPs in a 2-LOD interval from the IBM (Intermated B73 x Mo17) and DRIL14 (B73(4) x Mo17) populations overlaid on a physical map of the maize genome. Chromosomes are denoted as vertical gray bars, with blue bars representing IBM QTL and orange bars denoting DRIL14 QTL.



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CHAPTER 3: USE OF THE NC344 X OH7B INTROGRESSION LINES TO IDENTIFY QUANTITATIVE TRAIT LOCI FOR GOSS'S WILT OF MAIZE

ABSTRACT

Little is known about the genetic architecture underlying Goss's wilt resistance. Since it reemergence in 2006, a number of studies have attempted to identify sources of quantitative resistance to the disease in maize. Unfortunately, these studies failed to identify any large effect QTL, nor have most of their findings been further verified in subsequent work. The objective of this study was to perform linkage mapping on the NC344 x Oh7B disease resistant introgression lines (DRIL78) in order to identify novel sources of resistance to Goss's wilt, as well as confirm putative QTL previously identified in the literature. We found five QTL in the DRIL78 population previously QTL identified by Cooper et al. (2018) and Singh et al. (2016). No novel sources of resistance were found. The QTL found in this study are in clean Oh7B background, and will allow future work to further dissect the underlying causal mechanisms.

INTRODUCTION

The geographical range of Goss's wilt is expanding, and control methods are limited to cultural practices and host resistance; no effective chemical controls exist (Mehl et al., 2015). The disease, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), causes foliar blight and vascular wilt symptoms on susceptible maize lines (Schuster, 1975;Vidaver and Mandel, 1974). First identified in Nebraska, the disease gained notoriety in the western Corn Belt throughout the 1970's (Vidaver et al., 1981;Vidaver and Mandel, 1974). After breeders developed resistant hybrids, incidence of the disease began to fall and remained low until the early 2000's (Jackson et al., 2007). In 2006, Goss's wilt reappeared in Nebraska with over 50 confirmed samples, compared to the 40 total samples seen throughout the state since 1998 (Jackson et al., 2007). The range of the disease currently reaches from Alberta, Canada through Louisiana (Howard et al., 2015;Jackson et al., 2007;Singh et al., 2016).

Yield losses from Goss's wilt can be quite severe. Losses of over 50% have been observed in susceptible fields that develop symptoms early in the season (Jackson et al., 2007). In the period from 2012-2015, an estimated 501 million bushels were lost to this disease, making it the 4th most harmful pathogen of maize during the time (Mueller et al., 2016). Severity of Goss' wilt is influenced by interactions between the host, the pathogen, and the environment. Together, these factors make up a pathological concept known as the disease triangle (Agrios, 2005). Attempts to control Goss's wilt must focus on altering any of the three points on this triangle.

The extreme weather events in the Corn Belt provide the ideal environment for *Cmn*. The pathogen is non-motile and must rely on wounding of the host plant for entry (Vidaver and Mandel, 1974). Hail storms and sandblasting winds are common throughout the central United

States and help facilitate this type of infection (Jackson et al., 2007). In addition, very little is known about *Cmn* virulence. No correlations exist between colony morphology and plant pathogenicity, and attempts to identify underlying virulence genes have been largely unsuccessful (Ahmad et al., 2015). With no way to change local weather patterns and little meaningful data on *Cmn* pathogenicity, attempts to control Goss's wilt must rely on improvement of host resistance.

No major resistance genes exist for Goss's wilt (Schaefer and Bernardo, 2013;Singh et al., 2016). Instead, resistance to *Cmn* is quantitative in nature; it is composed of numerous, small effect, quantitative trait loci (QTL) (Treat and Tracy, 1990). Previous studies have successfully identified a number of QTL associated with Goss's wilt. Association mapping was performed on a panel of historically significant Minnesotan maize lines. Eight QTLs, found on chromosomes 1, 4, 5, 7, and 9, were identified and proposed as potential sources of quantitative resistance (Schaefer and Bernardo, 2013). This study represented the first example of QTL mapping for Goss's wilt in maize. In the following years, a joint linkage mapping study on three recombinant inbred line (RIL) populations, B73 x Oh43, B73 x HP301, and B73 x P39, was conducted. Putative QTLs were found on every chromosome aside from chromosome 8. More recently, linkage mapping on the Intermated B73 x Mo17 and related near isogenic lines revealed QTL overlapping with the Singh et al. (2016) and Schaefer and Bernardo (2013) results, and, in addition, identified the first QTL for Goss's wilt on chromosome 8 (Cooper et al., 2018).

Unfortunately, these studies had their own challenges as well. The association mapping by Schaefer and Bernardo (2013) was only conducted in one year, and therefore requires further validation. The joint linkage mapping of the B73 derived populations found many potential resistance QTL, but each were very small in effect and not useful for marker assisted selection in

their current, unstacked state (Singh et al., 2016). Finally, the IBM and related near isogenic lines were only able to find a QTL on chromosome 8 in one year of their dataset, not in the multi-year analysis (Cooper et al., 2018).

To confirm these previous findings, we performed linkage mapping on the NC344(4) x Oh7B disease resistance introgression lines (DRIL78) (Lopez, 2016). This population was generated via three generations of backcrosses, followed by four consecutive generations of self-pollination (Lopez, 2016). The parents were selected from the Goodman Diversity panel based on multiple disease resistance (MDR) and multiple disease susceptibility (MDS) characteristics (Flint-Garcia et al., 2005). Diseases considered in line selection included northern corn leaf blight, southern corn leaf blight, and gray leaf spot (Lopez, 2016). Oh7B has shown moderate susceptibility for Goss's wilt and NC344 displays moderate resistance. The diverse parental phenotypes used to create the DRIL78s provide a clear contrast between resistant and susceptible introgression lines. This allows for accurate phenotyping for lines containing Goss's wilt QTL and will provide loci that may serve useful in future attempts to determine underlying casual resistance mechanisms.

The objective of our study was to identify novel sources of allelic diversity for Goss's wilt and confirm putative QTL identified in previous literature. In addition, we sought to distinguish clean introgressions of the resistant NC344 genome in an Oh7B background that may prove useful for future molecular studies.

MATERIALS AND METHODS

Germplasm: The DRIL78 mapping population was used to identify Goss's wilt QTL (Lopez, 2016). The population was created by backcrossing the F1 of the donor NC344 and the recurrent

Oh7B parent back to the Oh7B recurrent parent for three generations. This was followed by four generations of self-pollinating via single seed descent to create a final population of $BC_3F_{4:5}$ lines (Lopez, 2016). Seed was obtained from Dr. Randall Wisser at the University of Delaware.

Field Design: The DRIL78 population was grown as single-row plots in 2016 at the Crop Science Research and Education Center in Urbana, IL (2016Urbana) and the 2017 summer field season in Urbana, IL (2017Urbana) and Monmouth, IL (2017Monmouth). Plots measured 3.2-meters with 0.76-meter alleys and had a row spacing of 0.762 meters. Plots were machine-planted at a density of 20 kernels/row. Standard agronomic practices for central Illinois were used. In 2016Urbana, one replication of 209 DRIL78 lines was evaluated for Goss's wilt. In 2017Urbana, two replications of 194 DRIL78s were phenotyped in Urbana, IL and two additional replications of 186 DRIL78s were evaluated in 2017Monmouth. Differences in the number of lines tested were due to seed and space availability. Experiments were laid out as an incomplete block design using the *agricolae* package (Mendiburu, 2017) in R statistical software version 3.3.2 (R Core Team, 2016). Included as check lines in each block were the resistant inbred lines NC344 or NC258 and the susceptible inbred line Oh7B.

Inoculation and Disease Rating: The Illinois *Cmn* strain *16Cmn001* was maintained in glycerol stocks stored at -80°C for use in the 2016 and 2017 field seasons. Inoculations were conducted as described in Cooper et al. (2018). Single colonies were grown on a shaker table in nutrient broth yeast extract for two to three days. A spectrophotometer was used to adjust a final inoculum concentration to 10^7 colony forming units per mL (OD_{600}=0.05) (Pataky, 1985). Inoculations were performed twice, one week apart, between the V4 and V7 stages using a pinprick inoculation method to simulate mechanical damage (Blanco et al., 1977;Chang et al., 1977). Disease ratings were taken three times on a per-plot basis, beginning two weeks after the initial

inoculation. A scale of 0%-100% with 5% intervals was used to visually score disease severity (Poland and Nelson, 2011). The area under disease progress curve (AUDPC) was calculated for each plot to represent disease severity throughout the season (Figure 2.1) (Wilcoxson et al., 1975).

Phenotypic Data Analysis: Linear models were run for the response variable AUDPC using lme4 package (Bates et al., 2015) in R statistical software version 3.3.2 (R Core Team, 2016). The genotype, environment, genotype-by-environment interaction, replication nested in environment, and block nested in replication nested in environment factors were all examined for significance. The final model for the combined DRIL78 dataset included genotype, environment, genotype-by-environment interaction, replication nested in environment, and block nested in replication nested in environment. The 2016Urbana model included only genotype, whereas the 2017Monmouth and 2017Urbana models included genotype, replication, and block nested in replication. Best linear unbiased predictors (BLUPs) were calculated for the combined year and the individual environment datasets (Table 2.1). Heritability was calculated using the PROC MIXED procedure of SAS software (SAS ver. 9.4, SAS Institute, Cary NC), as described by Holland et al. (2003). A Pearson's correlation coefficient was obtained between Goss's wilt BLUPs across environments using the PROC CORR procedure of SAS software (SAS version 9.4, SAS Institute).

Linkage Mapping: A total of 240 single nucleotide polymorphisms were used as genetic markers for QTL mapping (Lopez, 2016). Genetic mapping was performed using the Chromosome Segment Substitution option in ICIMapping version 4.0.6.0 (Meng et al., 2015). This option allows for the comparison of unique donor chromosome introgressions to the recurrent parent. A likelihood ratio is then used to identify the segments statistically associated

with the trait of interest. The step-wise regression likelihood ratio test was performed using the RSTEP-LRT-ADD function and QTL with a LOD score greater than 3.00 were identified.

RESULTS AND DISCUSSION

Characterization of Germplasm: Oh7B and NC344 were selected from the Goodman Diversity panel based on their reaction to multiple disease. Oh7B was chosen because it displays MDS to northern corn leaf blight, southern corn leaf blight, and gray leaf spot. In contrast, NC344 has MDR for these three diseases (Lopez, 2016). Our goal was to determine whether this MDR and MDS also pertained to Goss's wilt.

The phenotypic distribution of the DRIL78 population is normally distributed around the recurrent, susceptible, Oh7B parent (Figure 2.1). The genome of a $BC_3F_{4:5}$ population is >90% of the recurrent parent due to the repeated backcrossing. Therefore the majority of the lines should display similar phenotypes as the recurrent parent (Jamann et al., 2015). A small proportion of lines exhibited resistance in accordance with the donor, resistant NC344 parent. In addition, transgressive segregation was observed in a fraction of susceptible lines. These lines displayed higher susceptibility than the Oh7B parent.

Phenotypes were consistent between environments. In the combined dataset, Oh7B had an average AUDPC of 520, and NC344 of 170. Pearson correlation coefficients between environments were all statistically significant (Table 2.2). 2016Urbana had a positive correlation of 0.543 with 2017Urbana and 0.603 with 2017Monmouth. 2017Urbana had a positive correlation of 0.655 with 2017Monmouth. A significant genotype-by-environment interaction was observed in the combined dataset. This is consistent with previous studies, which also

observed highly significant genotype-by-environment interactions (Ngong-Nassah et al., 1992;Treat and Tracy, 1990).

Heritabilities for this population were moderately high. The plot heritability was 0.42 (Standard Error 0.036) and the family heritability was 0.76 (Standard Error 0.029). These results are consistent with heritabilities found in other Goss's wilt studies. Singh et al. (2016) found family-mean heritabilities ranging from 0.60 to 0.62 and Cooper et al. (2018) reported family-mean heritabilities between 0.53 and 0.63.

Linkage Mapping: We were successful in identifying putative QTL on five chromosomes – 1.05, 2.07, 3.06, 7.04, and 9.03. Confidence intervals were delimited by selecting the first markers on either side of the significant SNP with a threshold less than our 3.00 LOD score. The QTL on chromosome 2.07 was the only significant QTL found in all three environments and the combined dataset. Like previous Goss's wilt QTL, it had a small effect size of only around 7-11% (Table 2.3). This QTL overlaps with the results found in 2.07 by Singh et al. (2016) and Cooper et al. (2018). Only the 2.07 QTL overlapped between our study and the B73 and Mo17 derived populations (Cooper et al., 2018). This may be due to the difference in alleles sampled. There is an evident lack of similarity between B73 and Mo17 alleles compared to Oh7B and NC344 alleles. In contrast, a number of close similarities were found between our results and the results of Singh et al. (2016). This can be attributed to the Oh43 inbred lines used in their joint linkage mapping, which is closely related to the DRIL78 parental line Oh7B. Another possibility is that the small population sizes and marker density in the B73 and Mo17 introgression lines reduced their power to detect other significant QTL.

The QTL found on chromosome 3.06, 7.04, 9.03 also correspond with previously identified Goss's wilt QTL (Singh et al., 2016). However, no QTL were seen to overlap with the

Minnesota association mapping trial performed by Schaefer and Bernardo (2013). The QTL on chromosome 1.05, found in the combined dataset and 2017Monmouth, had two peak SNPs next to each other with LOD scores of 24.1 and 12.8. The proximity and significance of these two markers indicates that this could be one large QTL. This QTL also explained the largest proportion of phenotypic variance observed (Table 2.3). Unfortunately, this QTL did not overlap with any other studies, and therefore needs further verification before it can be used for breeding.

None of our QTL overlapped with the regions associated with resistance to other diseases, including northern corn leaf blight, southern corn leaf blight or gray leaf spot (Lopez, 2016). These results indicate that resistance to Goss's wilt does is not related to resistance to any of these diseases in this population. In contrast, Cooper et al. (2018) found that Goss's wilt severity in the B73 x Mo17 derived populations was significantly associated with northern corn leaf blight, but not with gray leaf spot or southern leaf blight. Further research is needed to determine if this association also exists in the DRIL78 population.

CONCLUSIONS

The DRIL78s are a collection of near isogenic lines derived from the resistant, donor, parent NC344 and the susceptible, recurrent, parent Oh7B. We were successful in identifying a number of Goss's wilt QTL in the DRIL78 population. QTL were found in bins 2.03, 3.06, 7.04, and 9.03 which confirmed QTL identified in previous studies (Cooper et al., 2018;Singh et al., 2016). No novel QTL were discovered, indicating that the potential of linkage mapping to identify novel allelic diversity for Goss's wilt may be almost spent. Future work should begin to focus on identifying causal genes within known QTL. Due to the clean introgressions it offers, the DRIL78 population will allow future work to examine the underlying causal mechanisms of the QTL found in this study. Future dissection of these QTL may offer clues into the underlying

pathogen virulence mechanisms or host defense systems for Goss's wilt. In conclusion, the QTL we have identified provide both confirm previous findings and offer new introgression lines to be used to dissect resistance mechanisms for Goss's wilt.

TABLES AND FIGURES

Table 2.1. Variance component estimates and their standard errors for factors included in the analysis for the DRIL78 (NC344(4) x Oh7B) population. All tabled variance component estimates were found to be significantly different than zero (P < 0.05).

	Variance	Standard Error
Combined		
Genotype	16443	128.23
Environment	20800	144.22
Genotype * Environment	4842	69.58
Replication(Environment)	1137	33.71
Block(Replication(Environment))	6053	77.8
Error	9646	98.21
2016-Urbana		
Genotype	2488	157.8
Error	11814	108.7
2017-Monmouth		
Genotype	23935	154.71
Replication	2690	51.86
Block(Replication)	5277	72.64
Error	11751	108.4
2017-Urbana		
Genotype	15811	125.7
Replication	711.7	26.68
Block(Replication)	9424	97.1
Error	11919	109.2

Table 2.2. Pearson correlation coefficients for Goss's wilt severity between 2016Urbana, 2017Urbana and 2017Monmouth. All coefficients were found to be significant at (P < 0.0001).

	2017Urbana	2017Monmouth
2016Urbana	0.543	0.603
2017Urbana		0.655

Table 2.3. Significant genetic markers for the DRIL78 (NC344(4) x Oh7B) population in the combined 2016&17 dataset, as well as individual years.

Dataset[†]	Chr.[‡]	Bin[§]	Peak Marker	Peak Position	2-LOD interval[¶]	LOD[#]	$R^{2‡‡}$	A[§§]
Combined	1	1.05	PHM12633.15	103835578	75.7-103.8	24.1042	39.7323	119.8655
Combined	1	1.05	PHM3463.18	107373210	107.4-119.7	12.8581	18.6399	-89.7924
Combined	2	2.07	PHM14412.4	203610640	197.6-212.0	8.1186	11.2036	-59.2559
Combined	3	3.06	PZA02402.1	171487608	170.2-188.0	5.0486	6.7964	-50.9722
2017Urbana	2	2.07	PHM14412.4	203610640	197.6-212.0	5.0941	9.587	-50.3995
2017Urbana	9	9.03	PZA00588.2	62366576	28.4-82.0	4.0001	7.3537	-47.2447
2017Monmouth	1	1.05	PHM12633.15	103835578	75.7-103.8	20.2144	45.0987	156.4043
2017Monmouth	1	1.05	PHM3463.18	107373210	107.4-119.7	10.6927	21.0141	-119.3963
2017Monmouth	2	2.07	PHM14412.4	203610640	197.6-212.0	4.1098	7.3916	-54.0658
2017Monmouth	7	7.04	PHM10225.15	167977360	155.4-168.7	2.9784	5.3664	-46.8954
2017Monmouth	9	9.03	PHM5185.13	18905238	156.2-268.3	3.9465	7.05	-72.3229
2016Urbana	2	2.07	PHM14412.4	203610640	197.6-212.0	4.1091	8.0882	-148.8606
2016Urbana	3	3.06	PZA02402.1	171487608	170.2-188.0	3.5101	6.9011	-148.7274

Dataset[†]: Year followed by location.

Chr.[‡]: Chromosome.

Bin[§]: Chromosome bin location of each QTL (Davis et al., 1999).

2-LOD interval[¶]: Physical map positions in Mb (RefGen_v3); For DRIL78 population only the physical position of peak marker is given (RefGen_v3).

LOD[#]: The log of odds (LOD) value at the position of the peak likelihood of the QTL.

Threshold[††]: LOD threshold to identify significant QTL corresponding to an empirical Type I error value of 0.10 produced from 1000 imputations. A LOD score of 3 was used to detect significant QTL in the DRIL14 population.

$R^{2‡‡}$: Proportion of phenotypic variance explained by the detected QTL.

A[§§]: Additive effect estimates of the detected QTL. Effects are in terms of the disease rating scale employed.

Figure 2.1. Phenotypic distribution of Goss's wilt in the DRIL78 (NC344(4) x Oh7B) population. Lines were assessed visually using a 0-100% scale, and the area under the disease progress curve values were calculated based on these visual scores. The phenotypic data shown is expressed as best linear unbiased predictors of the area under disease progress curve including the intercept.

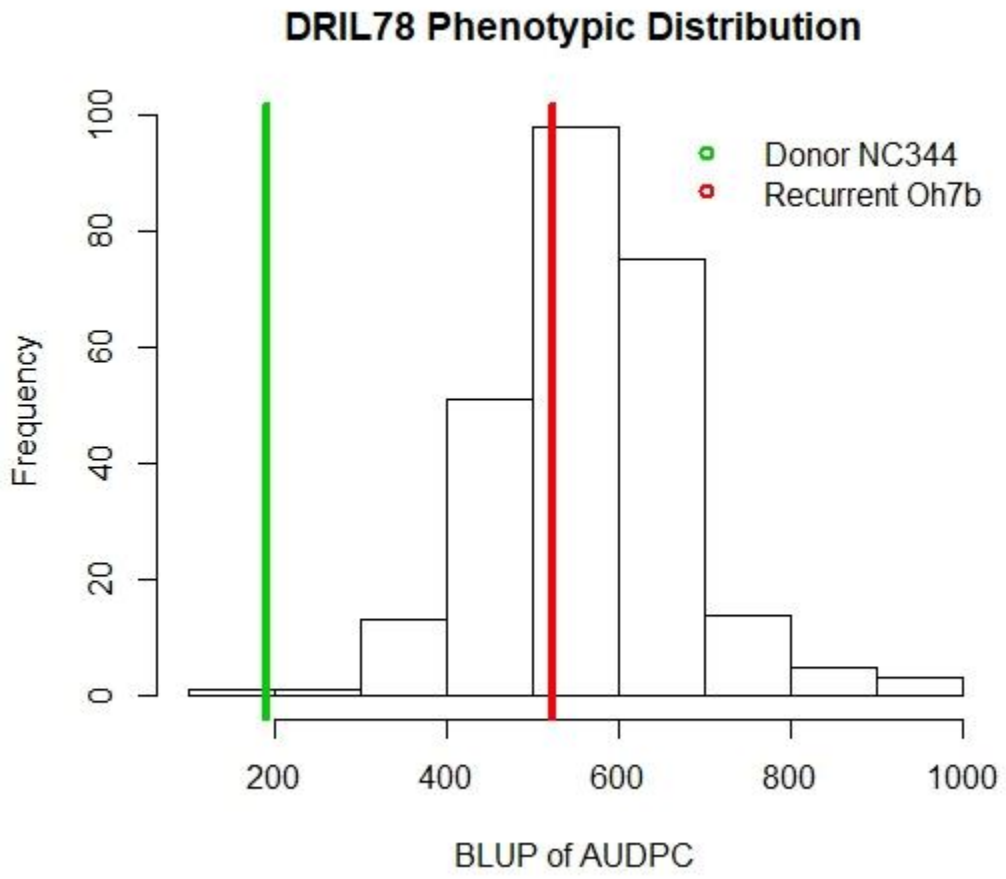
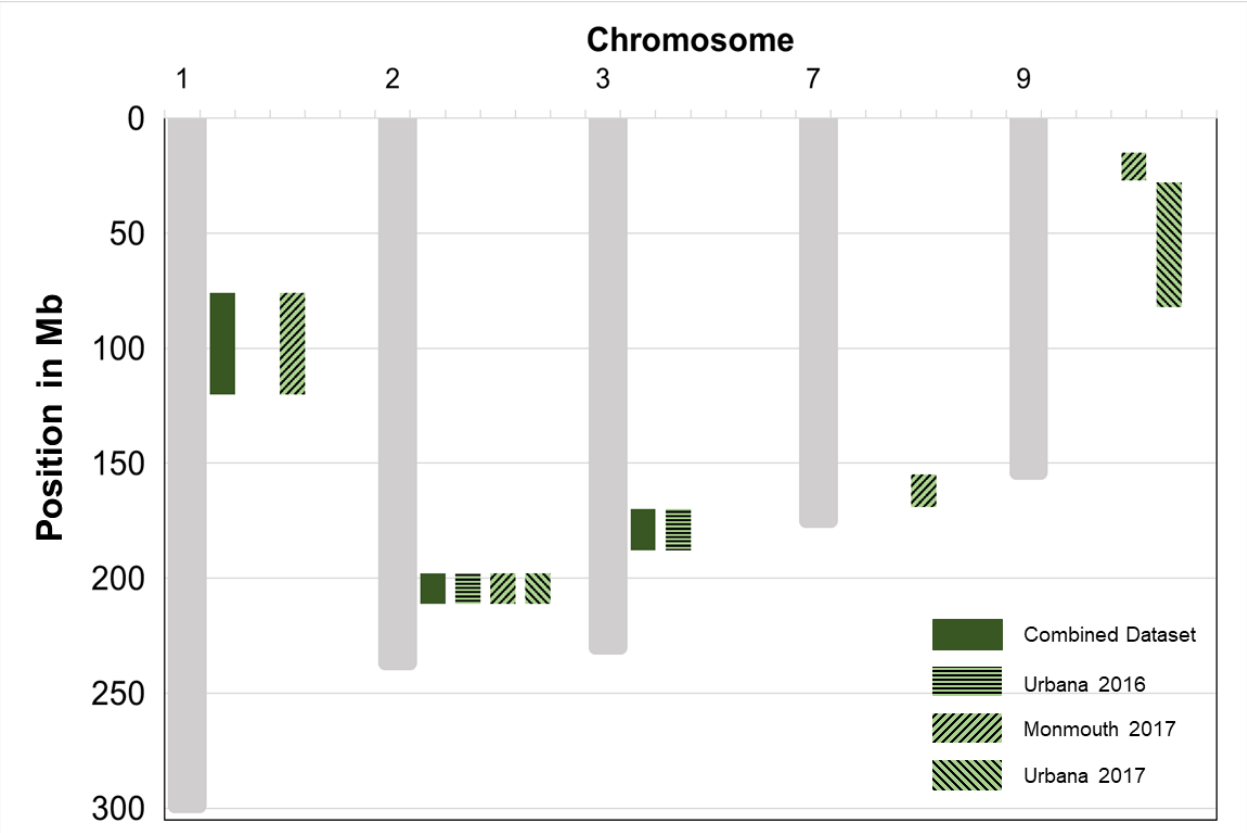


Figure 2.2. Peak SNPs in a 2-LOD interval from the DRIL78 (NC344(4) x Oh7B) population overlaid on a physical map of the maize genome. Chromosomes are denoted as vertical gray bars, with green bars representing QTL.



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CHAPTER 4: GENOME-WIDE ANALYSIS AND GENOMIC PREDICTION OF GOSS'S WILT RESISTANCE IN MAIZE

ABSTRACT

Goss's wilt is one of the most important foliar diseases of maize. To date, neither large-effect resistance genes have been identified nor does practical chemical control exist. The importance of discovering durable host resistance necessitates additional genetic mapping for this disease. Unfortunately, due to the biology of the pathogen and the highly significant genotype-by-environment interaction effect observed with Goss's wilt, consistent phenotyping across multiple years poses a hurdle for genetic studies and conventional breeding methods. The objective of this study was to perform a genome-wide association study to identify regions of the genome associated with Goss's wilt resistance, as well as use genomic prediction models to evaluate the use of genomic selection in predicting Goss's wilt phenotypes in a panel of diverse maize lines. Using genome-wide association mapping, we were unable to identify any variants significantly associated with Goss's wilt. However, using genomic prediction, we were able to train a model with an accuracy of 0.69. In addition, when evaluating the accuracy of our prediction model under reduced marker density, it was shown that only 10,000 single nucleotide polymorphisms, or ~20% of our total marker set, was necessary to achieve our model's prediction accuracy. This is the first report of genomic prediction for a bacterial disease of maize and these results highlight the potential of genomic selection for disease resistance in maize.

INTRODUCTION

Since its discovery in 1969, no source of complete genetic control has been found for Goss's wilt. The disease, caused by the gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), produces foliar blight lesions and vascular wilt symptoms in susceptible maize varieties (Schuster, 1975). The bacterium overwinters in infected crop residue on the soil surface, where it relies on splashing rain for dissemination (Schuster, 1975). The pathogen is non-motile (Vidaver and Mandel, 1974) and most likely to infect through wounded or damaged tissue; however, disease development has been observed in unwounded plants under high humidity conditions (Mallowa et al., 2016).

Goss's wilt is endemic from Louisiana to Alberta, Canada (Howard et al., 2015;Jackson et al., 2007;Singh et al., 2015). Yield losses greater than 40% have been observed in susceptible maize hybrids due to Goss's wilt (Carson and Wicks, 1991). An estimated 501 million bushels of maize were lost to Goss's wilt in the United States and Canada between 2012 and 2015, making it the fourth most severe disease during this period (Mueller et al., 2016). With limited success, cultural, chemical, and genetic control strategies have all been employed to curb Goss's wilt occurrence. No chemical control methods have been shown to decrease Goss's wilt occurrence at a statistically significant level (Mehl et al., 2015).

As with many plant pathogens, genetic resistance remains the best control strategy for Goss's wilt. Linkage mapping, which uses populations derived from controlled crosses to identify quantitative trait loci (QTL) associated with a trait of interest, has been used to identify several regions of the maize genome associated with Goss's wilt resistance. Singh et al. (2016) conducted joint linkage mapping for Goss's wilt resistance in three recombinant inbred line populations (B73 x Oh43, B73 x HP301, and B73 x P39). Using these populations, 19 putative

QTL on all chromosomes excluding 8 were identified; however, the effect size of each QTL was small, and none contributed >6% of the total observed phenotypic variance (Singh et al., 2016). An evaluation of the intermated B73 x Mo17 population identified seven putative QTL on chromosomes 1, 2, 7, 8, and 10, both confirming Singh et al.'s findings and presenting the first source of resistance on chromosome 8 (Cooper et al., 2018). In addition, the QTL on chromosome 1 overlaps with the known multiple disease resistance locus *qMDR1.06,* which has been associated with reduced incidence of Stewart's wilt, northern leaf blight, southern leaf blight, and a number of other maize diseases (Chung et al., 2010;Jamann et al., 2014;Wisser et al., 2006).

Another method of genetic mapping is association mapping, which takes advantage of historical recombination to identify markers in linkage disequilibrium with a trait of interest (Myles et al., 2009). The diverse subpopulations of maize, including stiff stalk, non-stiff stalk, popcorn, sweet corn, tropical, and mixed varieties, offer high allelic diversity that can be unlocked by association mapping (Flint-Garcia et al., 2005). In addition, high heritability has been reported for Goss's wilt resistance (Schaefer and Bernardo, 2013;Singh et al., 2016). This makes Goss's wilt ideal for multi-year, multi-environment trials required for association mapping. When a genome-wide association study (GWAS) for Goss's wilt was conducted on a panel of historically important maize lines from Minnesota, eight small-effect QTL were identified (Schaefer and Bernardo, 2013).

Ideally, favorable alleles of the QTL identified for Goss's wilt resistance in maize could be directly incorporated into maize breeding programs via marker-assisted selection (MAS). However, given the absence of simply inherited resistance genes for Goss's wilt and the lack of large-effect QTL, the use of MAS is not ideal for this disease. The genetic architecture for

Goss's wilt appears to be polygenic in nature, consisting of multiple loci, each with a small effect (Cooper et al., 2018;Schaefer and Bernardo, 2013;Singh et al., 2016). To effectively account for these multiple small-effect loci in a breeding program for resistance to Goss's wilt, the efficacy of genomic selection (GS) needs to be explored. Using genome-wide molecular makers to predict the phenotypic value of an individual based on all genotypic effects (Meuwissen et al., 2001), GS is based on the theory that quantitative traits are highly polygenic and thus their variation is best captured by modeling of all genome-wide markers. If all genetic variance can be explained by available marker data, then it becomes possible to quantify additive contribution of numerous, small effect loci to the phenotypic variation (Goddard and Hayes, 2007).

The ability of GS to predict trait values is evaluated by dividing a population into a "training" and a "prediction" population; both of which are genotyped by the same set of markers. The "training" population uses both phenotypic and genotypic data to fit a GS model where the phenotype of interest is the response variable and all markers throughout the genome are the explanatory variables (Meuwissen et al., 2001). This prediction model is then used to calculate a genomic estimated breeding value (GEBV) for the "prediction" population based only on the marker data (Meuwissen et al., 2001). This method of continually splitting the population into training and prediction subsets is known as k-fold cross-validation (Mostellar and Tukey, 1968). If a sufficiently large correlation between the actual trait values and the GEBVs are observed across all prediction subsets, then GS can be used to immediately and significantly increase selection gains per unit time and expedite the entire breeding cycle (Heffner et al., 2010;Wong and Bernardo, 2008).

Genomic selection has been used with varying success for a number of plant diseases, most notably for modeling resistance to wheat stem rust (Poland and Rutkoski, 2016;Rutkoski et

al., 2011). It was estimated that a prediction accuracy of 0.56-0.62 could reduce breeding cycles in wheat by up to two-fold (Rutkoski et al., 2012;Rutkoski et al., 2011). Similar studies utilizing GS for Fusarium head blight resistance (FHB) of wheat and barley found prediction accuracies between 0.41 and 0.68 using k-fold cross-validation (Lorenz et al., 2012;Rutkoski et al., 2012). Lorenz et al. (2012) found genomic predictions comparable to observed phenotypic means, and estimated that the cost of phenotyping for FHB was four times the cost of genotyping.

In maize, GS has been evaluated for improvement of resistance to many major pathogens, including Gibberella ear rot, northern leaf blight, southern leaf blight, and gray leaf spot. Riedelsheimer et al. (2013) used GS to predict disease severity of Gibberella ear rot and deoxynivalenol concentration in five double haploid families. Validation was high within full-sib families, (0.65-0.70), but fell for both severity (0.25-0.60) and deoxynivalenol concentration (0.05-0.70) when comparing across families (Riedelsheimer et al., 2013). A similar study by Technow et al. (2013) evaluated the use of GS to predict northern leaf blight severity. Two distinct heterotic maize inbred heterotic groups were examined, dent and flint, from the University of Hohenheim breeding program. Validation within each group was high (0.64-0.71) but when attempting to predict GEBVs across heterotic groups this accuracy dropped sharply (0.11-0.29) (Technow et al., 2013). In an evaluation of the nested association mapping populations, southern leaf blight displayed a prediction accuracy of 0.50-0.52 (Bian and Holland, 2017). However, in that same population, the prediction accuracy of gray leaf spot was only 0.22-0.25 (Bian and Holland, 2017). No hypothesis was given regarding the reduced power of the gray leaf spot prediction model, and this study shows that GS may not be equally effective for all diseases.

Goss's wilt is a strong candidate for GS for a number of reasons. The genetic architecture of Goss's wilt resistance is polygenic and no large-effect loci have been identified (Cooper et al., 2018;Schaefer and Bernardo, 2013;Singh et al., 2016). Additionally, the inoculation of Goss's wilt represents a significant challenge in obtaining accurate and consistent phenotypic data. Phenotyping for Goss's wilt is labor-intensive. The non-motile nature of the pathogen necessitates individual wounding and application of inoculum to each plant, which also increases the error observed within the experiment. In areas where Goss's wilt has not yet spread, an accurate genomic prediction model would allow screening of future commercial lines without the risk of introducing Goss's wilt to the local community. Genomic selection could be also implemented in pre-breeding to remove highly susceptible lines before selections begin (Poland and Rutkoski, 2016).

The combined use of association mapping and genomic prediction may offer the best strategy for identifying loci associated with Goss's wilt resistance and testing whether populations can be improved using genomic selection. Statistically significant QTL identified using association mapping would offer insight into the genetic mechanisms governing resistance, while GS would increase the efficiency of breeding for resistance in the absence of large-effect QTL. The objectives of this study were to (i) perform a GWAS on the Goodman maize diversity panel to identify putative Goss's wilt quantitative trait nucleotides (QTN) and (ii) test the accuracy of GS for population improvement to Goss's wilt.

MATERIALS AND METHODS

Field Design: The Goodman maize diversity panel (Flint-Garcia et al., 2005) was grown at the Crop Science Research and Education Center in Urbana, IL in single-row plots during the 2016 and 2017 summer field seasons. Each plot was 3.2-meters long, with 0.76-meter alleys and a row

spacing of 0.762 meters. Plots were machine-planted at a density of 20 kernels/row, and standard agronomic practices for central Illinois were followed. Initial seed for the diversity panel was obtained from the Germplasm Resources Information Network (GRIN). In 2016, disease ratings were obtained for two replications of 300 lines of the diversity panel. In 2017, disease ratings were obtained for two replications of 223 lines of the diversity panel. The difference in the number of lines tested in 2016 and 2017 was due to seed availability. An incomplete block design was implemented in the *agricolae* package (Mendiburu, 2017) of R version 3.3.2 (R Core Team, 2016) using resistant (FR4326) and susceptible (CQ183 and CQ184A) check lines in each block. Blocks in 2016 contained 18 lines, and blocks in 2017 contained 17 lines. A total of four replications were evaluated across two years.

Phenotyping: *Cmn* strain *16Cmn001* was maintained in glycerol stocks stored at -80°C for use in the 2016 and 2017 field seasons. Inoculations were conducted as described in Cooper et al. (2018). Briefly, single colonies were grown in nutrient broth yeast extract (NBY) on a shaker at room temperature for two to three days. The final bacterial cell concentration was adjusted to 10^7 colony forming units per mL using a spectrophotometer (OD_{600}=0.05) (Pataky, 1985). Inoculations were performed twice, one week apart, between the V4 and V7 stages using a modified pinprick inoculation method to simulate mechanical damage (Blanco et al., 1977;Chang et al., 1977). Disease ratings were performed three to four times, approximately every two weeks after initial inoculation using the methods described in Cooper et al. (2018). Inbreds were scored on a per-plot basis using a 0-100% scale with 5% intervals (Cooper et al., 2018). Ratings represented the total percent of infected leaf area, with 0% representing no symptoms and 100% denoting complete plant death (Poland and Nelson, 2011). Using the formula $A_k = \sum_{i=1}^{N_i-1}(\frac{y_i+y_{i+1}}{2})(t_{i+1}-t_i)$, where *y* refers to individual disease scores and *t* equals

time between ratings, the area under disease progress curve (AUDPC) was calculated for each plot to represent disease progression throughout the season (Wilcoxson et al., 1975). Days to anthesis notes were taken on a plot basis and the date was recorded when 50% of the tassels were shedding pollen.

Phenotypic Data Analysis: A $\log_{10}$ transformation was performed on the raw AUDPC scores to normalize the data. A linear model was then run in R/lme4 (Bates et al., 2015) and best linear unbiased predictors (BLUPs) were calculated. All factors were fit as random effects. Variance components of significant factors in BLUP calculations were obtained using R/lme4 (Bates et al., 2015). For the 2016-2017 combined dataset genotype, year, the genotype-by-year interaction, replication, and block were included in the model (Table 3.1). To account for a potentially large genotype-by-year interaction, BLUPs were also calculated for each year individually. A Scheffe's multiple comparison test was conducted on the raw AUDPC scores to discern phenotypic differences between the subpopulations (Scheffe, 1959). Heritability was calculated using the PROC MIXED function in SAS software (SAS version 9.4, SAS Institute, Cary, NC) as described by Holland et al. (2003). Pearson's correlation coefficients were obtained using the PROC CORR function in SAS software (SAS version 9.4, SAS Institute).

Association Mapping: Three separate association mapping analyses were run, one for the combined 2016 and 2017 dataset and one for each year individually. The BLUPs for disease resistance of each line were used as the phenotypic dataset for association mapping; the combined BLUPs across 2016 and 2017 were used for the multi-year analysis, while the BLUPs calculated for each individual year used for the single year analyses. The genotype-by-sequencing (GBS) single nucleotide polymorphisms (SNPs) of the USA national maize inbred seed bank, the Illumina MaizeSNP50 BeadChip (55K), and the 4K SNP marker set available on

the Panzea database (4K) (http://www.panzea.org) were combined and used in this analysis for a total of 416,376 markers (Cook et al., 2012;Romay et al., 2013;Zhao et al., 2006). A compressed unified mixed linear model (Zhang et al., 2010) was implemented in the R package Genome Association and Prediction Integrated Tool (Lipka et al., 2012). Principal component analysis was conducted by Lipka et al. (2013) using the 34,368 non-industry SNPs from the Illumina MaizeSNP50 BeadChip 55K marker set. To account for population substructure at an appropriate level, Bayesian information criterion (BIC; (Schwarz, 1978)) -based backwards elimination was used to select between zero to three of these first three principal components to include as fixed-effect covariates in the model. A kinship matrix was derived in GAPIT from the Illumina MaizeSNP50 BeadChip 55K to account for relatedness between the inbreds (Lipka et al., 2012). The Benjamini and Hochberg (1995) procedure was used to control the false discovery rate (FDR) at 10%; thus any SNPs with FDR-adjusted *P*-values of less than or equal to 0.10 were declared to be significantly associated with resistance to Goss's wilt. Manhattan and QQ plots were created in R/qqman (Turner, 2014) using the GAPIT results.

Genomic Prediction: Ridge regression best linear unbiased prediction (RR-BLUP) was performed using the rrBLUP package (Endelman, 2011) in R version 3.3.2 (R Core Team, 2016) using the BLUPs calculated for the diversity panel. In RR-BLUP, a mixed linear model is fit in which all markers are considered random effects (Whittaker et al., 2000). Each marker contributes an additive effect equal to the genetic variance divided by the total number of markers. Additionally, zero covariance between markers is assumed. RR-BLUP predicts GEBVs by estimating marker effects from a training population and then multiplying the effects of each marker by the prediction genotype to approximate phenotypic breeding values (Whittaker et al., 2000).

The 55K marker dataset, filtered to exclude markers with a minor allele frequency less than 0.05, was used for genomic prediction. A five-fold cross validation scheme with 100 iterations was used to generate the training and prediction populations. The prediction accuracy was calculated using the formula: $P = r^2(GEBV:PEBV)/\sqrt{h^2}$. This represents the average correlation between the GEBVs and observed phenotypically estimated breeding values (PEBVs) divided by the square root of the plot heritability (h^2). Subsets consisting of 100, 1000, 5000, 10000, and 25000 markers from the 55K marker dataset were randomly generated, and prediction accuracies across 100 iterations of a five-fold cross validation scheme were averaged to assess the impact of maker density and genomic prediction accuracy. Dunnett's (1955) multiple comparisons were performed between each marker density and the full 55K marker set using R/multcomp (Hothorn et al., 2008).

The linear function y ~ x was fit using the "lm" function in base R version 3.3.2 (R Core Team, 2016) to plot the relationship between GEBVs and phenotypically estimated breeding values (PEBVs). The slope and intercept of this linear regression was used to estimate our prediction bias (Arruda et al., 2015;Zhang et al., 2014). The linear relationship and confidence interval was plotted using R/ggplot2 (Wickham, 2009).

RESULTS AND DISCUSSION

Characterization of Germplasm: The Goodman maize diversity panel is composed of temperate, tropical, sweet, and popcorn lines and encompasses 75% of the allelic diversity of maize (Romay et al., 2013). The diversity panel had a wide range of disease scores, from 0-100% infected leaf area (Figure 3.1). Severe Goss's wilt symptoms, including vascular wilt, foliar necrosis, stunting, lodging, and premature death were observed in susceptible lines. Moderately

resistant lines displayed foliar lesions beyond inoculated leaves. Only highly resistant inbreds were able to limit disease incidence to inoculated leaves.

Disease severity was compared between subpopulations within the diversity panel. Tropical and sub-tropical varieties displayed the highest levels of susceptibility to Goss's wilt (Figure 3.2). Stiff and non-stiff stalk lines, while moderately resistant on average, both displayed long tails of outlying susceptibility. While trends did exist, all subpopulations exhibited a wide range of resistant and susceptible lines. In a Scheffe's multiple comparison test, tropical/sub-tropical, mixed, stiff stalk, popcorn, and sweet corn were found to compose one distinct statistical group. Stiff stalk, popcorn, sweet corn, and non-stiff stalk formed the second group. Overall, non-stiff stalk maize lines contain the greatest resistance to Goss's wilt. However, due large to the number of outliers in all subpopulations, it would be unwise to rule out identifying resistance alleles from any class.

Disease incidence was more severe in the 2016 growing season than in 2017. To assess reproducibility, Pearson's correlation coefficients were calculated for each replication across years. Replications within 2016 had a correlation coefficient of 0.782 (P <0.0001), and replications within 2017 had a correlation coefficient of 0.796 (P <0.0001). The correlation coefficients between years was 0.584 (P <0.0001). Hot and dry weather conditions during inoculations in 2017 may account for some of the differences observed between years.

Heritability was calculated using the method described by Holland et al. (2003). For the 2016-2017 combined dataset, a plot heritability of 0.638 with a standard error of 0.034 and a family heritability of 0.782 (standard error of 0.028) was estimated. Previous studies have reported plot and family heritabilities for Goss's wilt ranging from 0.24-0.35 and 0.53-0.62, respectively (Cooper et al., 2018;Singh et al., 2016). The highest heritability estimates have been

observed in crosses between diverse phenotypes (Ngong-Nassah et al., 1992). The high heritabilities displayed within the diversity panel indicate that a large proportion of overall variation in Goss's wilt resistance within the population can be explained by genetic differences among individuals. There was also an environmental component for disease resistance. The genotype-by-environment interaction within the diversity panel accounted for >7% of the total phenotypic variation and was significant at $P<0.001$.

Association Mapping: The ANOVA revealed a large genotype-by-year interaction, which demonstrates environmental conditions play a role in disease resistance (Table 3.1). Therefore, we chose to perform association mapping on both the combined and the individual year datasets. Despite the heritable nature of our trait and evident diversity of our population, no significant SNPs were detected in the combined or individual year datasets (Figure 3.3). Due to the allelic diversity present in the panel and the range of resistance and susceptibility within each subpopulation, allele frequencies may have been too low to detect significant SNPs. In addition, Goss's wilt resistance is polygenic in nature. While QTL may be present within the genome, their effect size may be too small to detect through conventional GWAS methods. Increased population size may allow additional detection of significant associations.

Maturity has been shown to play a large role in Goss's wilt development. Two week-old seedlings display the most susceptibility, with increased resistance achieved as the plants mature (Calub et al., 1974). Tropical lines within the diversity panel mature slower than temperate lines that are adapted to the Illinois climate. The increased susceptibility of young tropical lines during inoculation may have confounded our attempts to accurately rate disease severity. Additionally, a Pearson correlation coefficient of -0.223 (P <0.0001) was observed between days to anthesis and Goss's wilt severity. As maturity decreases, Goss's wilt severity increases, and vice-versa.

However, even when days to anthesis was included as a covariate in our BLUP calculations, no significant GWAS SNPs were identified.

Genomic Prediction: Using RR-BLUP, a direct correlation of 0.553±4.4e-05 was obtained for Goss's wilt using the 55K marker set (Table 3.2), translating to a prediction accuracy of 0.69. In comparison, the largest published QTL for Goss's wilt resistance explains less than 10% of phenotypic variance (Cooper et al., 2018;Schaefer and Bernardo, 2013;Singh et al., 2016). Performing precise and consistent Goss's wilt inoculations poses a significant challenge in obtaining accurate phenotyping data. The non-motile nature of the pathogen requires individual wounding and application of inoculum to each plant. Furthermore, senescence of the inoculated leaves can impair disease ratings later in the season. These factors and the high prediction accuracies displayed in our prediction model provide a convincing argument for the use of genomic prediction for breeding for Goss's wilt resistance.

Different maturation rates between temperate and tropical inbreds may have influenced disease severity, biasing our prediction model. To test for this, days to anthesis was included as a covariate in our BLUP calculations, and prediction accuracy was compared between models with and without days to anthesis. Although maturity was significantly associated with Goss's wilt severity, including it as a covariate did not significantly alter our 0.69 prediction accuracy. Therefore, it was excluded from our final results.

Due to the absence of significant QTL for Goss's wilt, RR-BLUP was the best model for genomic prediction. Other studies have attempted to incorporate GWAS results into their prediction model to increase accuracy. Gowda et al. (2015) examined the efficacy of GWAS and GS on lethal necrosis in a panel of Sub-Saharan maize lines. Twenty-four putative SNPs were identified in the GWAS which, when added to the prediction model, caused only a slight

increase in the overall prediction accuracy (Gowda et al., 2015). This confirms the hypothesis that prediction accuracy is mainly associated with the many, small-effect QTL spread across the genome and does not require large-effect QTL to be effective.

RR-BLUP has been shown to respond well under reduced marker densities (Habier et al., 2007). To evaluate the optimal marker density for GS of Goss's wilt, genomic prediction was performed on 100 random samples of 100, 1,000, 5,000, 10,000, and 25,000 SNPs from the 55K marker set. At 5,000 (10% of total marker dataset) markers, the gain in prediction accuracy due to increased marker coverage begins to lessen (Figure 3.4). At 10,000 (20% of total dataset) and 25,000 (50% of total dataset) SNPs, prediction accuracy is no longer significantly different then predictions made using the full 51,471 SNP marker set (Table 3.2). There is a clear point of diminishing returns when increasing marker density for GS no longer provides substantial additional prediction accuracy. This point appears to be between 5,000 and 10,000 SNPs. This indicates that exhaustive genotyping is not necessary for genomic prediction of Goss's wilt resistance. These results correlate with previous studies, which found that only 1,000 out of 14,000 (Gowda et al., 2015) and 500 out of 20,000 (Cao et al., 2017) SNPs were necessary to achieve comparable accuracies to the full SNP analysis.

The bias of our predictions was plotted as a simple linear regression model with PEBV as the response variable and GEBV as the explanatory variable. The results of this fitted model suggest a strong linear relationship between these variables (Figure 3.5). Values were plotted evenly along the fitted regression line of $y = 0.99x + 0.02$, with a narrow confidence interval near the middle of the distribution and slightly more variable values near each end (Figure 3.4). The formula was fit to our data. An intercept of zero and a slope of one indicate an unbiased prediction model (Arruda et al., 2015). The confidence interval of our intercept was [-0.31, 0.35]

and the confidence interval for our slope was [0.88, 1.1]. These results indicate that our model was both accurate and unbiased.

The Goodman maize diversity panel is far outside the normal range of breeding material for Illinois, however conclusions from this panel will translate well to germplasm in actual breeding programs. A study by Yu et al. (2016) developed prediction models for biomass traits including yield, plant height, root lodging, and stalk number, based off of 962 sorghum accessions from 33 countries, covering five races. The large allelic diversity and sup-population structure of this panel is very similar to the diversity found in the Goodman panel (Flint-Garcia et al., 2005). The prediction models were then applied to 580 exotic sorghum lines that had been previously collected but not phenotyped for the same biomass traits. Prediction accuracies of 0.76 for yield, 0.32 for height, 0.78 for root lodging, and 0.42 for stalk number were obtained (Xiaoqing et al., 2016). This shows that genomic selection models can be applied across populations, and highlights the ability of diverse germplasm collections to predict complex phenotypic traits.

Moderate to high prediction accuracies were obtained for Goss's wilt when comparing our results to previous studies. Goss's wilt achieved similar accuracies to northern corn leaf blight (0.64-0.71), Gibberella ear/stalk rot (0.65-0.70), and tar spot complex (0.55-0.74) (Cao et al., 2017;Riedelsheimer et al., 2013;Technow et al., 2013). Prediction accuracy for Goss's wilt was higher than southern leaf blight (0.50-0.52), maize lethal necrosis (0.36-0.56), and gray leaf spot (0.22-0.25) (Bian and Holland, 2017;Gowda et al., 2015). Out of the available literature, fungal pathogens appear to have the highest and most stable prediction accuracies (Cao et al., 2017;Technow et al., 2013). Current research also shows high prediction accuracies for viral diseases, but with lower precision between populations (Gowda et al., 2015). Goss's wilt is the

first assessment of genomic prediction accuracy for a bacterial pathogen. Our results indicate that genomic prediction accuracy is strong for bacterial diseases, and should be considered for other high-impact diseases.

CONCLUSIONS

Genomic selection is an emerging method used by breeders to circumvent long and costly phenotyping and can be effective when other methods of marker-assisted breeding fail (Heffner et al., 2010;Meuwissen et al., 2001). In addition, GS has merit when dealing with emerging diseases under quarantine or other federal regulation (Poland and Rutkoski, 2016). In such instances, GS allows for the development of resistant varieties without the potential release of a pathogen to limited geographical ranges. In this study, we were able to achieve a prediction accuracy of 0.69 for Goss's wilt in the Goodman diversity panel. Therefore, given the difficulty of phenotyping and the lack of large-effect QTL for Goss's wilt (Cooper et al., 2018;Schaefer and Bernardo, 2013;Singh et al., 2016), GS may provide the most promising option. In conclusion, where GWAS for Goss's wilt proved unsuccessful at identifying targets for MAS, GS has emerged as successful alternative for increasing Goss's wilt resistance in maize.

TABLES AND FIGURES

Table 3.1. Variance component estimates and standard errors for factors included in the combined and individual year Goodman maize diversity panel analysis. All factors were significant at $\alpha = 0.05$.

	Variance	Standard Error
2016-2017		
Genotype	0.041	0.201
Year	0.051	0.226
Genotype*Year	0.009	0.093
Block(Replication(Year))	0.010	0.102
Error	0.017	0.129
2016		
Genotype	0.040	0.200
Replication	1.0E-04	0.014
Block(Replication)	0.002	0.041
Error	0.0122	0.109
2017		
Genotype	0.065	0.254
Replication	0.009	0.096
Block(Replication)	0.021	0.143
Error	0.023	0.150

Table 3.2. Average predictive ability, standard deviation, prediction accuracy, and Dunnett's comparison to full 55K marker set of prediction accuracy for 100 iterations of genomic prediction using five-fold cross validation in rrBLUP under variable marker densities.

Number of Markers	r^2	Standard Error	$r^2/\sqrt{h^2}$	Pr(>|t|)
100	0.359	0.006	0.449	<0.001***
1,000	0.495	0.003	0.619	<0.001***
5,000	0.538	0.002	0.673	<0.001***
10,000	0.546	0.001	0.673	0.182
25,000	0.551	6.9E-04	0.690	0.921
51,471	0.553	4.4E-05	0.693	NA

r^2: Predictive ability as a Pearson's correlation coefficient

$r^2/\sqrt{h^2}$: Prediction accuracy

*** Significant at $P < 0.001$

Figure 3.1. Phenotypic distribution of Goss's wilt resistance in Goodman maize diversity panel. Raw area under disease progress curves (AUDPC) values revealed a tail of susceptibility within the diversity panel. A $\log_{10}$ transformation was performed on the best linear unbiased predictions (BLUPs) to normalize the data before a genome-wide association study was conducted.

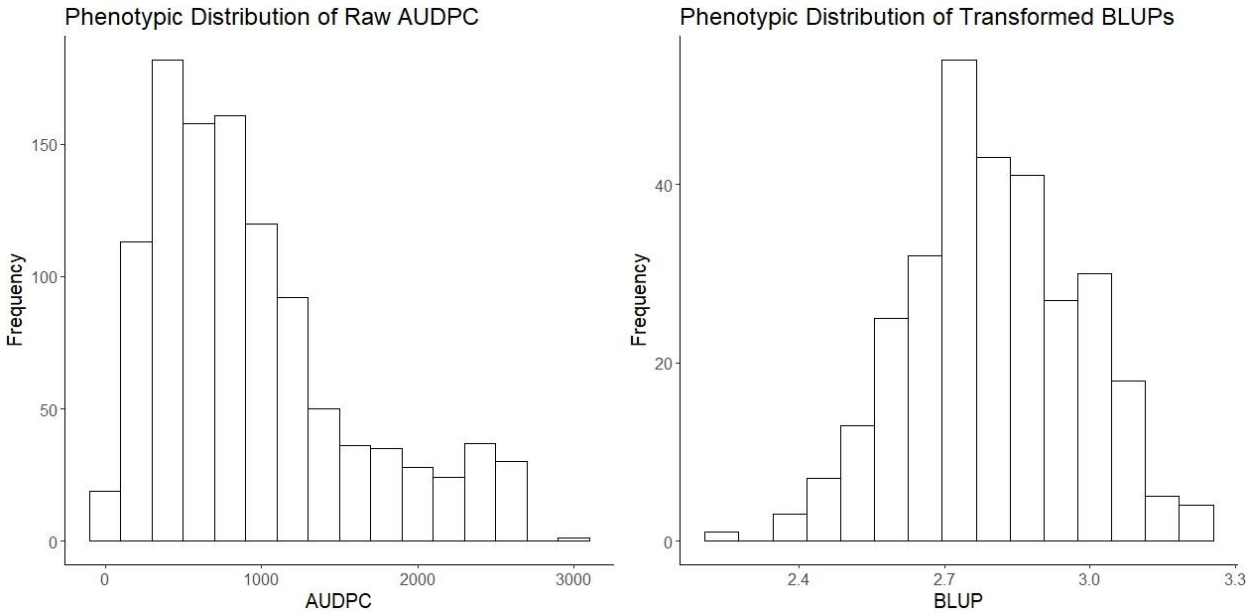


Figure 3.2. Goss's wilt untransformed area under disease progress curve (AUDPC) values between subpopulations. In a Scheffe's multiple comparison test, tropical/sub-tropical, mixed, stiff stalk, popcorn, and sweet corn were found to compose one distinct statistical group. Stiff stalk, popcorn, sweet corn, and non-stiff stalk formed the second group.

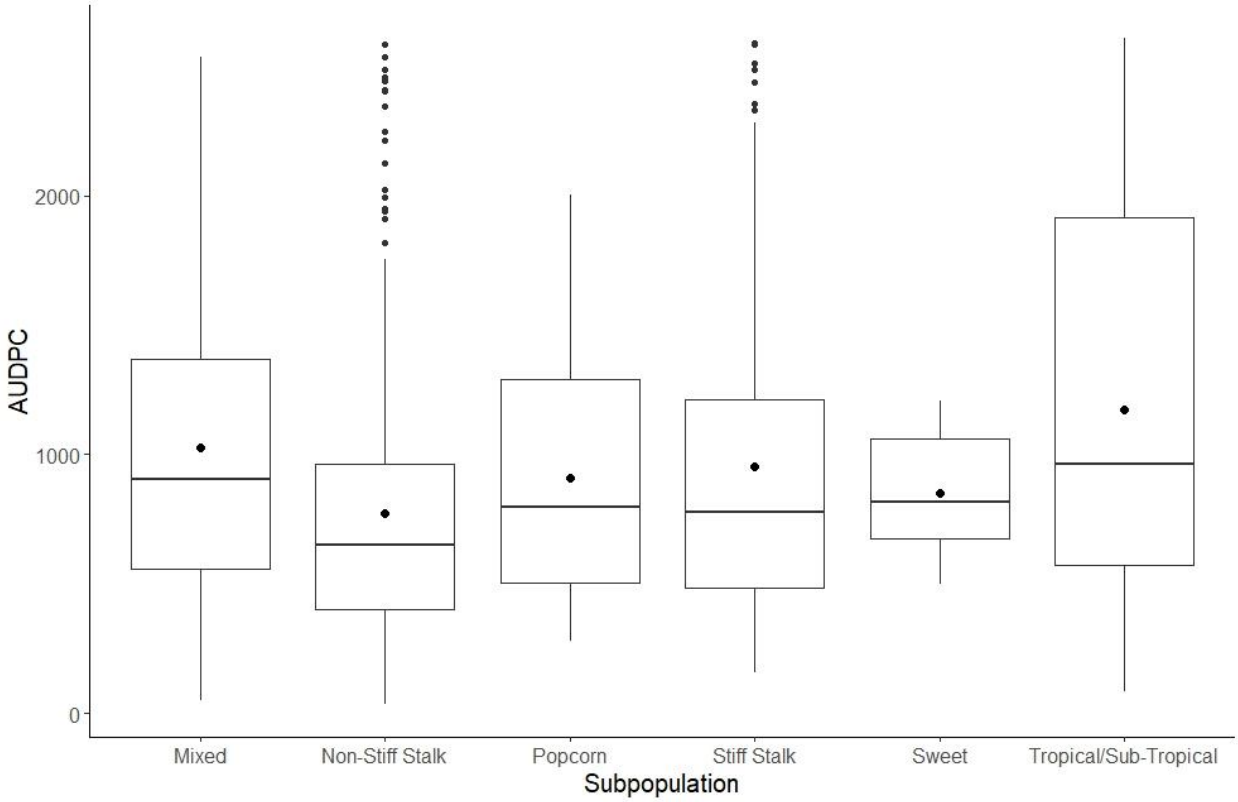


Figure 3.3. Quantile-quantile and Manhattan plots for the 2016-2017 combined dataset and the individual year analyses. A false discovery rate of 10% was used to determine significant single nucleotide polymorphisms (SNPs). No significant quantitative trait loci (QTL) were detected.

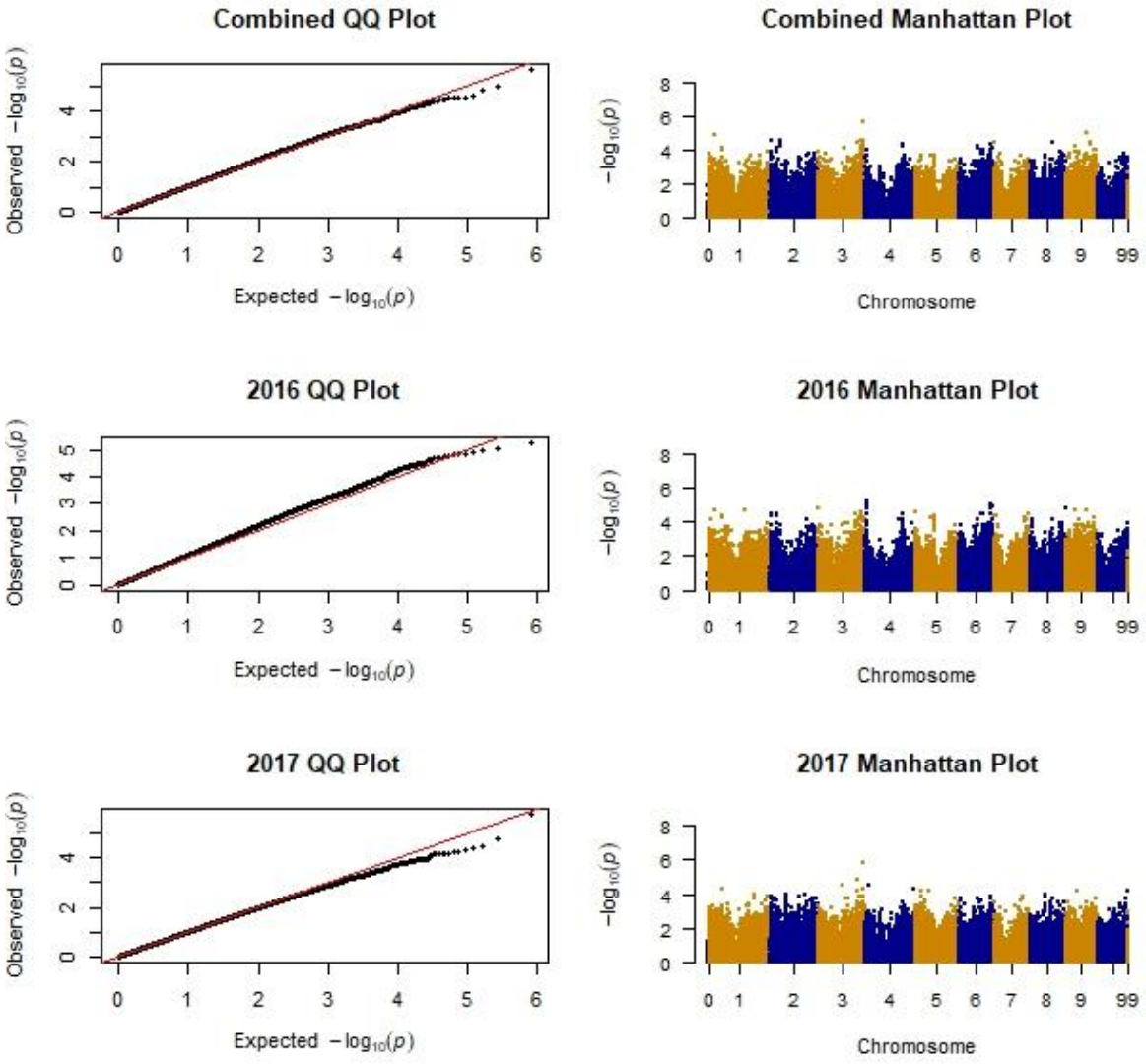


Figure 3.4. Effect of marker density on Pearson's correlation representing predictive ability for 100 iterations of genomic prediction for obtained from ridge regression best liner unbiased predictions (RR-BLUP) using five-fold cross validation. Datasets including 10,000, 25,000, and 51,471 markers all achieved comparable prediction accuracies.

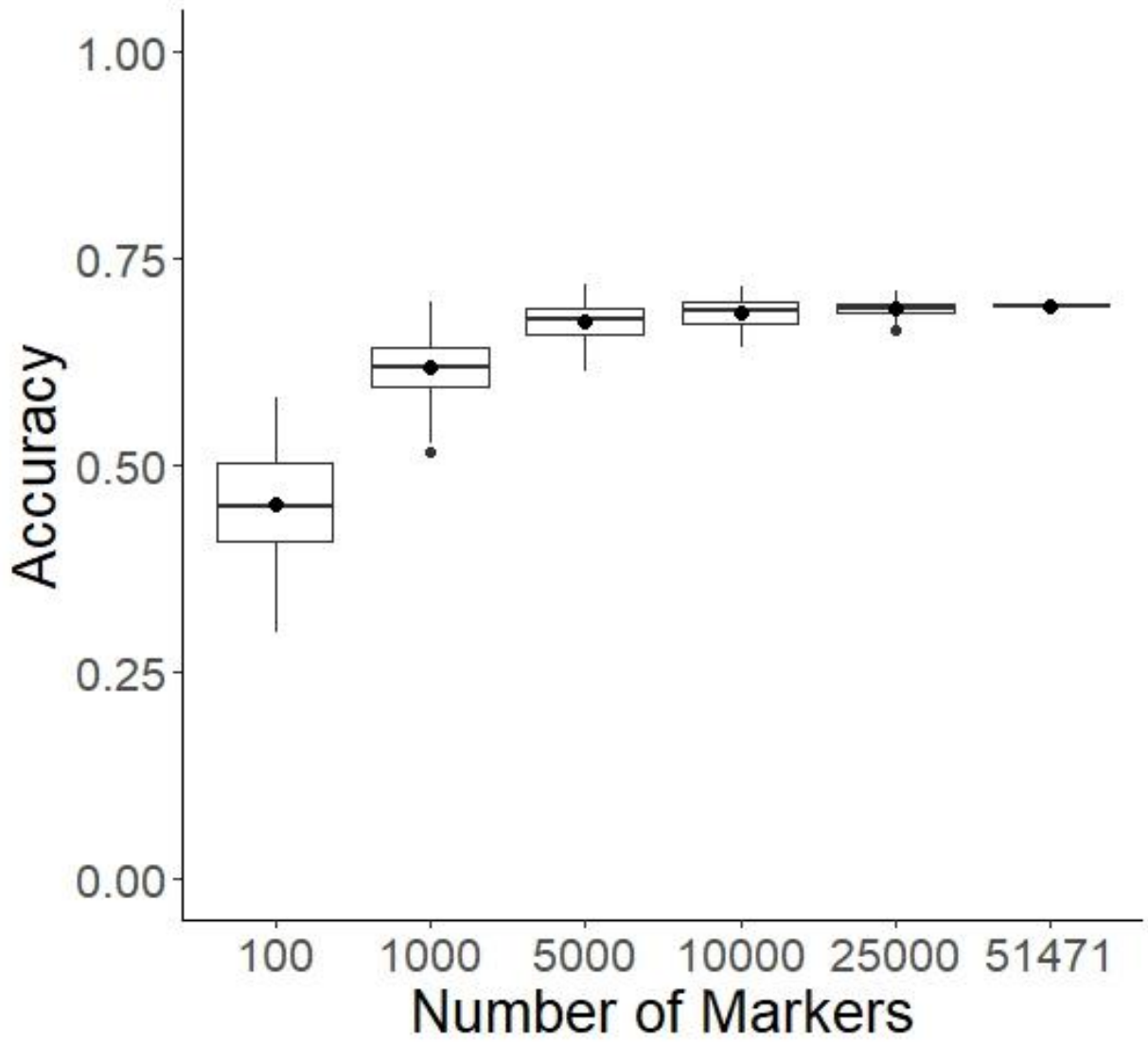
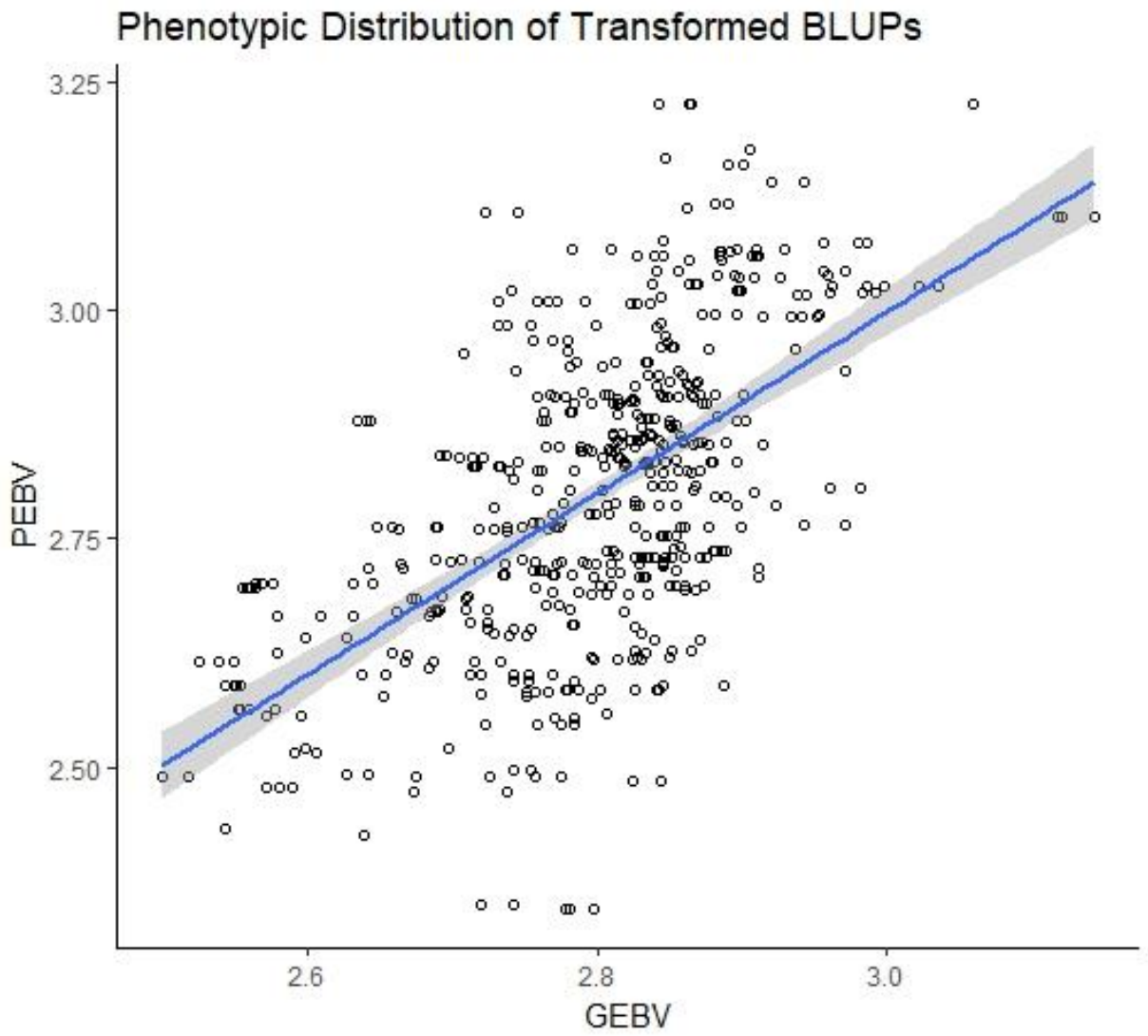


Figure 3.5. Relationship between phenotypically estimated breeding values (PEBVs) and genomic-estimated breeding values (GEBVs) obtained from ridge regression best linear unbiased prediction (RR-BLUP) using five-fold cross validation. The linear nature of our model y = 0.99x + 0.02 indicates an unbiased prediction model.



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APPENDIX A: SCALE FOR PHENOTYPING OF GOSS'S WILT

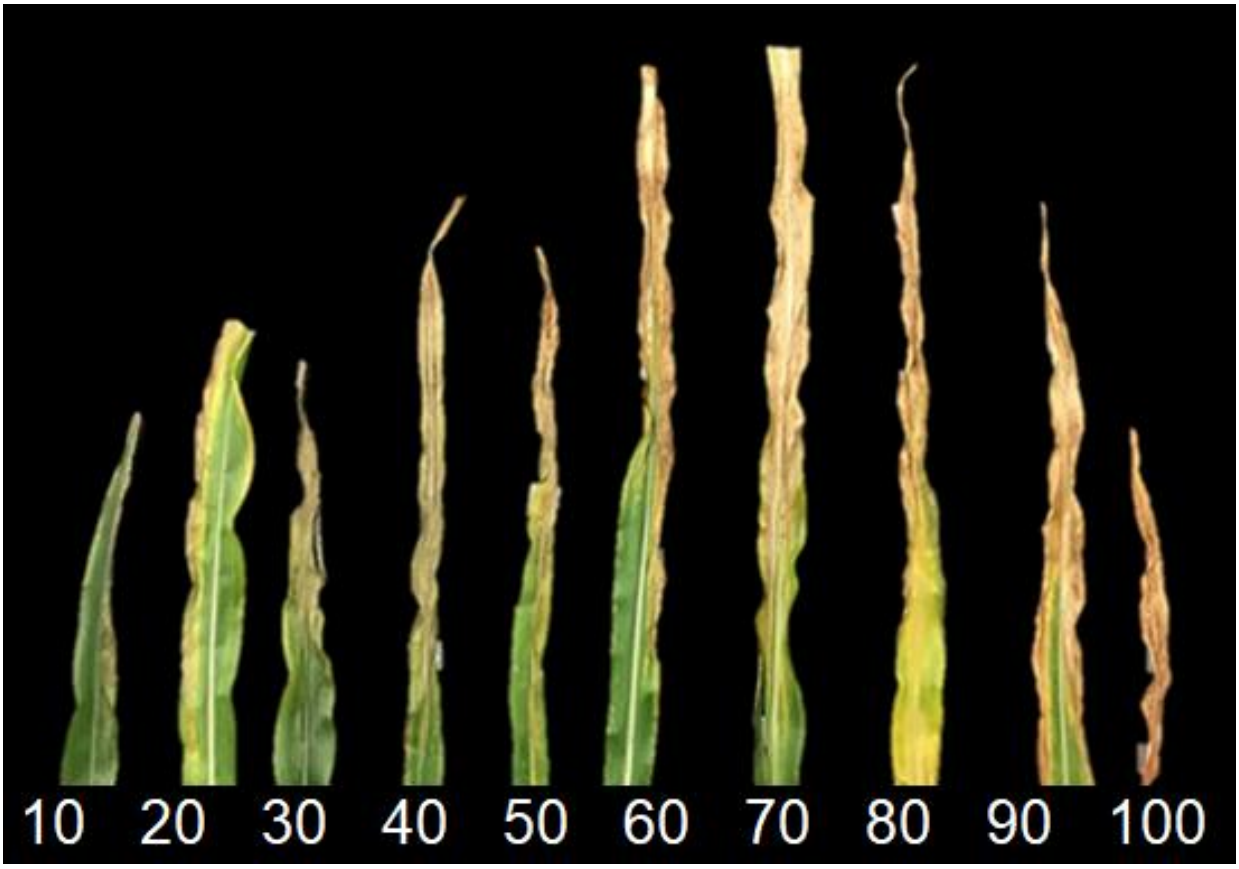

Figure A.1: Representative photograph for assigning diseased leaf area percentages to leaves infected by *Clavibacter michiganensis* subsp. *nebraskensis*. Numbers indicate the percentage of leaf area that is diseased.